Ophiostoma africanum sp. nov., and a key to ophiostomatoid species from *Protea* infructescences

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Two distinct and unrelated groups of ophiostomatoid fungi are associated with *Protea* infructescences. Two species, *Gondwanamyces proteae* and *G. capensis*, have unique anamorphs that reside in *Knoxdaviesia*. This anamorph genus is infrequently found amongst species of *Ceratocystis sensu lato*. The second group includes *Ophiostoma splendens* and *O. protearum* that have *Sporothrix* anamorphs typical of *Ophiostoma*. During recent collections of *Protea gaguedi* infructescences from the Northern Province in South Africa, an apparently new species of *Ophiostoma* with a *Sporothrix* anamorph was discovered. It can be distinguished from *O. splendens* and *O. protearum* based on its short ostiolar hyphae and its associated plant host. This teleomorph is described as *O. africanum* and the anamorph as *S. africanum*. Five species of ophiostomatoid fungi are now known from *Protea* infructescences and a key is presented for them.

INTRODUCTION

The ophiostomatoid fungi are ascomycetes sharing the morphological characteristics of spherical ascocarps with long necks and insect-associated habits (Upadhyay 1981, Wingfield, Seifert & Webber 1993). This group of fungi was previously placed in the genus *Ceratocystis*, but is now distributed amongst three genera including *Ceratocystis s. str.*, *Ophiostoma* and *Gondwanamyces*. Although morphologically similar, these taxa differ in cell wall composition (Rosinski & Campana 1964, Smith, Patik & Rosinksi 1967, Spencer & Gorin 1971, Jewell 1974, Weijman & de Hoog 1975; Marais 1996), anamorphs (Hunt 1956, de Hoog & Scheffer 1984, Mouton, Wingfield & Van Wyk 1994), and cycloheximide tolerance (Harrington 1981, de Hoog & Scheffer 1984, Marais 1996). These genera have also been shown to be phylogenetically unrelated based on sequence data and other molecular evidence (Hausner, Reid & Klassen 1993a, Spatafora & Blackwell 1994, Wingfield *et al*. 1994, Marais *et al*. 1998).

In recent years, two unique ophiostomatoid species have been recorded from *Protea* infructescences in the Western Cape Province of South Africa. Of these, *Gondwanamyces proteae* has falcate ascospores (Wingfield, Van Wyk & Marasas 1988) and *G. capensis* has allantoid ascospores (Wingfield & Van Wyk 1993). Hausner *et al*. (1993a) have suggested that *Ceratocystiopsis*, which was originally defined based on its falcate ascospores, cannot be maintained and that most of those species would better reside in *Ophiostoma*. Based on partial rDNA sequences of both the small and large subunit genes, these authors could not place *G. proteae* (as *Ceratocystiopsis proteae*) in either *Ophiostoma* or *Ceratocystis*. Using RFLP analysis of the rRNA operon of *G. protea* and *G. capensis* (Marais *et al*. 1998), it was shown that these two species are closely related to each other, but unrelated to species of *Ceratocystis s. str.* or *Ophiostoma*. A new genus, *Gondwanamyces*, was, therefore, established to accommodate them (Marais *et al*. 1998).

Two other ophiostomatoid fungi, *Ophiostoma splendens* (Marais & Wingfield 1994) and *O. protearum* (Marais & Wingfield 1997) have also been found in *Protea* infructescences. These species have *Sporothrix* states, which is a typical feature of *Ophiostoma*. Based on cycloheximide tolerance, *O. splendens* and *O. protearum* are also similar to the other species of *Ophiostoma*. It, therefore, appears that two unrelated ophiostomatoid groups have developed in *Protea* infructescences, although their respective origins remain unknown.

During the course of continuing surveys of *Protea* species in South Africa, an ophiostomatoid fungus was discovered in *P. gaguedi* infructescences. This fungus has a *Sporothrix* anamorph and, therefore, appears to be related to *Ophiostoma s. str.* and not to those species from *Protea* now accommodated in *Gondwanamyces*. The aim of this study was to describe this new fungus from *P. gaguedi*, and provide a key distinguishing the five species of ophiostomatoid fungi occurring in *Protea* infructescences.

MATERIALS AND METHODS

Protea gaguedi infructescences were collected from the Blyde River Canyon area of the Northern Province of South Africa (Fig. 1). Ascospore masses were removed from the apices of ophiostomatoid perithecia occurring on individual flowers and were transferred to 2% malt extract agar (MEA; 20 g Difco malt extract + 20 g Difco Bacto agar l^{-1} dH_2O). Cultures were incubated at 20 °C and microscopically examined after fruiting structures developed. The *Sporothrix* state was described from the culture, but as only protoperithecia developed in culture, the teleomorph was described from host tissue. Measurements of morphological structures were taken from 50 similar characteristic structures by using light microscopy. The results are expressed as minimum – maximum measurement (average).

The anamorph was examined using scanning electron microscopy (SEM). Blocks were cut from the agar (5–7 mm^3), fixed in glutaraldehyde, treated with 1% osmium tetroxide in a 0.1 M phosphate buffer (pH 7) and dehydrated in a graded acetone series. Specimens were then critical-point-dried, mounted, coated with gold/palladium and viewed with a JSM 6400 scanning electron microscope.

Growth of the *Sporothrix* at different temperatures was determined in the following way. Conidia were removed from a 1 week old culture using a sterile needle with a small piece of agar at the tip. The spore mass was placed on the agar surface in Petri dishes containing 20 ml MEA. Cultures were placed at temperatures ranging from 5 to 35 ° with 5 ° increments and incubated for 8 d in the dark. Six measurements were made by taking two diameter readings at right angles from each other, from each of three separate cultures. Results are presented as an average of the six readings.

Cycloheximide tolerance was tested by placing conidia from a 1 week old culture on MEA containing different concentrations of cycloheximide (0, 0.05, 0.1, 0.5, 1.0 and 2.5 g l^{-1}). Three plates of each cycloheximide concentration were inoculated and incubated for 8 d at 25 ° in the dark. Measurements were taken in the same way as those for the growth studies described above.

Dried plant material, representing the teleomorph as well as dried cultures of the anamorph, have been deposited in the National Collection of Fungi, Plant Protection Research Institute, Pretoria (PREM). Living cultures were deposited in the collection of The Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria.

TAXONOMY

A comparison of the *Ophiostoma* from *P. gaguedi* with the other four ophiostomatoid species associated with *Protea* species, showed that this fungus was morphologically distinct and that it differs from all previously described ophiostomatoid fungi in this niche and, indeed, from all other *Ophiostoma* species previously described. It is, therefore, described as new.

Ophiostoma africanum Marais & M. J. Wingf., **sp. nov.**

Perithecia superficialia, maturescentia solum in contexto hospitis, globosa inornata, basibus atris, 105–139 (121) µm diametro, colla atra laevia, 150–200 (168) µm longa, 23–35 (29) µm lata ad basim et 8–13 (10) µm ad apicem, terminantia in hyphis ostiolaribus brevibus, 5–8 (6) µm longa. In cultura protoperithecia sola formantur. Asci evanescentes. Ascosporae unicellulares hyalinae lunatae sine vaginis 3.1–4.3 (3.7) × 1.2 µm.

Perithecia superficial, maturing only on host tissue, globose, unornamented, with black bases, 105–139 (121) µm diam.

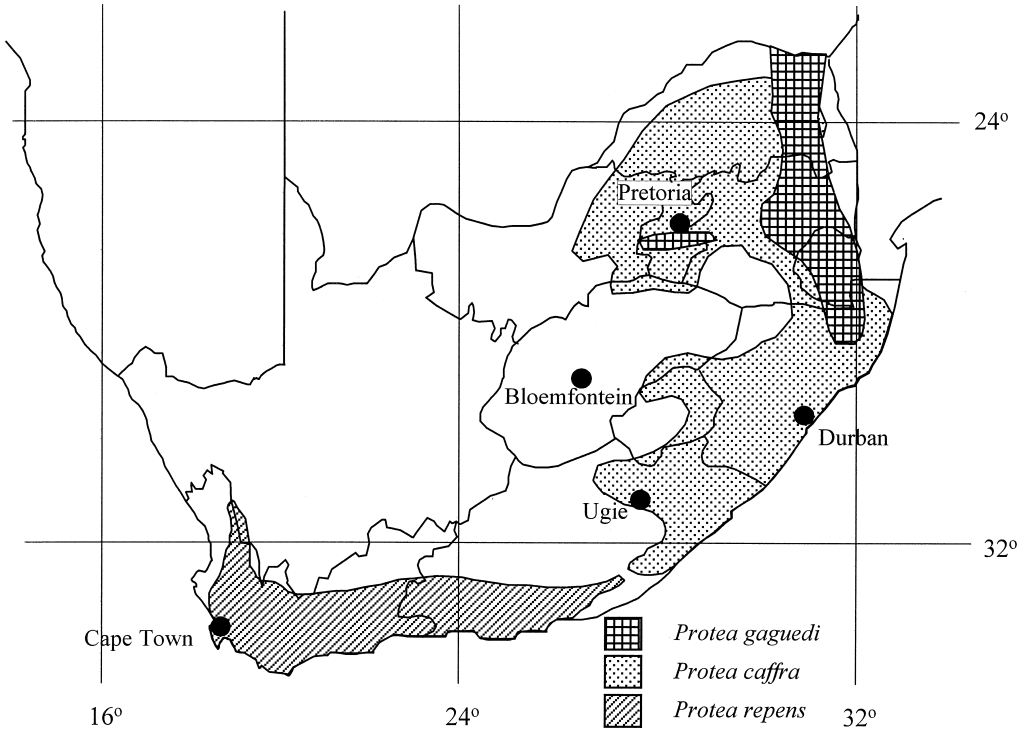
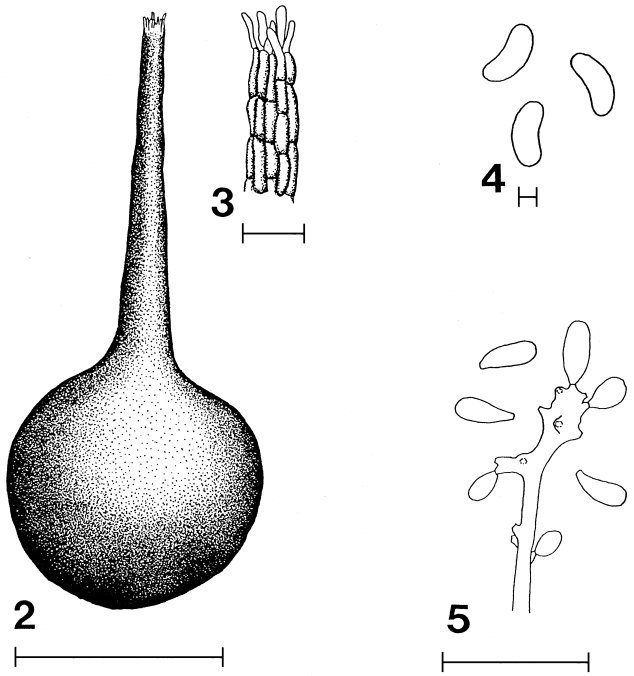


Fig. 1. Distribution of *Protea* species associated with ophiostomatoid fungi in South Africa.



Figs 2–5. *Ophiostoma africanum*. **Fig. 2.** Perithecium with a moderately long neck and unornamented base; bar = 100 µm. **Fig. 3.** Apex of perithecium with short ostiolar hyphae; bar = 10 µm. **Fig. 4.** Lunate ascospores without sheaths; bar = 1 µm. **Fig. 5.** *Sporothrix* anamorph with denticulate conidiogenous cells and smooth-walled clavate, hyaline conidia; bar = 10 µm.

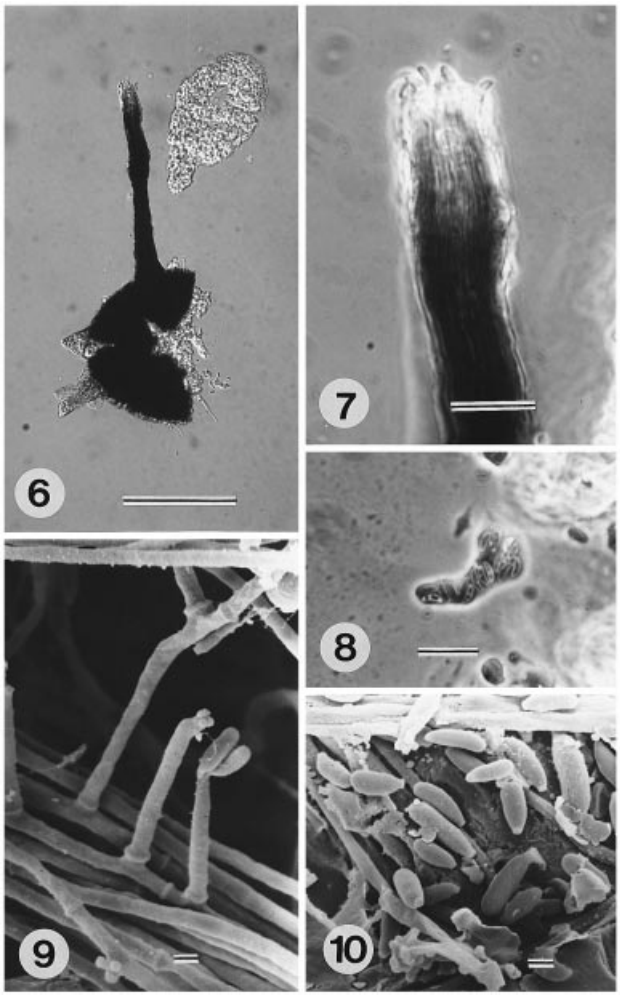
(Figs 2, 6); necks black, smooth-walled, 150–200 (168) µm long, 23–35 (29) µm wide at base and 8–13 (10) µm at the apex, terminating in short ostiolar hyphae, 5–8 (6) µm long (Fig. 3). In culture, only protoperithecia formed. *Asci* evanescent. *Ascospores* one-celled, hyaline, lunate, unsheathed, 3.1–4.3 (3.7) × 1.2 µm (Figs 4, 8).

Specimens examined: **South Africa**: Mpumalanga Province, Blyde River Canyon, on flowers in infructescences of *P. gaguedi* infested with insects, 22 June 1986, *M. J. Wingfield* (PREM 51892 – holotypus); *loc. cit.*, on insect-infested *P. gaguedi* flowers, 22 June 1986, *M. J. Wingfield* (PREM 51894); *loc. cit.*, on insect infested *P. gaguedi* flowers, January 1986, *M. J. Wingfield* (PREM 51896).

Sporothrix africanum Marais & M. J. Wingf., **sp. nov.**

Coloniae diam. 2.61 cm in MEA post 8 dies ad 25 °. Incrementum deminutum ad temperaturas supra vel infra 25 °. Sporulatio profusa in 2% MEA. Conidiophora micronematosa mononematosa hyalina spetata, 0.8–1.5 (1.2) µm lata et 10–62 (37) µm longa, gerentia cellas conidiogenas proliferantes sympodice per conidiotione, denticulis 0.3–0.6 (0.5) µm longis. Conidia quoque nata sunt in hyphis ipsis. Conidia holoblastica, hyalina, unicellularia, clavata, glabra, tenuitunicata, 1.3 × 4.1 µm, formata singulatim aggregescentia in mucosis massis.

Colonies 2.6 cm on MEA after 8 days at 25 °. Growth reduced at temperatures below and above 25 °. Sporulation profuse on 2% MEA. *Conidiophores* micronematous, mononematous, hyaline, septate, 0.8–1.5 (1.2) µm thick and 10–62 (37) µm long, bearing conidiogenous cells that proliferate sympodially during conidiation, becoming denticulate; denticles 0.3–0.6 (0.5) µm long. Conidia also produced directly on hyphae (Figs 5, 9). *Conidia* holoblastic, hyaline, 1-celled, clavate, smooth, thin-walled, 1.3 × 4.1 µm, formed singly becoming aggregated in slimy masses (Fig. 10).

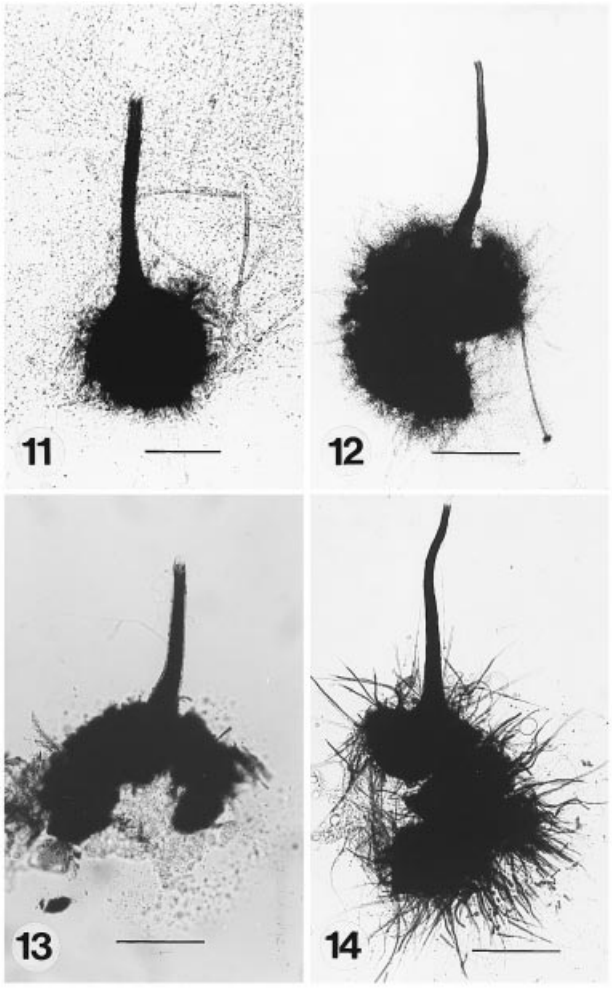


Figs 6–10. *Ophiostoma africanum*. **Fig. 6.** Perithecium broken at the base; bar = 100 µm. **Fig. 7.** Short convergent ostiolar hyphae at the apex of the neck; bar = 10 µm. **Fig. 8.** Lunate ascospores without sheaths; bar = 10 µm. **Fig. 9.** *Sporothrix* anamorph with denticulate conidiogenous cells; bar = 1 µm. **Fig. 10.** Smooth-walled clavate, hyaline conidia; bar = 1 µm.

Specimens examined; **South Africa**: Mpumalanga Province, Blyde River Canyon, on flowers in infructescences of *P. gaguedi* infested with insects, 22 June 1986, *M. J. Wingfield* (PREM 51893 – holotypus); *loc. cit.*, on insect-infested *P. gaguedi* flowers, 22 June 1986, *M. J. Wingfield* (PREM 51895); *loc. cit.*, on insect infested *P. gaguedi* flowers, January 1986, *M. J. Wingfield* (PREM 51897).

The mean colony diameter of *O. africanum* declined from 2.6 cm without cycloheximide to 1.9 cm on 2.5 g l^{-1} cycloheximide. Tolerance to high concentrations of cycloheximide is typical of species in the genus *Ophiostoma*.

O. africanum shares the same unusual niche as other ophiostomatoid species associated with *Protea* infructescences. However, it differs morphologically from the other species. *Gondwanamyces proteae* and *G. capensis* are characterised by having a *Knoxdaviesia* anamorph (Figs 19–20). In contrast, *O.*



Figs 11–14. Perithecial ascomata of four ophiostomatoid fungi from *Protea* infructescences. **Fig. 11.** *Gondwanamyces protea*; bar = 100 µm. **Fig. 12.** *G. capensis*; bar = 100 µm. **Fig. 13.** *Ophiostoma splendens*; bar = 100 µm. **Fig. 14.** *O. protearum*; bar = 100 µm. The bases of Figs 12–14 are damaged.

africanum has a *Sporothrix* anamorph indicating a closer affinity to *O. splendens* and *O. protearum*. *Ophiostoma splendens* lacks ostiolar hyphae, which are well developed in *O. africanum*. It also has allantoid ascospores which are different from the lunate ascospores of *O. protearum* and *O. africanum* (Fig. 17). *Ophiostoma protearum* differs from *O. splendens* and *O. africanum* in having prominent hyphal ornamentation at the perithecial bases and ostiolar hyphae (Fig. 14).

O. africanum is morphologically most similar to *Ceratocystis denticulata*, *Ceratocystiopsis crenulata*, *O. nigrocarpum* and *O. ulmi*. *Ceratocystis denticulata* has a *Sporothrix* anamorph (indicating that it should be transferred to *Ophiostoma*) and perithecial neck lengths that fall within the same range as those of *O. africanum*. *Ceratocystis denticulata*, differs however, in having allantoid ascospores and no ostiolar hyphae. *Ceratocystiopsis crenulata* has neck lengths that average 155 µm, but has divergent ostiolar hyphae, falcate ascospores and a *Hyalorhinocladiella* anamorph. *Ophiostoma nigrocarpum* has an average neck length of 173 µm, lunate ascospores and a *Sporothrix* anamorph, but lacks ostiolar hyphae. *O. ulmi* has a *Sporothrix* anamorph and a neck length averaging 200 µm but is also characterised by the presence of a *Pesotum* anamorph, which is absent in *O. africanum*.

DISCUSSION

The five ophiostomatoid species now known from *Protea* species are widely distributed in South Africa and occur abundantly in the infructescences of these plants (Fig. 1). They are, indeed, the most common fungi found in this niche. *Gondwanamyces proteae* is associated only with *P. repens* infructescences and is thus restricted to the parts of the country where this plant is found. This includes the Western Cape Province as well as the southern parts of the Eastern Cape Province. *Gondwanamyces capensis* has been found associated with *P. longifolia*, *P. neriifolia*, and *P. lepidocarpodendron*, but never with *P. repens*. These *Protea* species occur in the same region as *P. repens*. Consequently, *G. capensis* shares the same geographical distribution as *G. proteae*. *Ophiostoma protearum* is found only on *Protea caffra* and has been collected in the Eastern Cape Province, Drakensberg mountains (Kwazulu-Natal Province), and near Pretoria (Gauteng). *Ophiostoma africanum*, described in this study, was isolated in the Northern Province from the infructescences of *P. gaguedi* (Fig 1). *Protea gaguedi* occurs as far north as the equator (Rourke 1980) but whether *O. africanum* occurs outside southern Africa remains unknown.

The ophiostomatoid fungi associated with *Protea* species can be separated into two groups, differentiated by their anamorphs (Figs 19–22). The first group includes three species most easily identified by their *Sporothorix* states (Figs 21–22). *Ophiostoma protearum* is distinguished from the other two species by the hyphal ornamentation at the base of the ascomata (Fig. 23). Ascospore lengths can be used to distinguish between *O. spelndens* and *O. africanum*. *O. splendens*

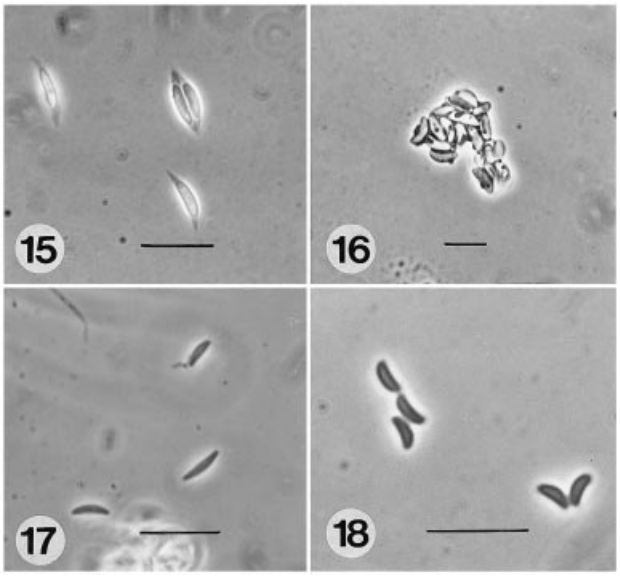
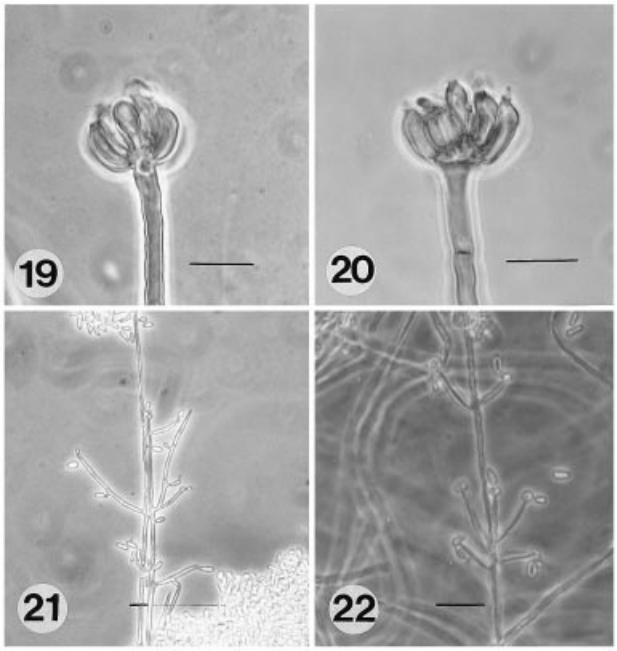


Fig. 15–18. Ascospores of four ophiostomatoid fungi from *Protea* infructescences. **Fig. 15.** *Gondwanamyces protea*; bar = 10 µm. **Fig. 16.** *G. capensis*; bar = 10 µm. **Fig. 17.** *Ophiostoma splendens*; bar = 10 µm. **Fig. 18.** *O. protearum*; bar = 10 µm.



Figs 19–22. Anamorph states of four ophiostomatoid fungi from *Protea* infructescences. **Fig. 19.** *Gondwanamyces protea*; bar = 10 μm. **Fig. 20.** *G. capensis*; bar = 10 μm. **Fig. 21.** *Ophiostoma splendens*; bar = 1 μm. **Fig. 22.** *O. protearum*; bar = 10 μm.

has ascospores that are on average 5.7 μm long (Marais & Wingfield 1994), while those of *O. africanum* are on average 3.3 μm long. In cases where no ascospores are produced, these two species can be distinguished by the presence of short ostiolar hyphae at the apex of the ascomata of *O. africanum* (Fig. 23).

Although *G. proteae* and *G. capensis* are closely related, they can nevertheless be distinguished from each other by their mature ascomata. The most obvious difference between these species are the non-sheathed, allantoid ascospores of *G. capensis* (Wingfield & Van Wyk 1993) (Fig. 16) and the apparently sheathed, elongate ascospores of *G. proteae* (Wingfield *et al*. 1988) (Fig. 15). In the absence of teleomorphs, it is more difficult to discriminate between these species. There are slight differences in the anamorphs such as the more robust rhizoids and larger conidiogenous cells and colarettes in *Knoxdaviesia capense* (Wingfield & van Wyk 1993). None of these characteristics are, however, obvious except where cultures of both species are available for comparison (Figs 19–20). Host specialisation of these two species also appears to be a valuable taxonomic characteristic.

On host tissue, the anamorphs of ophiostomatoid fungi from *Protea* infructescences are seldom visible. In such cases, ascomatal characteristics are essential for the identification of the species. Only one species, *Ophiostoma protearum*, is characterised by the presence of hyphal ornamentation at the perithecium base. Although *G. proteae* and *G. capensis* have some hyphal growth around the ascomatal bases (Figs 11–12), it is not as obvious as in *O. protearum* (Fig. 14). Wingfield *et al*. (1988) and Wingfield & Van Wyk (1993) described *G. proteae* and *G. capensis*, respectively, as having unornamented ascomatal bases but we now recognise that this was not accurate.

Four of the five ophiostomatoid species from *Protea* infructescences are characterised by the presence of ostiolar hyphae at the apex of the perithecial neck. *O. splendens* is the only species lacking ostiolar hyphae (Marais & Wingfield 1994). *O. protearum* has the most prominent ostiolar hyphae and these average 17 μm long (Marais & Wingfield 1997) while *O. africanum* has ostiolar hyphae that average 6 μm long. Both *G. proteae* and *G. capensis* have indistinct ostiolar hyphae that are shorter than 5 μm.

Gondwanamyces proteae is characterised by apparently sheathed ascospores. Wingfield & Van Wyk (1993) noted that the sheaths are not rigid and cold represent remains of the perithecial cavity, which is unlike those in other species of *Ophiostoma* (Van Wyk & Wingfield 1991) and *Ceratocystis s. str*. (Van Wyk, Wingfield & Van Wyk 1991). In the absence of a sheath, the ascospores of *G. proteae* are approximately the same size as those of *G. capensis*. There is, however, a substantial difference in the sizes of ascospores in these fungi and those with *Sporothrix* states. Ascospores in *G. proteae* and *G. capensis* average 10 μm and 6 μm long, respectively. The average length of ascospores in *O. splendens* is 5.7 μm, which comes closest to those of *G. capensis*. There is also a clear difference between the shapes of the ascospores of these two species (Fig. 23). *Ophiostoma protearum* and *O. africanum* have ascospores averaging 3.3 and 3.7 μm, respectively. The width of ascospores can also be used to distinguish among species of *Gondwanamyces* and *Ophiostoma* that are associated with *Protea* infructescences. *G. proteae* and *G. capensis* have ascospores much wider than those of the *Ophiostoma* species (Fig. 23). The shape and width of the conidia can also be used to separate species of *Gondwanamyces* and *Ophiostoma* from *Protea* species (Fig. 23).

The five ophiostomatoid species associated with *Protea* infructescences apparently represent two distinct phylogenetic lineages (Marais *et al*. 1998). One group, which includes *Gondwanamyces proteae* and *G. capensis*, appears to have developed independently from other ophiostomatoid species. The other group, including *Ophiostoma splendens*, *O. protearum* and *O. africanum*, resemble species in *Ophiostoma*. Based on RFLP analysis (Marais *et al*. 1998) and cycloheximide sensitivity (Wingfield *et al*. 1988, Wingfield & Van Wyk 1993) species with *Knoxdaviesia* anamorphs are more closely related to *Ceratocystis s. str*. than to species of *Ophiostoma*. There is, however, no evidence of any other species in *Ceratocystis* that share morphological characteristics with those species having *Knoxdaviesia* anamorphs.

That the five species of ophiostomatoid fungi associated with *Protea* infructescences are apparently host specific suggests that these organisms have developed distinctive relationships with their hosts. At present, the basis of this host specificity is not known. As previously speculated (Marais & Wingfield 1994), this could be due to the nutritional characteristics specific to certain *Protea* species or, more likely, specific insect vectors associated with these plants. All morphological characteristics of these fungi suggest that they are associated with insects and determining the nature of this relationship promises to be intriguing.

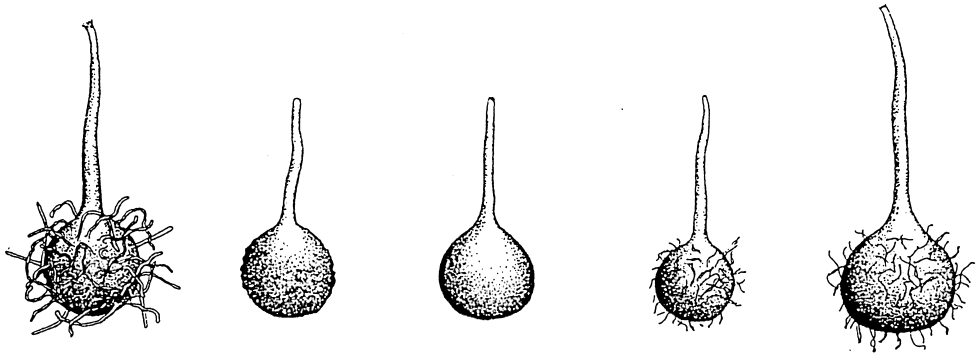
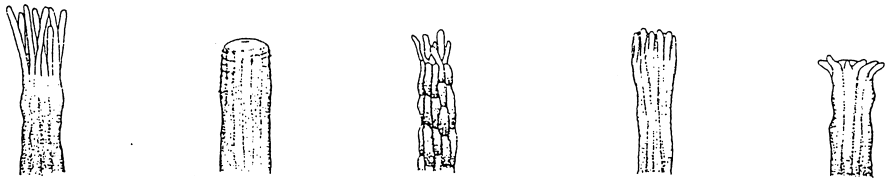
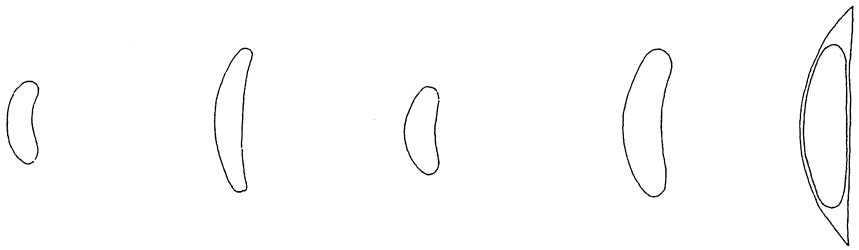
All measures in µm	*Ophiostoma protearum*	*Ophiostoma splendens*	*Ophiostoma africanum*	*Gondwanamyces capensis*	*Gondwanamyces proteae*
Ascomata: 100 µm					
Base size (diam.)	93-167 (130)	100-150 (122)	105-139 (121)	68-194 (106)	99-216 (151)
Ornamentation:	Yes	No	No	Yes	Yes
Neck length:	217-267 (245)	143-217 (168)	150-200 (168)	102-270 (194)	168-354 (285)
Neck at apex: 10 µm					
Length of ostiolar hyphae:	14-19 (17)	Absent	5-8 (6)	Indistinct	2-7 (2)
Ascospores: 1 µm					
Ascospore length	3.1-3.8 (3.3)	5.5-6.0 (5.7)	3.1-4.3 (3.7)	4.5-8 (6)	7-13 (10)
Ascospore width:	1.2	1-1.2 (1.1)	1.2	2-3.5 (2.8)	2-4 (3)
Anamorph states: *Sporothrix*: 10 µm *Knoxdaviesia*: 10 µm					
Conidial length:	3.9	5.9	4.1	4-7 (5)	4-7 (5)
Conidial width:	1.4	1.7	1.3	3-5 (3.8)	2-4 (3)
Conidiophore length:	6.2-16.0 (10.8)	8.7-21.7 (12.6)	10-62 (37)	33-93 (65.6)	69-198 (132)

Fig. 23. A comparison of teleomorph and anamorph characteristics of five ophiostomatoid fungi from *Protea* infructescences.

Key to ophiostomatoid fungi from *Protea* spp.

1	Teleomorph present	2
	Teleomorph absent	6
2(1)	Ornamentation at the base of the perithecium	3
	Ornamentation lacking at the base of the perithecium	5
3(2)	Ostiolar hyphae longer than 10 µm.	**Ophiostoma protearum**
	Ostiolar hyphae shorter than 10 µm	4
4(3)	Ascospores allantoid	**Gondwanamyces capensis**
	Ascospores faclate.	**Gondwanamyces proteae**
5(2)	Ascospores longer than 5 µm.	**Ophiostoma splendens**
	Ascospores shorter than 5 µm	**Ophiostoma africanum**
6(1)	*Knoxdaviesia* anamorph present	7
	Sporothrix anamorph present	8
7(6)	Associated with *Protea repens* infructescences	**Gondwanamyces proteae**
	Associated with protea infructescences other than *P. repens*	**Gondwanamyces capensis**
8(6)	Associated with protea infructescences other than *Protea caffra* and *P. gaguedi*	**Ophiostoma splendens**
	Associated with *Protea caffra* infructescences	**Ophiostoma protearum**
	Associated with *Protea gaguedi* infructescences	**Ophiostoma africanum**

ACKNOWLEDGEMENTS

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REFERENCES

Harrington, T. C. (1981) Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. *Mycologia* **73**: 1123–1129.

Hausner, G., Reid, J. & Klassen, G. R. (1993a) *Ceratocystiopsis*: a reappraisal based on molecular criteria. *Mycological Research* **97**: 625–633.

Hausner, G., Reid, J. & Klassen, G. R. (1993b) On the subdivision of *Ceratocystis s. l.*, based on partial ribosomal DNA sequences. *Canadian Journal of Botany* **71**: 52–63.

Hoog de, G. S. & Scheffer, R. J. (1984) *Ceratocystis* versus *Ophiostoma*: a reappraisal. *Mycologia* **76**: 292–299.

Hunt, J. (1956) Taxonomy of the genus *Ceratocystis*. *Lloydia* **19**: 1–58.

Jewell, T. R. (1974) A quantitative study of cellulose distribution in *Ceratocystis* and *Europhium*. *Mycologia* **66**: 139–146.

Marais, G. J. (1996) *Fungi associated with infructescences of Protea species with special reference to the Ophiostomatales*. PhD thesis, University of the Free State, Bloemfontein.

Marais, G. J. & Wingfield, M. J. (1994) Fungi associated with infructescences of *Protea* species in South Africa, including a new species of *Ophiostoma*. *Mycological Research* **98**: 369–374.

Marais, G. J. & Wingfield, M. J. (1997) *Ophiostoma protearum* sp. nov. associated with *Protea caffra* infructescences. *Canadian Journal of Botany* **75**: 362–367.

Marais, G. J., Wingfield, M. J., Viljoen, C. D. & Wingfield, B. D. (1998) A new ophiostomatoid genus from *Protea* infructescences. *Mycologia* **90**: 136–142.

Mouton, M., Wingfield, M. J. & Van Wyk, P. S. (1994) Conidium development in anamorphs of *Ceratocystis sensu lato*: a review. *South African Journal of Science* **90**: 293–298.

Rosinski, M. A. & Campana, R. J. (1964) Chemical analysis of the cell wall of *Ceratocystis ulmi*. *Mycologia* **56**: 738–744.

Rourke, J. P. (1980) *Die Proteas van Suid Afrika*. Table Mountain Publishers Incorporated, Cape Town.

Smith, M. J., Patik, C. M. & Rosinski, M. A. (1967) A comparison of cellulose production in the genus *Ceratocystis*. *Mycologia* **59**: 965–969.

Spatafora, J. W. & Blackwell, M. (1994) The polyphyletic origins of ophiostomatoid fungi. *Mycological Research* **98**: 1–9.

Spencer, J. F. T. & Gorin, P. A. J. (1971) Systematics of the genera *Ceratocystis* and *Graphium*. Proton magnetic resonance spectra of the mannose-containing polysaccharides as an aid in classification. *Mycologia* **63**: 387–402.

Van Wyk, P. W. J. & Wingfield, M. J. (1991) Ultrastructure of ascosporogenesis in *Ophiostoma davidsonii*. *Mycological Research* **95**: 725–730.

Van Wyk, P. W. J., Wingfield, M. J. & Van Wyk, P. S. (1991) Ascospore development in *Ceratocystis moniliformis*. *Mycological Research* **95**: 96–103.

Upadhyay, H. P. (1981) *A Monograph of Ceratocystis and Ceratocystiopsis*. University of Georgia Press, Athens, GA.

Weijman, A. C. M. & de Hoog, G. S. (1975) On the subdivision of the genus *Ceratocystis*. *Antonie Van Leeuwenhoek* **41**: 353–360.

Wingfield, B. D., Grant, W. S. Wolfaardt, J. F. & Wingfield, M. J. (1994) Ribosomal RNA sequence phylogeny is not congruent with ascospore morphology among species in *Ceratocystis sensu stricto*. *Molecular and Biological Evolution* **11**: 376–383.

Wingfield, M. J., Seifert, K. A. & Webber, J. F. (eds) (1993) *Ceratocystis and Ophiostoma: taxonomy, ecology & pathogenicity*. APS Press, St Paul, MA.

Wingfield, M. J., Van Wyk, P. S. & Marasas, W. F. O. (1988) *Ceratocystiopsis proteae* sp. nov., with a new anamorph genus. Mycologia **80**: 23–30.

Wingfield, M. J. & Van Wyk, P. S. (1993) A new species of *Ophiostoma* from *Protea* infructescences in South Africa. *Mycological Research* **97**: 709–716.

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