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Fungi occurring on Proteaceae in Australia: selected foliicolous species

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Abstract

This paper represents the first of a series dealing with fungi associated with Proteaceae cultivated in Australia. Several leaf pathogens are newly recorded, including *Botryosphaeria proteae*, *Coleroa senniana*, *Kabatiella proteae*, *Mycosphaerella jonkershoekensis* and *Mycosphaerella lateralis* from *Protea* spp. The host range of *Phyllosticta telopea* is expanded to also include *Grevillea*, *Leucadendron* and *Leucospermum*, and a Pestalotiopsis leaf spot known previously from Africa only is also reported on *Protea* and *Telopea*. *Cercostigmina protearum* var. *hakeae*, known from *Hakea* in South Africa, is also newly reported on *Grevillea* in Australia. New species described include *Leptosphaeria telopeae* on *Telopea*, *Mycosphaerella buckinghamiae* on *Buckinghamia* and *Ramularia proteae* on *Protea*.

Introduction

Given their beauty, unique appearance and relatively long shelf life, flowers of the Proteaceae represent a sought after crop for the export market (Crous and Palm 1999). One of the major impediments for exporting this crop, however, is their diseases. Although several of these may cause significant losses to plants in the field and nurseries, others are considered to be more important because they are regarded as actionable quarantine organisms. However, pathogenicity studies have shown that most of these pathogens are highly specific to certain species of Proteaceae only (Taylor and Crous 1998; Denman *et al.* 1999; Taylor *et al.* 1999), and in this regard pose no threat to countries importing these flowers. Furthermore, many of these pathogens have in the past already been introduced to other countries as endophytes or latent pathogens with seed or cuttings. For the host specificity reasons stated above, these introductions are usually only of concern to others farming Proteaceae in the area. However, the presence of these fungal pathogens is considered a threat to the cultivation of Proteaceae in the centres of origin (Australia and South Africa), or elsewhere, where they are being developed as new crops (United States of America (USA) – California, Hawaii; Europe – Portugal, Spain; Middle East – Israel).

In South Africa, the most important commercially cultivated genera are *Protea* (L.) L., *Leucospermum* R. Br. and *Leucadendron* R. Br. These genera have also been introduced to Australia and are cultivated extensively. However, in Australia there is a much wider diversity of Proteaceae, and also *Telopea* R. Br., *Banksia* L. f. and *Grevillea* R. Br. ex Knight, among others, are commercially cultivated. In recent years it has become apparent that several pathogens of Proteaceae have been introduced to Australia from South Africa, while many other fungi, unique to Australian Proteaceae, remain to be described. The present study forms the first in a series dealing with the fungi occurring on Proteaceae in Australia.

Methods

Leaf and stem samples of diseased Proteaceae were collected and the associated fungi cultured. Symptomatic material devoid of fruiting structures was either placed in damp chambers, or

stored dry in plastic bags at 4°C for approximately one month (Taylor and Crous 2000). Single-conidium colonies were established on 2% malt-extract agar (MEA; Biolab, Midrand, South Africa), supplemented with 0.1 g/L streptomycin sulphate, then transferred to divided plates containing MEA and carnation-leaf-piece agar (CLA; Fisher *et al.* 1982) to encourage sporulation. Single-ascospore colonies were established using the technique described by Crous *et al.* (1991). Cultures were incubated for 14–28 days at 25°C under continuous near-ultraviolet light to enhance sporulation. Colony growth was measured, characteristics noted and the colour rated using cultures incubated for 7–14 days at 25°C in the dark (Rayner 1970). For microscopic examination fungal structures and conidia were mounted in lactophenol and measurements made at 1000× magnification. The 95% confidence intervals were determined from 30 observations and the minimum and maximum ranges given in parentheses. Herbarium specimens are lodged at NSW Agriculture Herbarium, Orange (DAR) and reference cultures are maintained in the culture collections of DAR and the Department of Plant Pathology, University of Stellenbosch (STE-U).

Results and Discussion

Botryosphaeria proteae (Wakefield) Denman & Crous, Mycologia 91: 511. 1999.
≡ *Phyllachora proteae* Wakefield, Kew Bull. 1922: 164. 1922.
Anamorph. *Fusicoccum proteae* Denman & Crous, Mycologia 91: 511. 1999.

The morphology and cultural characteristics of this species were described in full by Denman *et al.* (1999). Australian collections sporulated well in culture and formed both conidium types on MEA. This is the first record of *B. proteae* occurring in Australia. This pathogen has recently been documented as a common endophyte of *Protea* spp. (Swart *et al.* 2000) and it is possible that it could have been introduced in this manner to Australia. Thus far it has been recorded on *Protea* and *Leucospermum* spp., and inoculations on other Proteaceae have proven unsuccessful (unpublished data), suggesting that it is a rather host-specific leaf pathogen.
Australian hosts: *Protea* spp.
Distribution: Australia (Tasmania), South Africa and the USA (Hawaii).
Specimens examined: Australia, Tasmania, Royal Tasmanian Botanic Gardens, Hobart, leaves of *Protea magnifica* Link, Aug. 1999, Alan Macfadyen, JT1009, DAR 74847; leaves of *Protea eximia* (Knight.) Fourc., Aug. 1999, Alan Macfadyen, JT1006, DAR 74848, STE-U 3142-3244; leaves of *Protea obtusifolia* H. Buek ex Meisn., Aug. 1999, Alan Macfadyen, JT1003, DAR 74849, STE-U 3076, 3149-3151.

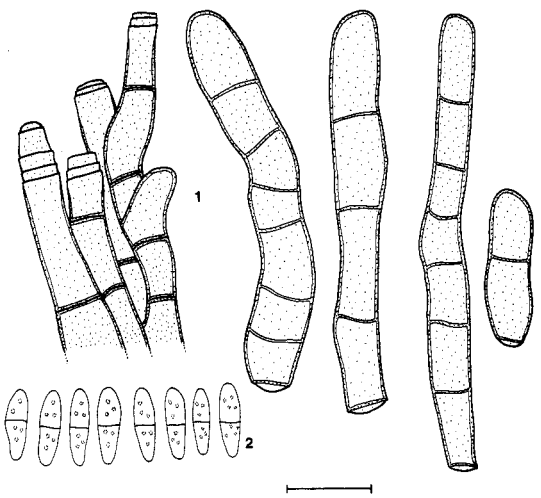
Coleroa senniana (Sacc.) Arx, Die Gattungen der Didymosporen Pyrenomyceten. Beiträge zur Kryptogamenflora der Schweiz 11: 418. 1962
≡ *Phaeosphaerella senniana* Sacc., Annls mycol. 8: 337. 1910.
≡ *Aphysa senniana* (Sacc.) Doidge, Bothalia 4: 213. 1941.
= *Thyriopsis proteae* Van der Byl, S. Afr. J. Sci. 26: 318. 1929.
Anamorph: Unknown.

The morphology of this pathogen was treated in full by Taylor and Crous (1998). By examining Australian herbarium specimens (BPI) of Proteaceae intercepted by APHIS at various ports of entry into the USA, we were aware that *C. senniana* occurred on *Banksia* in Australia (Taylor and Crous 1998). In the present study, we found that this pathogen was also well represented on *Protea* spp. in Australia. This was also supported by *Protea* specimens with *Coleroa* fruiting bodies lodged in DAR (72413, 74598). Although *C. senniana* is a well-known pathogen of these hosts in South Africa, it has never been fully documented in Australia. This pathogen is common and well established on Proteaceae in southern Africa, and it is thus possible that like *B. proteae*, it has also been introduced to Australia.
Australian hosts: *Banksia* and *Protea* spp.
Distribution: Australia (NSW, Tasmania), Eritrea, New Zealand, South Africa.
Specimens examined: Australia, Tasmania, Royal Tasmanian Botanic Gardens, Hobart, on leaves of *P. magnifica*, Aug. 1999, Alan Macfadyen, JT 1008, DAR 74850; Tasmania, Royal Tasmanian Botanic Gardens, Hobart, on leaves of *Protea* cv. Pink Ice, Aug. 1999, Alan Macfadyen, JT 1005, DAR 74856; Tasmania, Royal Tasmanian Botanic Gardens, Hobart, on leaves of *Protea* sp., Aug.

1999, Alan Macfadyen, JT 1007, DAR 74854; NSW, Royal Botanic Garden, Sydney, on leaves of *Protea* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 924, DAR 74851; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea pudens* Rourke, Aug. 1999, P.W. Crous & B. Summerell, JT 966, DAR 74852; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea susannae* Phill., Aug. 1999, P.W. Crous & B. Summerell, JT 970, DAR 74853; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea lorifolia* (Salisb. ex Knight) Fourc., Aug. 1999, P.W. Crous & B. Summerell, JT 941, DAR 74855; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea lepidocarpodendron* (L.) L., Aug. 1999, P.W. Crous & B. Summerell, JT 994, DAR 74857; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea magnifica*, Aug. 1999, P.W. Crous & B. Summerell, JT 945, DAR 74858; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 910, DAR 74859; NSW, Royal Botanic Garden, Sydney, on leaves of *Protea* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 954, DAR 74860.

Cercostigmina protearum* var. *hakeae U. Braun & Crous, Sydowia 46: 206. 1994. Figures 1, 2
Teleomorph: Undescribed *Mycosphaerella* sp.

Leaf spots amphigenous, irregular to sub-circular, 6–10 mm diameter, grey on the adaxial surface, with a dark brown border, and grey to light brown on the abaxial surface with an indistinct border. *Mycelium* internal and external, light brown, consisting of septate, branched, smooth to finely verruculose hyphae, 3–4 µm wide. *Caespituli* acervular to sporodochial, predominantly epiphyllous on leaves, up to 250 µm wide and 150 µm high. *Conidiophores* aggregated in dense fascicles arising from the upper cells of a dark brown stroma up to 200 µm wide and 80 µm high; conidiophores medium brown, smooth to finely verruculose, 1–5 septate, subcylindrical, straight to geniculate-sinuous, unbranched or branched above, 20–60 × 4–7 µm. *Conidiogenous cells* terminal, unbranched, light brown, smooth, tapering to flat-tipped apices, rarely proliferating sympodially, predominantly proliferating 1–5 times percurrently, 10–30 × 4–5 µm. *Conidia* solitary, subcylindrical with irregular swellings, apex obtuse, base truncate, straight to curved, (1–)3–6(–12) septate, (20–)35–50(–100) × 4–5(–6) µm; hila inconspicuous with a minute marginal frill.



Figures 1, 2 *Cercostigmina protearum* var. *hakeae* and its *Mycosphaerella* teleomorph. **1.** Conidiophores and conidia. **2.** Ascospores. Bar = 10 µm.

Presently only one cercosporoid species is known from *Grevillea*, namely *Pseudocercospora agharkarii* (Chidd) Crous & M.E. Palm, which has subcylindrical, olivaceous conidia that are (1–)3–6(–11) septate, (35–)45–70(–95) × (3–)3.5–4.5(–5) µm. *Cercostigmina protearum* var. *hakeae* is distinct from this species in having darker, wider conidia with marginal basal frills, and conidiogenous cells that proliferate percurrently.

Two collections were made from *Grevillea* spp., namely JT926 and JT 873. One collection (JT 926) had caespituli that were frequently found to occur on immature pseudothecia. This specimen also closely matched the type specimen of *C. protearum* var. *hakeae* (PREM 51117). The second collection (JT 873), differed in that the conidia were slightly darker brown, and frequently up to 6 µm wide. Further collections are required, however, to establish if this is a distinct species, and for the present, we are of the opinion that it can be accommodated within *C. protearum* var. *hakeae*. Some mature pseudothecia were also observed on this specimen. Pseudothecia were globose, brown, and up to 100 µm wide. Ascospores were fusoid, guttulate, not constricted at the septum and widest in the middle of the apical cell (7–)8–9(–10) × 2–3 µm. Insufficient material was available to fully describe the teleomorph of this fungus, and further collections would be required to do so. *Hakea* is a weed that was introduced to

South Africa from Australia and it appears that this pathogen accompanied it to Africa.
Cultural characteristics: Colonies reaching 6 mm diameter after 14 days at 25°C; margins uneven, colonies erumpent, olivaceous grey (23""'i) on the surface, greenish black (33""'k) underneath, with sparse aerial mycelium.
Australian hosts: *Grevillea* spp.
Distribution: Australia (NSW), South Africa.
Specimens examined: Australia, NSW, Mount Annan Botanic Gardens, on leaves of *Grevillea* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 926, DAR 74861, STE-U 2968; Mount Tomah Botanic Gardens, on leaves of *Grevillea* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 873, DAR 74862, STE-U 3145-3148, 3366-3368; South Africa, Northern Province, Louis Trichardt, Hangklip Forest Station, *Hakea salicifolia* (Vent.) B.L. Burt. (= *H. saligna* Knight), Apr. 1988, C. Roux, PREM 51117 (holotype).

Kabatiella proteae J.E. Taylor & Crous, Mycol Res. 104: 619. 2000. Figures 3–5
Teleomorph: Unknown.

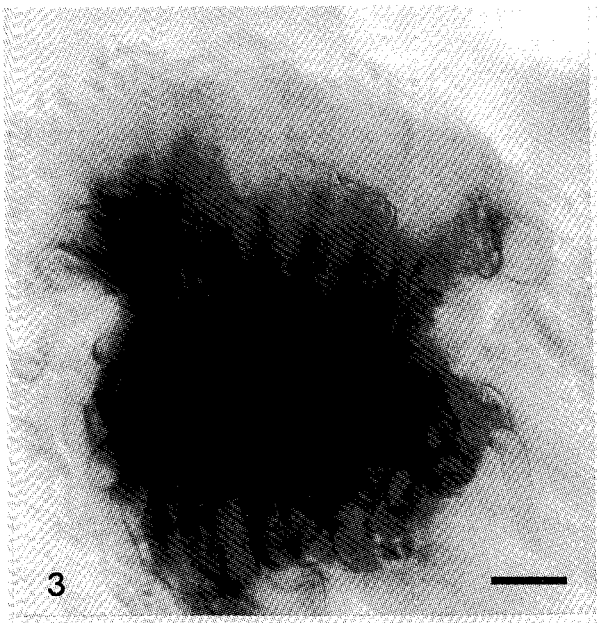
The morphology of this pathogen was recently dealt with by Taylor and Crous (2000), who described it from leaf spots on *Protea cynaroides* from the Western Cape of South Africa. The present collection represents the first record of this pathogen from Australia, where it occurred in association with leaf spots of *Mycosphaerella jonkershoekensis*. The morphology of the Australian collection compared well with that of the South African specimen (Taylor and Crous 2000). In culture, conidia were solitary, aseptate, ellipsoidal, with a minute truncate base that was visible on larger conidia, hyaline, thin-walled, (4–)6–7(–9) × (2–)2.5–3(–3.5) µm. As no cultures were previously available, a cultural description is provided below.
Cultural characteristics: Colonies reaching 76 mm diameter after 14 days at 25°C; colonies with radial striations appearing in the agar and visible from underneath; aerial mycelium moderate to sparse, olivaceous grey (23""'i) on the surface, olivaceous grey to iron grey (23""'i – 23""'k) underneath.
Australian host: Protea cv. Pink Ice.
Distribution: Australia (NSW) and South Africa.
Specimen examined: Australia, NSW, Oakdale, 430 Steveys Forest Rd., on leaves of *Protea* cv. Pink Ice, Jul. 1999, L. Taylor, WAR 40A-D, JT 1045, DAR 74863, STE-U 2868.

Leptosphaeria telopeae Crous & Summerell, sp. nov. Figures 6–10
Anamorph: Unknown.

Ascomata dispersa, globosa, demum erumpentia usque 200 µm diam.; pariete stratis 4–6 texturae angularis. *Asci* numerosi, sessiles, cylindrico-clavati, 35–50 × 6–8 µm. *Ascosporae* fusoideae vel ellipsoideae, 3-septatae; luteo-brunneae, laeves, (12–)13–14(–15) × 3–3.5(–4) µm.

Leaf spots amphigenous, 7–20 mm diameter, subcircular to circular, grey on surface, brown underneath with a thin, raised, dark brown border. *Mycelium* internal, comprising pale brown, septate hyphae, 2–3 µm wide; external hyphae dark brown, up to 6 µm wide. *Ascomata* evenly dispersed, predominantly epiphyllous, globose, immersed, becoming slightly erumpent, up to 200 µm diameter, central ostiole up to 40 µm diameter; wall consisting of 4–6 layers of brown *textura angularis*, outer region of brown sclerenchymatic cells. *Pseudoparaphyses* numerous, 1–3 µm diameter, hyaline, septate. *Asci* 8-spored, numerous, sessile, cylindrical-clavate, thin-walled, apex rounded with a visible apical chamber, 35–50 x 6–8 µm. *Ascospores* 2–3 seriate, fusoid-ellipsoidal, straight to slightly curved with obtuse ends, 3-septate, primary septum median, subapical cell becoming slightly swollen, slightly constricted at septa, yellow-brown, smooth, (12–)13–14(–15) × 3–3.5(–4) µm. Ascospores becoming 5–6 µm wide upon germination; germinating primarily from both ends, with germ tubes parallel to the long axis of the spore; ascospores becoming more prominently constricted at septa, but remaining smooth and light brown in colour.
Holotype: Australia, NSW, Mangrove Mountain, on leaves of *Telopea* sp., Aug. 1999, P.W. Crous &

Figures 3–9 *Kabatiella proteae* and *Leptosphaeria telopeae*. **3–5.** *K. proteae*. **3, 4.** Erumpent conidiomata on carnation leaf agar. **5.** Ellipsoidal conidia. **6–9.** *Leptosphaeria telopeae*. **6.** Vertical section through a pseudothecium. **7–9.** Asci and ascospores. Bars = 10 µm.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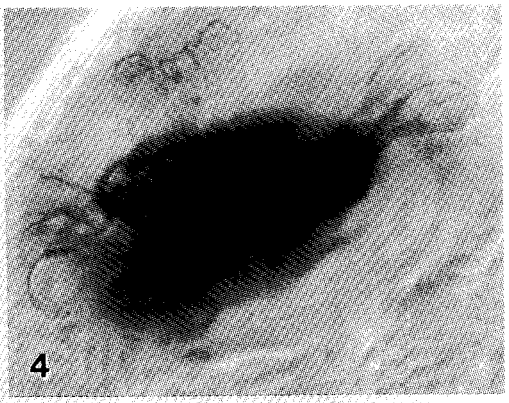
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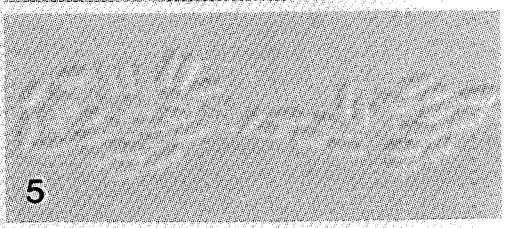
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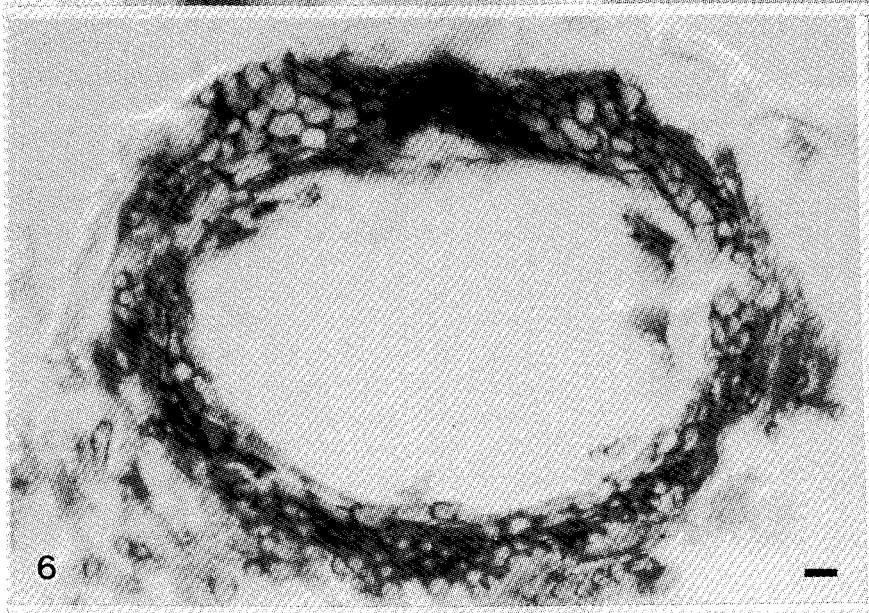
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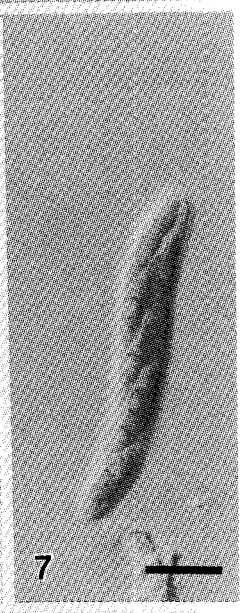
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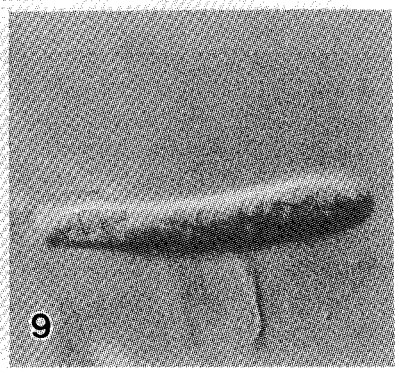
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B. Summerell, JT 890, DAR 74864, culture ex-type STE-U 2921.
Cultural characteristics: Colonies reaching 8 mm diameter after 14 days at 25°C; colonies erumpent, margins uneven, aerial mycelium moderate, surface off-white, underneath ochreous (15'b).
Australian host: *Telopea speciosissima* R. Br.
Distribution: Australia (NSW).

Two species of *Leptosphaeria* are presently known from Proteaceae, namely *L. protearum* Syd. (ascospores 23–29 × 6–7 µm) which has a *Coniothyrium* Corda anamorph, and *L. leucadendri* Crous & M.A. Palm (ascospores 20–28 × 5–7 µm), which has a *Sclerostagonospora* Höhn. anamorph (Crous and Palm 1999). *Leptosphaeria telopeae,* which has no known anamorph, is clearly distinct by having much smaller ascospores 12–15 × 3–4 µm than the two species discussed above.

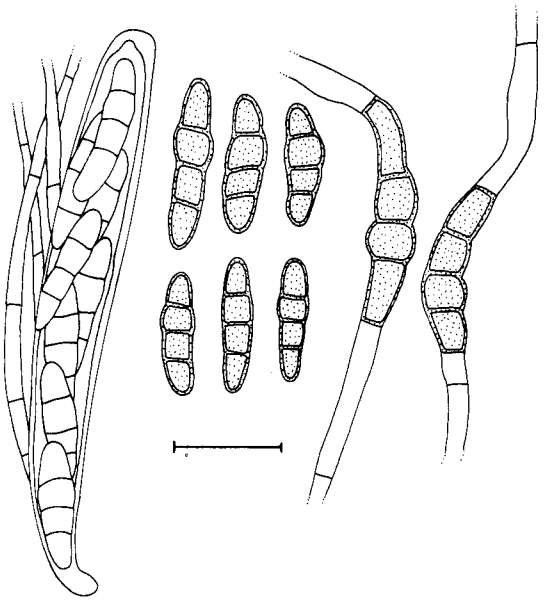


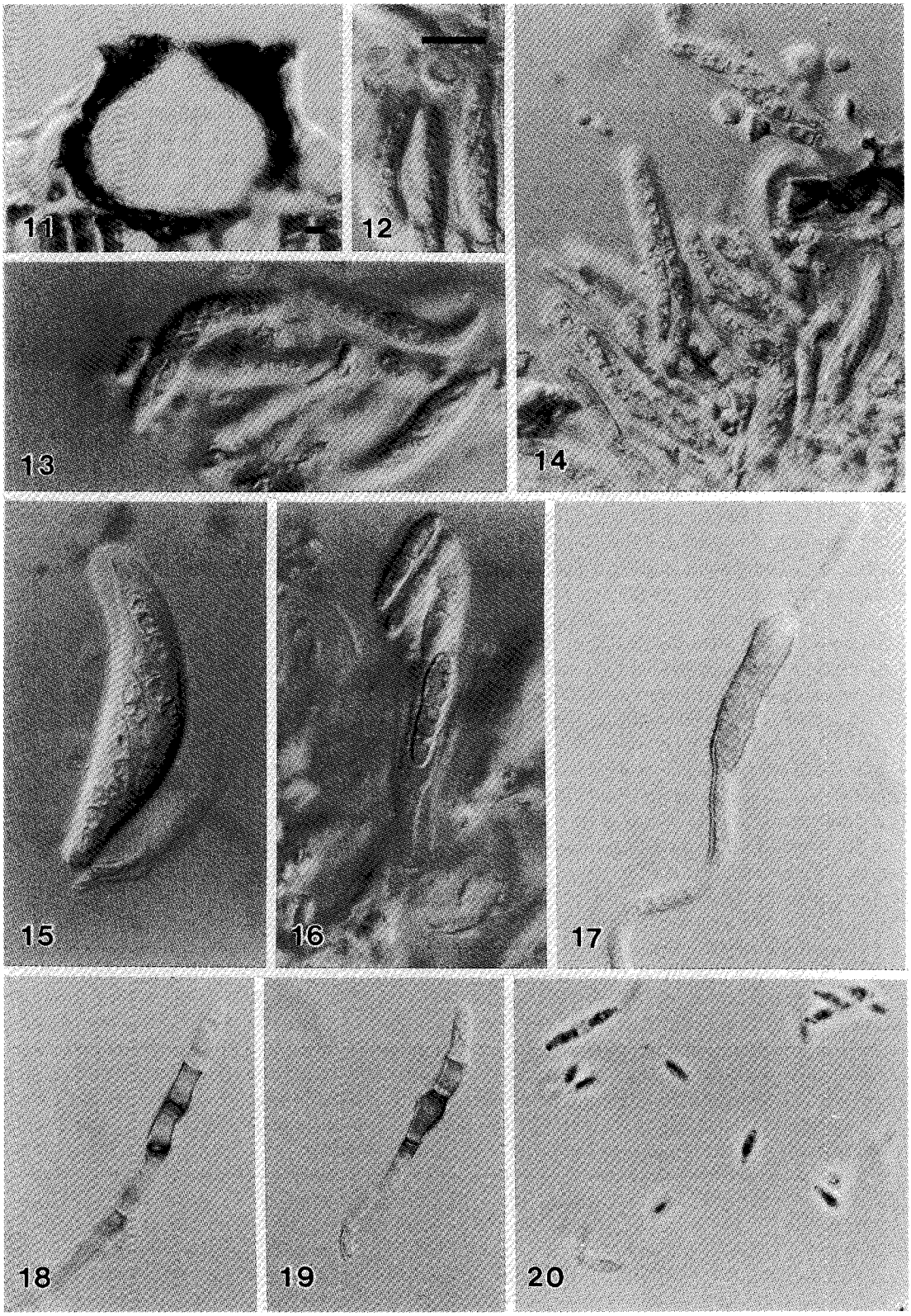
Figure 10 Asci intermingled with pseudoparaphyses (left), ascospores (middle) and germinating ascospores (right) of *Leptosphaeria telopeae*. Bar = 10 µm.

Mycosphaerella buckinghamiae Crous & Summerell, sp. nov. Figures 11–14, 21, 22
Anamorph: Unknown.

Pseudothecia epiphylla, nigra, subepidermalia, erumpentia, globosa ad subglobosa, 100–200 µm diam. *Asci* fasciculati, bitunicati, obovoidei ad ellipsoidei, recti vel curvati, 30–40 × 6–8 µm. *Ascosporae* tri- ad multiseriatae, imbricatae, hyalinae, guttulatae, parietibus tennibus, rectae, fusoideo-ellipsoideae, apicibus obtusis, latissimae in medium cellulae apicalis, mediano 1-septatae, (9–)10–12(–13) 2.5–3(–3.5) µm.

Leaf spots amphigenous, irregular to sub-circular, 2–12 mm diameter, dark brown with raised, brown borders and a thin red-purple margin on the abaxial surface. *Pseudothecia* predominantly epiphyllous, black, subepidermal, becoming erumpent, globose, 100–200 µm diameter; apical ostiole up to 30 µm wide, with 1–3 layers of pseudoparenchymatal cells forming an apical stroma, as typically observed for species giving rise to cercosporoid anamorphs; wall consisting of 2–3 layers of medium brown *textura angularis*; ostiolar periphyses 6–10 × 2–2.5 µm. *Asci* aparaphysate, fasciculate, bitunicate, sub-sessile, narrowly ellipsoidal to obovoid, straight to slightly curved, 8 spored, 30–40 × 6–8 µm; apical chamber not clearly visible. *Ascospores* tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, fusoid-ellipsoidal with obtuse ends, widest just above the septum, medianly 1 septate, constricted at the septum, tapering towards both ends, slightly more prominently towards the lower end (9–)10–12(–13) 2.5–3(–3.5) µm. Ascospores germinate from both ends, with germ tubes growing parallel to the long axis of the spore, spore body becoming 4–5 µm wide, constricted at the primary septum.
Holotype: Australia, NSW, Mangrove Mountain, on leaves of *Buckinghamia* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 902, DAR 74865, cultures ex-type, STE-U 3006, 3007.
Cultural characteristics: Colonies reaching 11 mm diameter after 14 days at 25°C; colonies

Figures 11–20 *Mycosphaerella buckinghamiae*, *M. jonkershoekensis* and *Ramularia proteae*. **11–14.** *M. buckinghamiae*. **11.** Vertical section through a pseudothecium. **12–14.** Asci with ascospores. **15–19.** *M. jonkershoekensis*. **15, 16.** Asci with ascospores. **17.** Germinating ascospore. **18, 19.** Swollen cells that develop on the terminal ends of hyphae in culture. **20.** Conidia of *R. proteae*. Bars = 10 µm.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erumpent, aerial mycelium sparse, margins smooth, lobed, surface of inner region off-white, intermediate region rose (1'b), outer region pale olivaceous grey (23""'d), greenish black (33""'k) underneath.
Australian host: *Buckinghamia* sp.
Distribution: Australia (NSW).

No species of *Mycosphaerella* is known from this host. Although pseudothecial anatomy suggested that a cercosporoid anamorph could form on the apical stroma under favourable conditions, none was induced in culture.

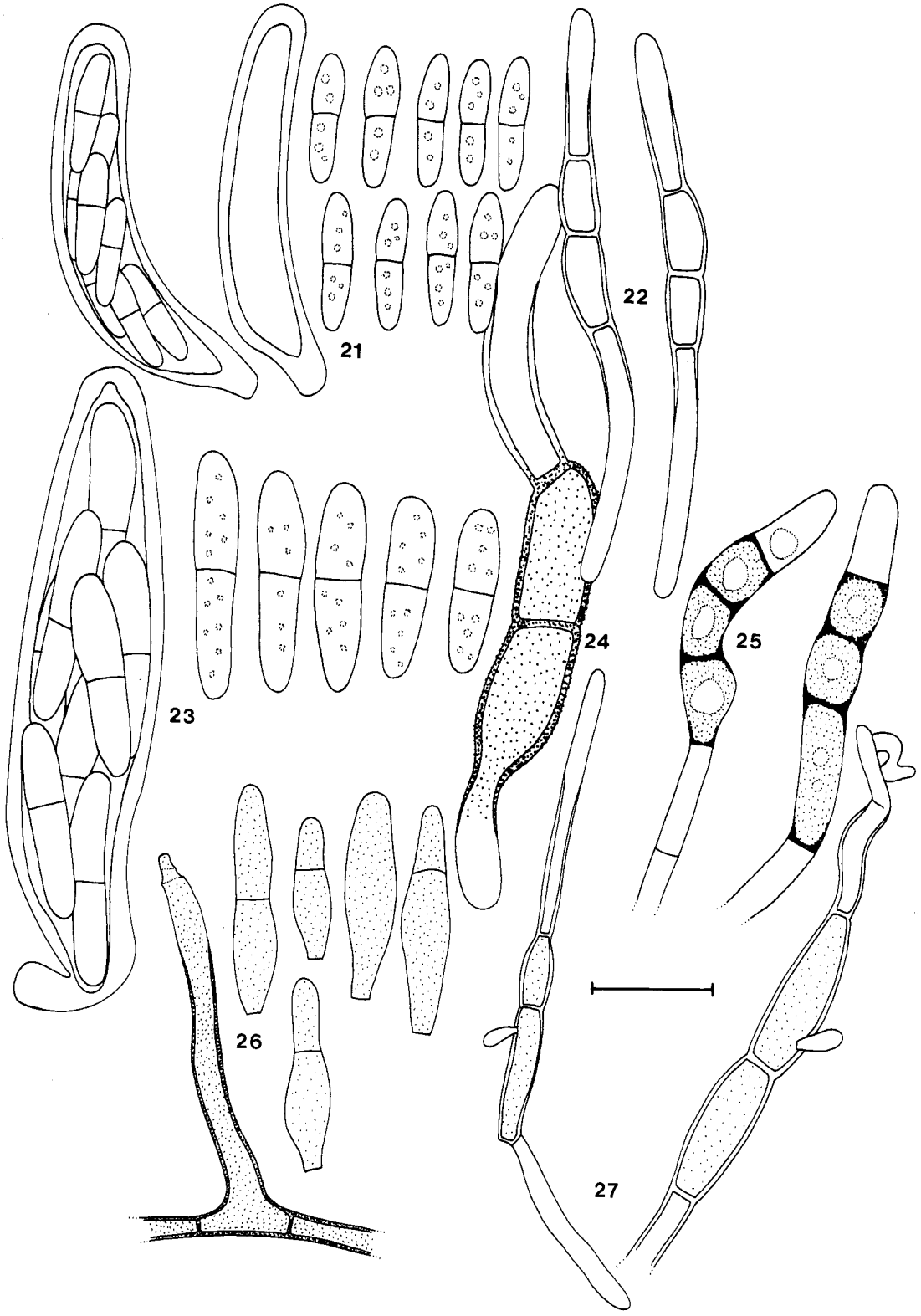
Mycosphaerella jonkershoekensis P.S. van Wyk, Marasas & Knox-Dav., Jl. S. Afr. Bot. 41: 234. 1975. Figures 15–19, 23–25
Anamorph: Unknown.

This species induces very typical grey-brown, circular leaf spots with raised, dark brown borders. Ascospores were fusoid, guttulate, and frequently had slightly longer basal cells (12–)16–20(–25) × 3.5–4(–5) µm, becoming slightly constricted at their septum, and pale brown with age. Upon germination ascospores became light brown, verruculose, 6–8 µm wide, and slightly constricted at the septum; germination was from both ends, with germ tubes growing more or less parallel to the long axis of the spore. When South African collections of *M. jonkershoekensis* were initially studied in culture (MEA), numerous cellular structures were observed to develop on the ends of hyphae. In the Australian cultures, the same structures once again developed, proving to be characteristic of the species. These structures are (3–)4(–5) cells in length, the terminal cell being hyaline, and the following 2–3 cells being medium brown and having darkened, thickened, refractive septa. Though tempting to speculate that these structures may function as conidial propagules, they were never observed to dehisce from the hyphae to be treated as such, and probably are chlamydospore-like, with a survival rather than a dispersal function. *M. jonkershoekensis* has thus far been known from South Africa only (Taylor and Crous 1998), and its occurrence on South African Proteaceae cultivated in Australia suggests, once again, that it has been introduced to this country.
Australian hosts: *Protea* spp.
Distribution: Australia (NSW), South Africa.
Specimens examined: Australia, NSW, Mount Tomah Botanic Gardens, on leaves of *Protea longifolia* Andr. [= *P. minor* (E. Phillips) Compton], Aug. 1999, P.W. Crous & B. Summerell, JT 967, DAR 74866; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea lepidocarpodendron*, Aug. 1999, P.W. Crous & B. Summerell, JT 993, DAR 74867, STE-U 3115-3117; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea* cv. Pink Ice, Aug. 1999, P.W. Crous & B. Summerell, JT 990, DAR 74868; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea* cv. Kurrajong Carnival, Aug. 1999, P.W. Crous & B. Summerell, JT 988, DAR 74869; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 987, DAR 74870; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea magnifica*, Aug. 1999, P.W. Crous & B. Summerell, JT 948, DAR 74871; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea magnifica*, Aug. 1999, P.W. Crous & B. Summerell, JT 965, DAR 74872; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea* cv. Kurrajong Carnival, Aug. 1999, P.W. Crous & B. Summerell, JT 953, DAR 74873.

Mycosphaerella lateralis Crous & M.J. Wingf., Mycologia 88: 454. 1996. Figures 26, 27
Anamorph: *Dissoconium dekkeri* de Hoog & Hijwegen, Mycol. Res. 95: 679. 1991.
= *Uwebraunia lateralis* Crous & M.J. Wingf., Mycologia 88: 454. 1996.

Although the genera *Dissoconium* De Hoog, Oorschot & Hijwegen and *Uwebraunia* Crous & M.J. Wingf. are morphologically similar (Crous *et al.* 1999), species of the latter are considered to be plant pathogenic, while those of *Dissoconium* are known to have wide host ranges, and to be antagonistic to, or mycoparasitic on other phyllosphere fungi (de Hoog *et al.* 1991; de Hoog and Takeo 1991). *D. dekkeri* is known from The Netherlands,

Figures 21–27 *Mycosphaerella buckinghamiae*, *M. jonkershoekensis* and *M. lateralis*. **21, 22.** *M. buckinghamiae*. **21.** Asci and ascospores. **22.** Germinating ascospores. **23–25.** *M. jonkershoekensis*. **23.** Asci and ascospores. **24.** Germinating ascospore. **25.** Swollen cells that develop at the terminal ends of hyphae in culture. **26, 27.** *M. lateralis*. **26.** Conidiophore and primary conidia. **27.** Germinating primary conidia with anastomosing secondary conidia on MEA. Bar = 10 µm.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Germany, New Zealand and Malaysia on hosts such as *Abies, Picea, Tsuga*, (Pinaceae), *Berberis* (Berberidaceae), *Buxus* (Buxaceae), *Calocedrus, Chamaecyparis, Juniperus*, *Thuja* (Cupressaceae), *Pieris* (Ericaceae), *Skimmia* (Rutaceae), *Taxus* (Taxaceae), and *Brassica* (Brassicaceae). It is also known from *Eucalyptus* spp. in South Africa, Zambia (Crous 1998), and Uruguay (unpublished data). It has never before been observed on any members of the Proteaceae. When lesions induced by *Mycosphaerella jonkershoekensis* were soaked for ascospore germination studies (Crous 1998), long, slender spores were also observed to germinate from both ends, with germ tubes curling in an irregular manner. Upon closer inspection, the typical microconidia of *Dissoconium* were observed to be attached to the primary conidia. After numerous slide preparations, no trace could be found of the *Mycosphaerella* state of *D. dekkeri*, and we thus had to conclude that this fungus was present as superficial mycelium on lesions induced by *M. jonkershoekensis*. The implications are, however, that sporulation and active conidium discharge (de Hoog *et al.* 1991; de Hoog and Takeo 1991) could occur within 24 h after soaked leaf sections were inverted over MEA plates. Little is known of its epidemiology, and further research needs to be focused on its possible hyperparasitic role on other leaf pathogens.
Australian hosts: *Protea* spp. and *Leucadendron* sp.
Distribution: Australia (NSW), Germany, Malaysia, New Zealand, South Africa, The Netherlands, Uruguay, Zambia.
Specimens examined: Australia, NSW, Mount Tomah Botanic Gardens, on leaves of *Protea* cv. Kurrajong Carnival, Aug. 1999, P.W. Crous & B. Summerell, DAR 74874, STE-U 3072; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea* sp., Aug. 1999, P.W. Crous & B. Summerell, STE-U 3070; NSW, Mount Tomah Botanic Gardens, on leaves of *Protea magnifica*, Aug. 1999, P.W. Crous & B. Summerell, STE-U 3008; NSW, Mount Tomah Botanic Gardens, on leaves of *Leucadendron* sp., Aug. 1999, P.W. Crous & B. Summerell, culture No. 94.

Pestalotiopsis sp.
Teleomorph: Unknown.

The Pestalotiopsis leaf spot is probably one of the most prominent leaf spots of *Telopea* spp. in NSW. This pathogen has recently been described in detail from host material and cultural studies by Swart *et al.* (1999). Because numerous untreated names are available in *Pestalotiopsis* Steyaert and *Pestalotia* De Not. that have to be considered for this pathogen (Nag Raj 1993), it was decided not to name this species, but to lodge specimens and cultures for later study, pending a revision of the group. This specific *Pestalotiopsis* sp. causes a similar disease of *Leucospermum* and *Protea* spp. in Zimbabwe. Although it has also been isolated from *Protea* spp. in South Africa, it has not been found to cause serious disease, and this is presumably due to differences in climatic conditions (Swart *et al.* 1999). The disease on *Telopea* is quite characteristic, in that the pathogen causes grey, circular, leaf spots, 5–40 mm diameter (distinct from the more brown lesions caused by *Leptosphaeria telopeae*), surrounded by a thin, raised, dark brown border. Conidiomata are predominantly epiphyllous, black, and rupture the epidermis by an irregular split. During the course of these investigations several other *Pestalotiopsis* spp. were also found to occur on these lesions. However, in the case of the latter, we suspect them to be more saprophytic. The pathogenic *Pestalotiopsis* sp. (Swart *et al.* 1999) can be distinguished by having two darker, verruculose upper cells, and 2–4 tubular apical appendages.
Australian hosts: *Protea* and *Telopea* spp.
Distribution: Australia (NSW), South Africa and Zimbabwe.
Specimens examined: Australia, NSW, Nunkeri Nature Flowers, on leaves of *Telopea speciosissima*, Aug. 1999, P.W. Crous & B. Summerell, JT 887, DAR 74875, STE-U 3016-3019; NSW, Nunkeri Nature Flowers, on leaves of *Protea* cv. Pink Ice, Aug. 1999, P.W. Crous & B. Summerell, JT 904, DAR 74876.

Phyllosticta telopeae H.Y. Yip, Mycol. Res. 93: 494. 1989.
Teleomorph: Undescribed *Guignardia* sp.

Yip (1989) described *P. telopea* from leaves of *Telopea speciossima* collected in Tasmania, Australia, where it causes a prominent leaf spot disease of this host. Since then, this fungus has frequently been intercepted at various ports of entry into the USA, and was generally considered to be of quarantine significance (reference specimens lodged at BPI). Species of *Phyllosticta* have always been

accepted to be host specific (Van der Aa 1973). Recently, however, molecular data have been obtained to suggest that this is not always the case (G. Carrol, personal communication), but that some species could in fact have wide host ranges and distribution. Preliminary results also suggest this to be the case with *P. telopeae*. This argument also restrained Crous and Palm (1999) from describing another Australian collection from *Protea* as new. As with the Pestalotiopsis leaf spot disease, we again suspect that climate plays a major determining role for the Phyllosticta leaf spot to develop if the fungus is present. Further molecular and pathological work is presently underway to determine the distribution and host range of *P. teleopeae*. Several strains formed a *Guignardia* teleomorph in culture. Based on the same argument above, however, we have refrained from describing it as new till more is known about its taxonomy and possible earlier synonyms.
Australian hosts: *Grevillea*, *Leucadendron*, *Leucospermum* and *Telopea* spp.
Distribution: Australia (NSW, Tasmania).
Specimens examined: Australia, NSW, Mount Tomah Botanic Gardens, on leaves of *Leucadendron* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 919, DAR 74877; NSW, Mount Tomah Botanic Gardens, on leaves of *Leucadendron* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 917, DAR 74878; NSW, Nunkeri Nature Flowers, on leaves of *Leucadendron* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 899, DAR 74879; NSW, Mount Annan Botanic Gardens, on leaves of *Telopea* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 921, DAR 74880, STE-U 2927, 2928; NSW, Mount Annan Botanic Gardens, on leaves of *Grevillea* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 974, DAR 74881, STE-U 2922, 2923; NSW, Nunkeri Nature Flowers, on leaves of *Leucospermum* sp., Aug. 1999, P.W. Crous & B. Summerell, JT 932, DAR 74822, STE-U 2984-2986.

Ramularia proteae Crous & Summerell, sp. nov. Figures 20, 28

Teleomorph: Unknown.

Maculae orbiculares vel suborbiculares, amphigenae, 5–15 mm diam. *Caespituli* amphigeni, griseo-albidi. *Mycelium* immersum; hyphae hyalinae, septatae, ramosae, 2–3.5 µm diam. *Conidiophora* erecta, subcylindrica vel geniculata-sinuosa, 3–20 × 2–4 µm, 0–2-septata, hyalina, levia. *Conidia* catenata vel ramicatenata, subcylindrica-fusiformia, 5–8(–10) × 1–1.5(–2) µm *in vivo*, 5–8(–11) × 2–2.5 µm *in vitro*, 0(–1) septate, hyalina, levia.

Leaf spots amphigenous, circular to subcircular, 5–15 mm diameter, pale to medium brown. *Caespituli* rarely observed, amphigenous, greyish-white, reduced to a few conidiophores. *Mycelium* internal and external, consisting of branched, septate, hyaline hyphae, 2–3.5 µm diameter, climbing leaf hairs. *Conidiophores* separate, rarely aggregated into poorly developed, loose fascicles, erect, subcylindrical, straight to geniculate-sinuous, rarely branched, 3–20 × 2–4 µm, 0 to 2 septate, hyaline, smooth. *Conidiogenous cells* hyaline, smooth, straight to geniculate, 3–15 × 2–3 µm; scars thickened, darkened and refractive. *Conidia* catenate, chains simple or branched, subcylindrical-fusoid, 5–8(–10) × 1–1.5(–2) µm *in vivo*, 5–8(–11) × 2–2.5 µm *in vitro*, 0(–1) septate, hyaline, smooth, with somewhat attenuated ends; hila slightly thickened, darkened and refractive.

No other species of *Ramularia* have thus far been recorded on Proteaceae. Although several species of *Pseudocercospora* and *Cercostigmina* with pigmented conidia are known from Proteaceae (Crous and Braun 1994; Crous and Palm 1999; Taylor and Crous 2000), *R. proteae* represents the only hyaline cercosporoid known from this family to date.
Holotype: Australia, Tasmania, Royal Tasmanian

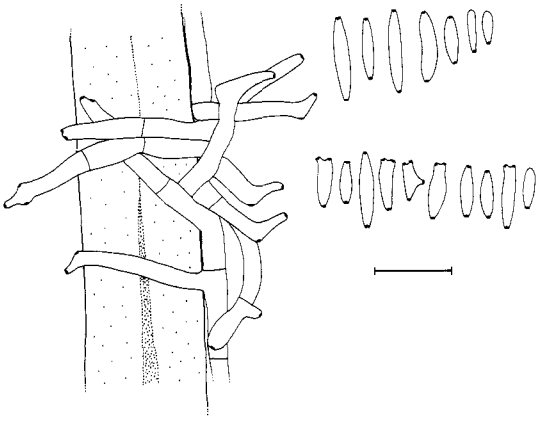

Figure 28 Conidiophores and conidia of *Ramularia proteae*. Bar = 10 µm.

Botanic Gardens, Hobart, on leaves of *Protea longifolia* Andr., Aug. 1999, Alan Macfadyen, DAR 74883, culture ex-type STE-U 3075.
Cultural characteristics: Colonies reaching 6 mm diameter after 14 days at 25°C; margins uneven, colonies erumpent, aerial mycelium absent, mouse grey (15""'i) on the surface, greyish sepia (17""i) underneath.
Host: *Protea longifolia*.
Distribution: Australia (Tasmania).

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