

Fungi occurring on Proteaceae. I.

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The present study has led to the description of several new fungi occurring on leaves of *Protea* L., *Leucospermum* R.Br., *Telopea* R.Br. and *Brabejum* L. collected from South Africa, Australia or New Zealand. *Cladophialophora proteae* L. Viljoen & Crous, *Coniothyrium nitidae* Crous & S. Denman, *Coniothyrium proteae* Crous & S. Denman, *Coniothyrium leucospermi* Crous & S. Denman, *Harknessia leucospermi* Crous & L. Viljoen, and *Septoria protearum* L. Viljoen & Crous spp. nov. are described from *Protea* and *Leucospermum* in South Africa, while *Phyllosticta owaniana* G. Winter is redescribed from leaves of *Brabejum stellatifolium* L. Furthermore, *Mycosphaerella telopeae* M. Palm & Crous sp. nov. is described from leaves of *Telopea* collected in New Zealand, while *Phyllosticta telopeae* H.Y. Yip, which also occurs on this host, is described in culture from Australian material.

Keywords: *Brabejum*, *Leucospermum*, *Protea*, *Telopea*, fungal systematics.

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Introduction

The Proteaceae, which is the most unique family of the Cape Floral Kingdom, comprises approximately 8600 species, the majority of which are found in the southern hemisphere. About 360 proteaceous species occur in South Africa, of which 330 species in 14 genera are confined to the South-Western Cape Province (fynbos biome) (Rebello 1995).

Proteaceae, which are grown for cut-flowers in South Africa, form part of a large, economically viable, and expanding industry. Based on a survey conducted during the 1993/1994 season, approximately 2507 ha of veld are cultivated, while an additional 600626 ha are natural fynbos vegetation. Export of fresh fynbos from the Western and Eastern Cape for the 1993/1994 season amounted to nearly R9 million (Malan 1995).

The proteaceous cut-flower industry has considerable potential, but diseases which blemish foliage and blooms are one of the problems which make this a high risk factor crop (Greenhalgh 1981). Since South Africa is the centre of origin for the majority of proteaceous plants, pests and diseases that have evolved with these hosts have become a serious problem not only in South Africa, but to the industry internationally (Knox-Davies 1981). Prior to 1970, the foliicolous fungi occurring on proteas in South Africa were poorly studied (Van Wyk 1973). The description of *Cercostigmia protearum* (Cooke) U. Braun & Crous (= *Cercospora protearum* Cooke) by Cooke (1883), represents the first reference of a leaf pathogen occurring on the genera *Protea*, *Leucadendron* and *Leucospermum*. However, since the 1970's, numerous diseases of the Proteaceae have been recorded and the causal fungi described (Van Wyk 1973; Van Wyk *et al.* 1975; Benic & Knox-Davies 1983; Van Wyk *et al.* 1985; Knox-Davies *et al.* 1987; Orffer & Knox-Davies 1989; Serfontein & Knox-Davies 1990). Since the Proteaceae are amongst the most endangered species of the Southern African flora, it is in the interest of the conservationist and the Protea industry to record all new diseases and potentially important pathogens. In the present study, nine new fungi, most being associated with leaf spots, are described from leaves of Proteaceae collected in South Africa, New Zealand and Australia.

Materials and methods

Symptomatic leaves and leaf litter samples were incubated in Petri dish moist chambers at 25°C on the laboratory bench to induce sporulation. Single conidium colonies were established on 2% malt extract agar (MEA) (Oxoid), then transferred to plates containing fresh MEA and carnation-leaf agar (CLA) (Fisher *et al.* 1982; Crous *et al.* 1992), and incubated at 25°C under continuous near-ultraviolet light. Linear growth of colonies growing on MEA at 25°C in the dark was measured after 1, 2 or 6 weeks, and colours determined according to the charts of Rayner (1970). Leaf lesions with which species of *Mycosphaerella* were associated were excised and single ascospore cultures established on MEA using the technique described by Crous *et al.* (1991). For microscopic examination the fungi were mounted in lactophenol and measurements made at 1000× magnification. Averages were derived from at least 30 observations, and the ranges are given in parentheses. Reference cultures are maintained in the culture collection of the Department of Plant Pathology at the University of Stellenbosch (STE-U).

Taxonomy

Cladophialophora proteae L. Viljoen & Crous sp. nov., Figures 1, 13, 14.

Conidiogenae cellulae integratae, protuberationes breves truncatas formantes, 2–3 × 1.5–2 µm, mycelio concoloratae, subcylindracea. Conidia in catenis longis acropetalis (ad 20), simplicia vel ramosa, subcylindracea ad oblongo-doliiformia, (9–)13–17(–22) × 2.5–3(–4) µm *in vitro*, 0–1(–2)-septata, pallide brunnea ad pallide olivacea, laevia, hilis subtruncatis ad truncatis, non crassis sed parum refractivis.

Sterile hyphae branched, septate, often forming strands, anastomosing, smooth to finely verruculose, frequently constricted at septa, olivaceous, 3–4 µm wide; hyphal cells in older cultures becoming swollen, up to 6 µm wide. Conidiophores reduced to conidiogenous cells. Conidiogenous cells integrated, forming short, truncate protuberances, 2–3 × 1.5–2 µm, concolorous with mycelium, subcylindrical. Conidia in long acropetal chains (up to 20), simple or branched, subcylindrical to oblong-doliiform, (9–)13–17(–22) × 2.5–3(–4) µm *in vitro*, 0–1(–2)-septate, light brown to pale olivaceous, smooth, hila subtruncate to truncate, not thickened, but somewhat refractive.

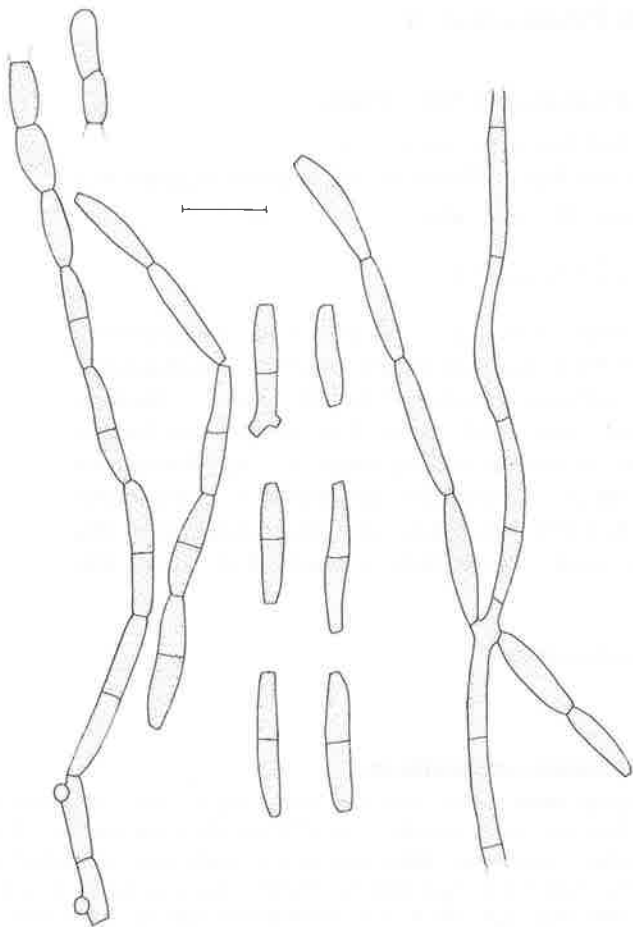


Figure 1 *Cladophialophora proteae*. Chains of 0–1-septate conidia formed on malt extract agar (bar = 10 μ m).

Cultural characteristics: Colonies erumpent, segmented, with smooth, sinuate margins; fuscous black 7"b (surface and bottom); aerial mycelium absent. Colonies reaching 5 mm diam. on MEA after 6 weeks in the dark at 25°C.

Specimen examined: South Africa, Western Cape Province, Stellenbosch, J.S. Marias Park isolated as endophyte from leaves of *Protea cynaroides* (L.) L. with *Batcheloromyces* lesions, L. Viljoen, Dec. 1996, PREM 55345 (holotype), cultures ex-type STE-U 1514–1516.

The genus *Cladophialophora* Borelli, which includes species associated with human disorders and others isolated as saprophytes or endophytes from plants, has teleomorphs placed in *Capronia* Sacc. in the Herpotrichiellaceae (Braun & Feiler 1995; De Hoog *et al.* 1995) and *Venturia* Sacc. in the Venturiaceae (Untereiner 1997). Strains isolated as endophytes from leaves of *Protea cynaroides* fit this generic complex. It appears that *Cladophialophora* is heterogeneous and it is possible that the saprophytic species will be placed in their own form genus, separate from the human pathogens. The present species is considered congeneric with *Cladophialophora* because of the flat, unthickened but somewhat refractive conidial scars, long conidial chains, and by being saprophytic. Morphologically *C. proteae* is most similar to *C. hachijoensis* (Matsush.) U. Braun & U. Feiler [conidia 1–3-septate, (4.5–)8–25(–35) \times (1.5–)2–4(–5) μ m], but *C. proteae* has slightly smaller conidia. Braun and Feiler (1995) depicted a lot of variation between strains presently treated as *C. hachijoensis*, and it is possible that there are even more distinct species within this complex.

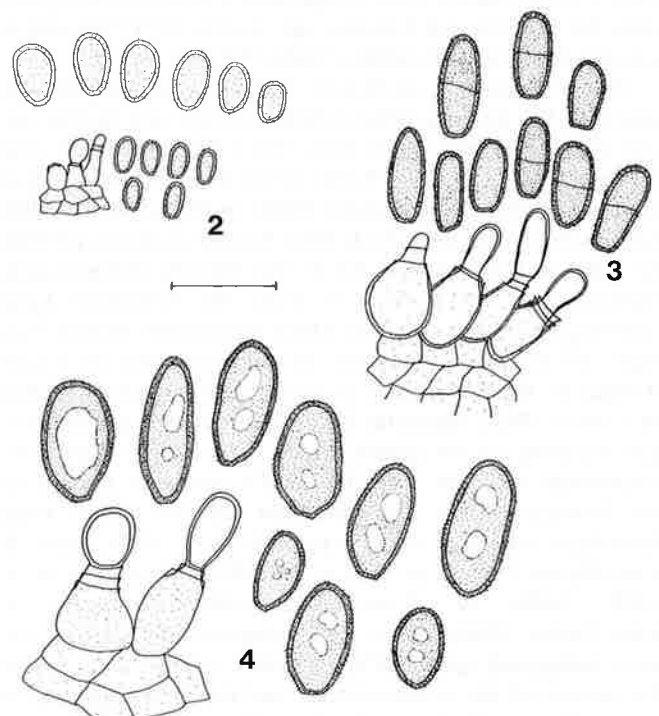
***Coniothyrium nitidae* Crous & S. Denman sp nov., Figures 3, 15.**

Conidiomata pycnidialia, subepidermalia, globosa, discreta, brunnea, ad 200 μ m diam., pariete in ex 3–4 stratis cellularum brunnearum texturae angularis constanti. Conidiogenae cellulae discretiae, laeves hyalinae ad pallide olivaceae, doliiformes ad ampulliformes, 1–4 plo enteroblastice et percurrenter proliferantes, 5–8 \times 5–10 μ m. Conidia pallide ad medio brunnea, parietibus verruculosi, 0–1-septata ellipsoidea ad subcylindracea, apice obtuso, base obtusirobundata ad truncata, (6.5–)8–9(–11) \times 3–4(–4.5) μ m *in vivo*, (5.5–)6–8(–9) \times 3–4 μ m *in vitro*.

Leaf spots light brown, amphigenous, variable in shape and size, frequently associated with tip die-back or situated along leaf margins. Mycelium immersed, septate, medium brown, finely verruculose, 3–4 μ m diam. *in vivo*, 2–5 μ m *in vitro*, finely verruculose, light to medium brown, forming intercalary and terminal chains of globose chlamydospores. Conidiomata pycnidial, subepidermal, globose, separate, brown, up to 200 μ m diam., wall consisting of 3–4 layers of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, smooth, hyaline to pale olivaceous, doliiform to ampulliform, proliferating 1–4 times enteroblastically and percurrently, 5–8 \times 5–10 μ m. Conidia medium brown, thick-walled, verruculose, 0–1-septate, ellipsoidal to subcylindrical, apex obtuse, base bluntly rounded to truncate, (6.5–)8–9(–11) \times 3–4(–4.5) μ m *in vivo*, (5.5–)6–8(–9) \times 3–4 μ m *in vitro*.

Cultural characteristics: Colonies with irregular margins; grey olivaceous 21"b to cinnamon 13"b (bottom); aerial mycelium moderate, dirty pink to white. Colonies reaching 32 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimen examined: South Africa, Western Cape Province, Hermanus, leaves of *Protea nitida* Mill., S. Denman, 29 Aug. 1996, PREM 55346 (holotype), cultures ex-type STE-U 1476–1478, 1531–1533.



Figures 2–4 Conidia and conidiogenous cells of *Coniothyrium* spp. *in vivo*. 2. *C. proteae*. 3. *C. nitidae*. 4. *C. leucospermi*. (bar = 10 μ m).

***Coniothyrium proteae* Crous & S. Denman sp. nov.**, Figures 2, 16.

Conidiomata pycnidialia, subepidermalia, globosa, discreta, brunnea, 60–120 µm in diam., pariete ex 2–3 stratis cellularum brunnearum texturae angularis constanti. Conidiogenae cellulae discretiae, laeves hyalinae, doliiformes ad ampulliformes 1–2 plo enteroblastice et percurrenter proliferantes, 3–8 × 3–4 µm. Conidia pallide ad medio brunnea, parietibus tenuibus, laevia ad subtiliter verruculosa, aseptata, ellipsoidea ad globosa, rare pyriformia, apice obtuso, base obtusirobundata ad truncate, (5–)5.5–7(–8) × 3.5–4 µm *in vivo*, (3–)3.5–4 × 2–2.5 µm *in vitro*.

Leaf spots light brown, amphigenous, variable in shape and size, frequently associated with tip die-back or situated along leaf margins. Mycelium immersed, septate, branched, pale to medium brown, smooth to finely verruculose, 3–4 µm diam. Conidiomata pycnidial, subepidermal, globose, separate, brown, 60–120 µm diam., wall consisting of 2–3 layers of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, smooth, hyaline, doliiform to ampulliform, proliferating 1–2 times enteroblastically and percurrently, 3–8 × 3–4 µm. Conidia light to medium brown, thin-walled, smooth to finely verruculose, aseptate, ellipsoidal to globose, rarely pyriform, apex obtuse, base bluntly rounded to truncate, (5–)5.5–7(–8) × 3.5–4 µm *in vivo*, (3–)3.5–4 × 2–2.5 µm *in vitro*.

Cultural characteristics: Colonies with smooth, sinuate margins, olivaceous grey 23^{""}i (bottom), smoke grey 21^{""}f (surface);

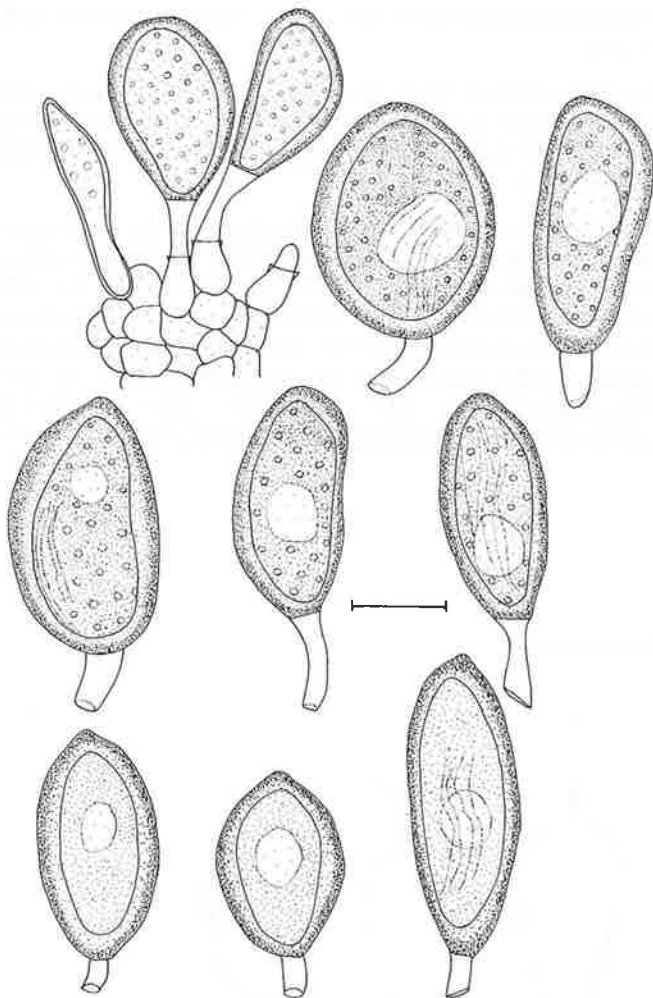


Figure 5 *Harknessia leucospermi*. Conidia and conidiogenous cells *in vivo* (bar = 10 µm).

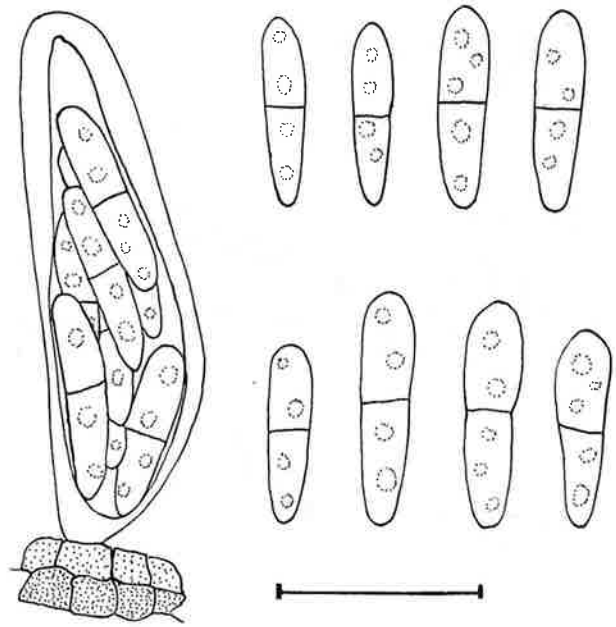


Figure 6 *Mycosphaerella telopeae*. Ascospores and ascus *in vivo* (bar = 10 µm).

aerial mycelium sparse to moderate. Colonies reaching 4 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimens examined: South Africa, Western Cape Province, Hermanus, leaves of *Protea nitida*, S. Denman, 29 Aug. 1996, PREM 55347 (holotype), cultures ex-type STE-U 14231425; Western Cape Province, Stellenbosch, leaves of *Protea mellifera*, L. Verwoerd, Apr. 1923, BPI 639096.

***Coniothyrium leucospermi* Crous & S. Denman sp. nov.**, Figures 4, 17.

Conidiomata pycnidialia, subepidermalia, amphigena, discreta, globosa atrobrunnea ad 200 µm in diam., pariete ex 3–4 stratis cellularum brunnearum texturae angularis. Conidogenae cellulae discretiae, laeves, pallide brunneae, doliiformes ad ampulliformes, 1–3 plo enteroblastice et percurrenter proliferantes, 9–11 × 5–7 µm. Conidia mediobrunnea, parietibus crassis, verruculosa, aseptata, ellipsoidea ad globosa, apice obtuso, base obtusirobundata ad truncate, 11–13 × 5–6 µm *in vivo*, (9–)10–13(–15) × 6–7 µm *in vitro*.

Leaf spots amphigenous, irregular, grey to light brown with a raised, dark brown border, frequently associated with tip blight of leaf margins. Conidiomata pycnidial, subepidermal, amphigenous, separate, globose, dark brown, up to 200 µm diam., wall consisting of 3–4 layers of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, smooth, light brown, doliiform to ampulliform, proliferating 1–3 times enteroblastically and percurrently, 9–11 × 5–7 µm. Conidia medium brown, thick-walled, verruculose, aseptate, ellipsoidal to globose, apex obtuse, base bluntly rounded to truncate, 11–13 × 5–6 µm *in vivo*, (9–)10–13(–15) × 6–7 µm *in vitro*.

Cultural characteristics: Colonies with smooth, regular margins, fucous black 7^{""}k (surface), olivaceous black 27^{""}m (bottom); aerial mycelium sparse. Colonies reaching 12–16 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimens examined: South Africa, Western Cape Province. Piketberg, leaves of *Leucospermum conocarpodendron* (L.) H. Buek., S. Denman, 29 Aug. 1996, PREM 55348 (holotype), cultures ex-type STE-U 1426–1428. Dominican Republic, leaves of *Leucospermum* sp., L. Schroeder, 7 Jul. 1986, BPI 1107823.

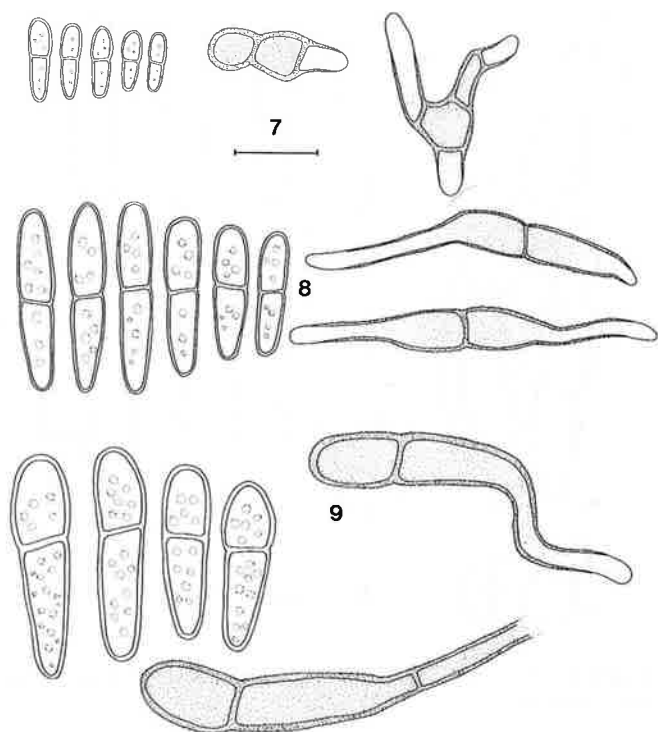


Figure 7-9 Ascospores and germinating ascospores of *Mycosphaerella* spp. on malt extract agar after 24 h. 7. *M. belulus*. 8. *M. jonkershoekensis*. 9. *M. protea*. (bar = 10 μ m).

Van Wyk (1973) listed several specimens from *Protea* and *Leucadendron* (PREM 44798, 44853, 44854) which he considered to represent a new species of *Coniothyrium*. An examination of these specimens found conidiomata to be present on PREM 44854 and 44798. Conidia were aseptate, finely verruculose, and resembled *C. protea* in shape, but were much larger [(7-)-8-9(-)-10) \times 4-5(-6) in PREM 44854; (5-)-7-8(-9) \times (2.5-)-4-5(-6) in PREM 44798]. Although it appears that these collections represent yet another distinct species, further collections and cultures are required to suitably characterise this pathogen.

As far as we could establish, only one other species of *Coniothyrium*, namely *C. proteae-abyssinicae* Bacc. has been described from these hosts. The latter species has conidia that differ in size (14.4 \times 3.2 μ m; Baccharini 1917) to those of the species described in the present study. It is important to note,

however, that major differences occurred in conidial shape and dimension in some species when cultured on agar, and these discrepancies will have to be carefully considered when comparing new species and isolates in the future.

***Harknessia leucospermi* Crous & L. Viljoen sp. nov.**, Figures 5, 18.

Conidiomata discreta, immersa, globosa ad subglobosa, unilocularia, subepidermalia, ad 350 μ m diam. Conidiogenae cellulae subcylindraceae ad lageniformes, hyalinae, laeves, 8-20 \times 2.5-5 μ m. Conidia holoblastica, late ventricosa, guttula media, granulata, laevia, irregulariter striata, apice obtusa ad obtusirobundato, base truncata (23-)-25-28(-32) \times (13-)-15-17(-18) μ m, appendicula basali hyalina non ramosa 4-8(-14) \times 2-2.5(-3) μ m.

Conidiomata separate, immersa, globose to subglobose, unilocular, subepidermal, up to 350 μ m diam. ostiole with light brown furfuraeous margin; basal and lateral walls 5-7 cells thick composed of *textura angularis*, brown, becoming hyaline towards the interior. Conidiophores reduced to conidiogenous cells. Conidiogenous cells subcylindrical to lageniform, hyaline, smooth, 8-20 \times 2.5-5 μ m. Conidia holoblastic, broadly ventricose with a central guttule, granular, smooth, irregularly striate, apex obtuse to bluntly apiculate, base truncate (23-)-25-28(-32) \times (13-)-15-17(-18) μ m, with a hyaline, unbranched basal appendage 4-8(-14) \times 2-2.5(-3) μ m *in vivo*; conidia broadly ellipsoid, apiculate, (23-)-25-27(-30) \times (12-)-13-15 μ m, basal appendage 3-10 \times 2-2.5 μ m *in vitro*.

Colony characteristics: Colonies with moderate, pale yellow aerial mycelium, luteus 21b (bottom); margins smooth to irregular. Colonies reaching 56 mm diam. on MEA after 1 week in the dark at 25°C.

Specimen examined: South Africa, Western Cape Province, Kirstenbosch, leaf litter of a *Leucospermum* sp., P.W. Crous, 20 May 1996, PREM 55349 (holotype), cultures ex-type STE-U 1372-1374, IMI 375227, ATCC 201156, CBS 778.97.

Harknessia leucospermi is morphologically similar to *H. eucalypti* Cooke apud Cooke and Harkn., which has broadly ventricose, apiculate conidia (19-)-2-25(-28) \times (11-)-13-15 μ m with restricted striations and basal appendages (6-)-8-13 \times 2-4 μ m, and *H. eucalyptorum* Crous *et al.*, which has broadly ventricose, aseptate conidia with blunt apices, (16-)-20-25(-29) \times (9-)-10-14(-16) μ m *in vivo*, (14.5-)-17-22(-24) \times (10.5-)-11-13(-14) μ m *in vitro*, basal appendages 3-16 μ m (Crous *et al.* 1993).

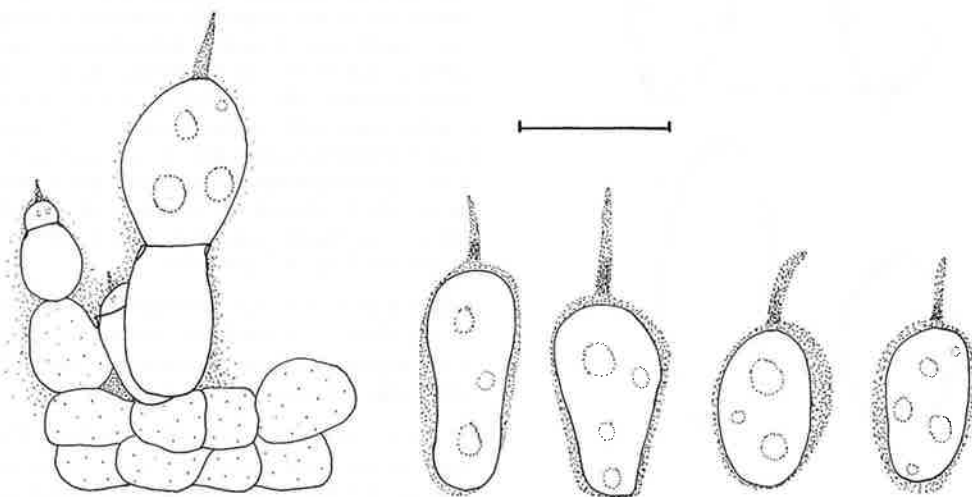


Figure 10 *Phyllosticta owaniana* sporulating on malt extract agar (bar = 10 μ m).

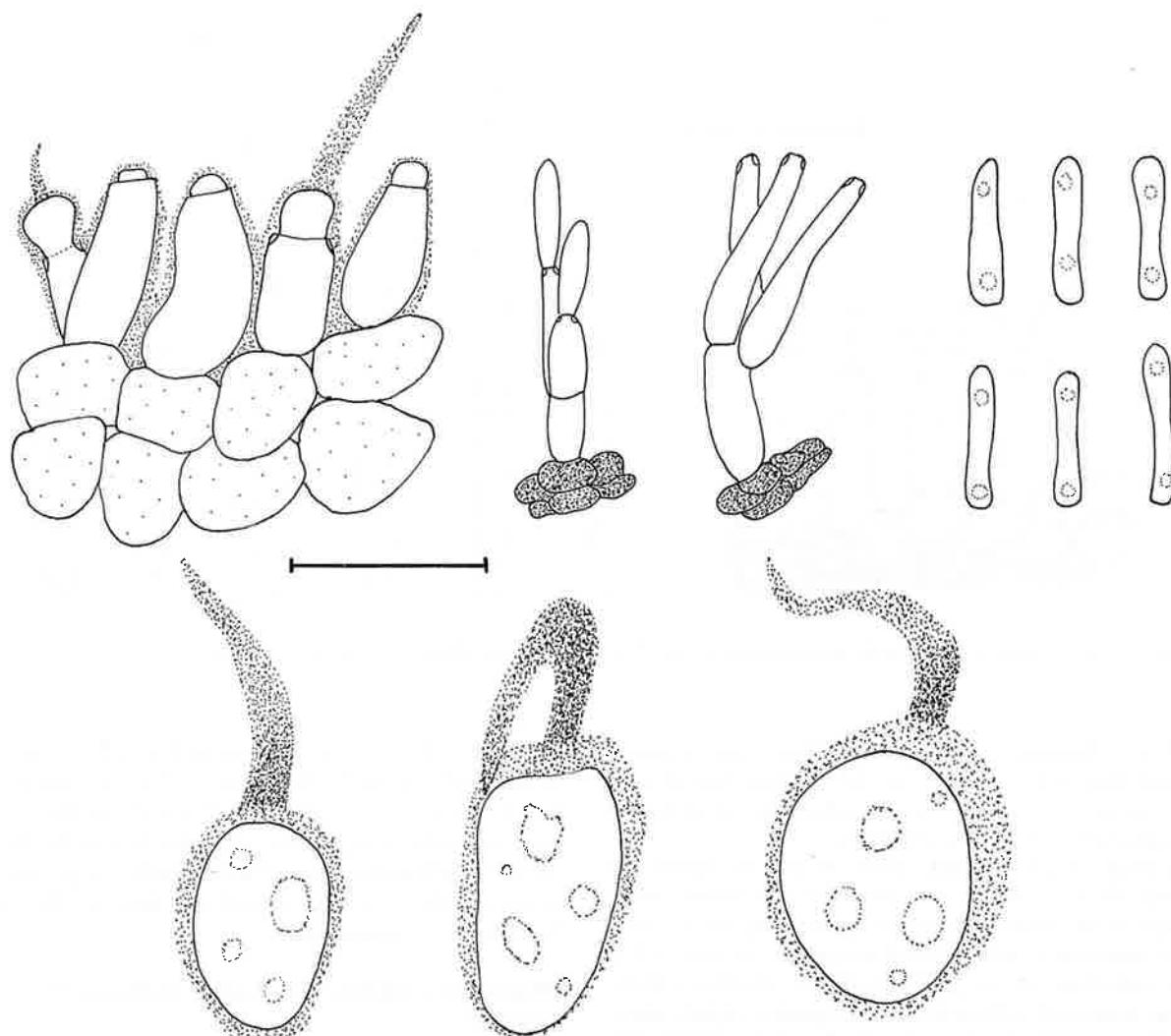


Figure 11 *Phyllosticta telopeae* sporulating on malt extract agar (bar = 10 μm).

Sutton and Pascoe (1989) reported *H. eucalypti* occurring on *Banksia marginata* Cav. and *Lambertia formosa* Sm., both hosts in the Proteaceae. Conidia of *H. leucospermi* are larger than those of *H. eucalypti* and *H. eucalyptorum*. On leaf tissue the conidia resemble those of *H. eucalyptorum* in being more bluntly apiculate. In culture, however, conidia become more broadly ellipsoidal and sharply apiculate, distinct from conidia of *H. eucalyptorum* formed in culture (Crous *et al.* 1993). Although Nag Raj (1993) illustrated conidia of *H. eucalypti* to be striate, striations were only observed in restricted areas on some conidia, and were not as prominent as in *H. leucospermi*. The latter feature was also found to be constant in culture for *H. leucospermi*.

***Mycosphaerella telopeae* M. Palm & Crous sp. nov.**, Figure 6.

Pseudothecia amphigena, sparse distributa, unica, nigra, erumpentia globosa ad 120 μm diam. Asci aparaphysati fasciculati bitunicati subsessiles obovoidei ad late ellipsoidei vel cylindracei, recti vel parum curvati, 8 sporis, 20–28 \times 8–10 μm . Ascospores multiseriatae, imbricatae, hyalinae, guttulate, parietibus tenuibus, rectae ad parum curvatae, fusoido-ellipsoideae apicibus obtusis, latissimae in medio cellulae apicalis, mediano 1-septatae, magis prominenter ad basim contractae (9–)10–11(–12) \times (2–)2.5(–3) μm .

Leaf spots circular, amphigenous, 1–4 mm diam., grey in centre, surrounded by a raised, dark brown border and a narrow chlorotic margin. Pseudothecia amphigenous, sparsely distributed, single, black, erumpent, globose, up to 120 μm diam.; apical papillate ostiole 5–10 μm in diam.;

wall consisting of 3–4 layers of medium brown *textura angularis*. Asci aparaphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid or cylindrical, straight or slightly curved, 8-spored, 20–28 \times 8–10 μm . Ascospores multiseriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, widest in the middle of the apical cell, medianly 1-septate, generally not constricted at septum, with some ascospores on the leaf surface appearing slightly constricted; ascospores tapering more prominently towards the lower end (9–)10–11(–12) \times (2–)2.5(–3) μm .

Specimens examined: New Zealand, leaf of *Telopea* sp., M. Abdelshife, 11 Sept. 1996, PREM 55350 (holotype); New Zealand, leaf of *Telopea* sp., M. Abdelshife, 5 Aug. 1996, BPI 806263; New Zealand, leaf of *Telopea* sp., M. Abdelshife, 6 Aug. 1996, BPI 806264.

Although no species of *Mycosphaerella* has been described from *Telopea* (Corlett 1991, 1995), three species are presently known from leaves of *Protea* (Proteaceae), namely *M. proteae* (Syd.) Arx, *M. jonkershoekensis* Van Wyk *et al.* and *M. bellulus* Crous and M.J Wingf. (Figures 7–9) (Crous & Wingfield 1993). Ascospores of *M. telopeae* are much smaller than those of the large-spored *M. proteae*, which measure 20–33 \times 6–8 (\bar{x} = 26 \times 7) μm . Morphologically ascospores of *M. telopeae* resemble those of *M. jonkershoekensis* which measure 11–23 \times 4–6 (\bar{x} = 18 \times 4.5) μm , but ascospores of the former differ in being much smaller and less prominently constricted at the septum. Ascospores of *M. telopeae* are slightly larger than those of *M. bellulus*, 7–11 \times 2–3 (\bar{x} = 9 \times 2.5) μm , and are not as

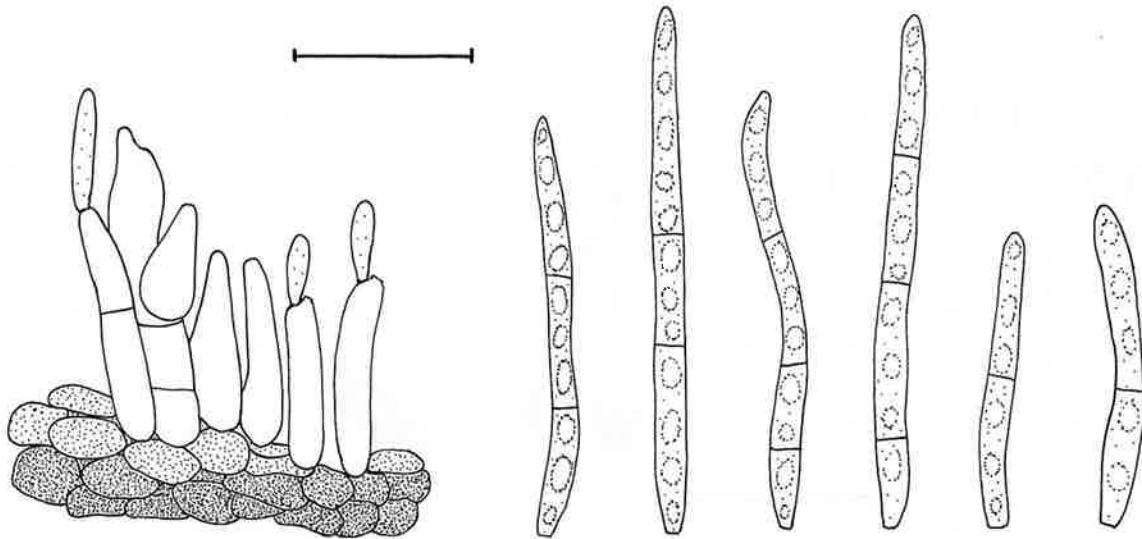


Figure 12 *Septoria protearum*. Conidia and conidiogenous cells formed on carnation leaf agar (bar = 10 μ m).

prominently constricted as in the latter species. The erumpent black pseudothecia of *M. telopea* are also distinct from those of *M. jonkershoekensis* and *M. bellulus*, which are subepidermal and generally not visible to the naked eye.

When Crous and Wingfield (1993) treated the species of *Mycosphaerella* occurring on *Protea*, little was known about their behaviour in culture. Subsequent to that study, fresh collections were obtained of all three of those species. As observed in the type collection of *M. jonkershoekensis* (PREM 44830), ascospores from fresh collections were frequently slightly olivaceous in their asci. When ascospores are shot out for germination on MEA (Crous *et al.* 1991), ascospores become verruculose, brown, and constricted at the septum. Ascospores germinate initially with germ tubes growing parallel to the long axis of the spore (Figure 8). After 48 h, however, ascospores have usually formed several germ tubes, and the germination is irregular. A peculiarity about *M. jonkershoekensis* is that ascospores germinate at 25°C, but die soon after germination if the plates are not incubated at 15°C for one to two weeks. After this initial phase the fungus will grow at most temperatures, and it is hypothesised that this low temperature requirement is a prerequisite for successful germination and infection of leaf tissue. The same phenomenon has also recently been reported for *M. juvenis* Crous and M.J. Wingf. on *Eucalyptus* (Crous & Wingfield 1996).

Ascospores of *M. bellulus* germinate with one to several germ tubes which grow irregularly to the long axis of the spore. As with *M. jonkershoekensis*, spores darken and become verruculose at germination (Figure 7). In the present study, *M. bellulus* was also isolated from leaf lesions of *Leucospermum* spp. (STE-U 1321–1323), and it appears to be a very common species of *Mycosphaerella* on *Protea* spp., frequently also occurring in association with *Leptosphaeria protearum* Syd.

After several unsuccessful attempts, ascospores of *M. proteae* were finally induced to germinate in culture. Unlike *M. jonkershoekensis* and *M. proteae*, ascospores could never be induced to shoot out onto the agar surface, and the epidermis had to be cut open to expose the pseudothecia. This difference, as well as the distinct lesions and red-purple discolouration of the leaf tissue suggest that *M. proteae* is a fungus quite unrelated to the other species dealt with above. In culture, germinating ascospores become constricted at their septum, brown in colour, and

germinate with one germ tube generally parallel to the long axis of the spore (Figure 9). Ascospores did not become as verruculose as those of *M. bellulus* and *M. jonkershoekensis*. Colonies were extremely slow growing, and after about 6 months at 25°C on MEA had hardly reached 5 mm in diam., suggesting that this fungus is more of an obligate pathogen than the other species of *Mycosphaerella* treated here.

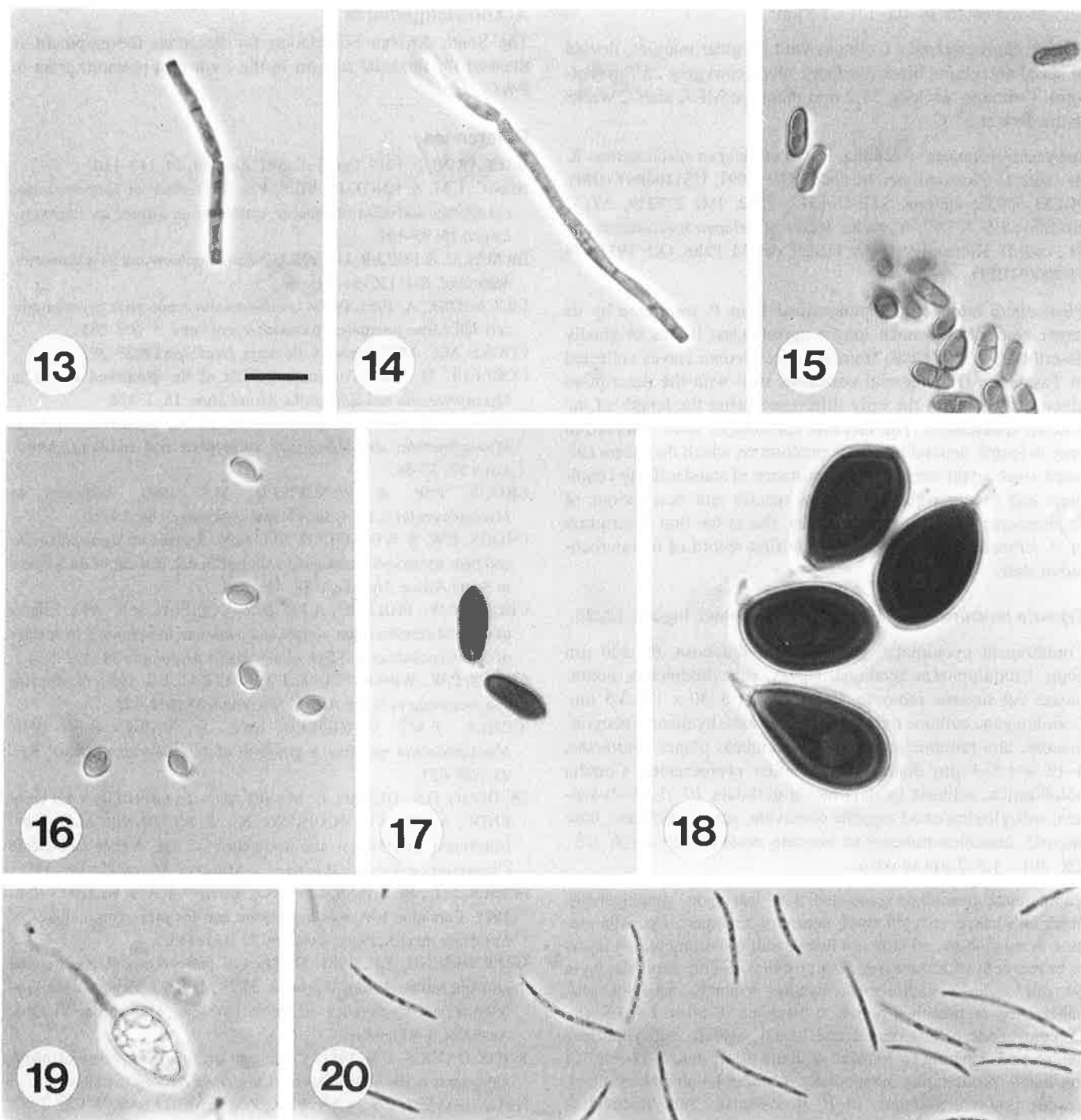
Phyllosticta owaniana G. Winter, Hedwigia 24: 3 1(1885) Figure 10.

Leaf spots amphigenous, circular, 0.5–5 mm diam., light brown, becoming darker brown towards the raised, dark brown border; margins chlorotic when present. Conidiomata pycnidial, predominantly epiphyllous, clearly visible to the naked eye, scattered, immersed, becoming erumpent, globose to subglobose, up to 120 μ m diam., unilocular, medium brown, ostiolate, becoming papillate; wall up to 15 μ m thick, of *textura angularis*, with brown cells becoming lighter towards the interior. Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth-walled, subcylindrical to dolii-form, 5–8 \times 3–6 μ m. Conidia obovoid to ovoid *in vivo*, ovoid *in vitro*, apex rounded, base truncate to rounded, hyaline, guttulate, (10–)12–14(–15) \times 7–8(–9) μ m *in vivo* and *in vitro*, enclosed in a mucous sheath 0.5–3 μ m thick, persistent on most conidia, bearing a single, unbranched, slightly tapering apical mucoid appendage 5–8(–14) \times 1–1.5 μ m.

Cultural characteristics: Colonies with irregular margins, devoid of aerial mycelium, black (surface), olivaceous black 27"m (bottom). Colonies reaching 9.5 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimen examined: South Africa, Western Cape Province, Jonkershoek leaf spots on *Brabejum stellatifolium* L., A. den Breejën, Mar. 1995, PREM 55351, cultures STE-U 1009–1010, IMI 375228, ATCC 201157, CBS 776.97.

Phyllosticta owaniana appears to be a well-established pathogen of *Brabejum stellatifolium* and is associated with prominent leaf spots on this plant throughout the Western Cape, where this host is planted as an ornamental. The present collection correlates well with the original description of Winter (1885), who cited conidia as being ovoid to subpyriform, 10–12 \times 8 μ m.



Figures 13–20 Conidia of microfungi occurring on Proteaceae. **13, 14.** Catenulate conidia of *Cladophialophora proteae*. **15.** *Coniothyrium nitidae*. **16.** *Coniothyrium proteae*. **17.** *Coniothyrium leucospermi*. **18.** *Harknessia leucospermi*. **19.** *Phyllosticta telopeae*. **20.** *Septoria protearum* (bar = 10 µm).

Phyllosticta telopeae H. Y. Yip, Mycol. Res. 93: 494 (1989), Figures 11, 19.

Leaf spots amphigenous, circular to somewhat irregular, often confined by leaf veins, 2–7 mm diam., grey-brown to grey olivaceous with a narrow, dark, slightly raised border on the adaxial surface. Conidiomata pycnidial, predominantly epiphyllous, clearly visible to the naked eye, scattered, immersed, becoming erumpent, globose to subglobose, up to 150 µm diam., unilocular, medium brown, ostiolate, becoming papillate; wall up to 15 µm thick of brown cells of *textura angularis*. Conidiophores reduced to conidiogenous cells. Macroconidiogenous cells hyaline, smooth-walled, subcylindrical to lageniform, 7–12 × 3–5 µm, often proliferating once enteroblasti-

cally and percurrently. Macroconidia ellipsoidal to obovoid with a rounded apex and a truncate base, rarely with a minute marginal frill, unicellular, hyaline, smooth-walled, guttulate, (12–)13–16(–18) × (7–)8–9 µm *in vitro*, 11.5–15.5 × 7–10 µm *in vivo*, enclosed in a thin mucoid sheath, 0.5–2 µm thick bearing a single, unbranched, attenuated apical mucoid appendage 10–20 × 2–3 µm on MEA (up to 40 µm long when cultured on a medium consisting of 2% malt extract, 2% V8 juice and 4% agar), (6.5–)20–100 × 2–3 µm *in vivo*. Microconidiophores subcylindrical, hyaline, 0–2-septate, branched above, 15–20 × 2–3 µm. Microconidiogenous cells subcylindrical, ampulliform to lageniform with minute periclinal thickening, hyaline, smooth-walled, 5–12 × 2–2.5 µm. Microconidia bacillar with a rounded apex and swollen, truncate base, unicellular, hyaline,

smooth-walled, (6-)8-10(-12) × 1.5 µm.

Colony characteristics: Colonies with irregular margins, devoid of aerial mycelium, black (surface), olivaceous grey 25^{mm}m (bottom). Colonies reaching 35.2 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimens examined: Australia, leaves of *Telopea speciosissima* R. Br., coll. D. Koizumi, det. M. Palm, Oct. 1991, US1108807 (BPI), PREM 55352, cultures STE-U 1517, 1522, IMI 375229, ATCC 201158, CBS 777.97; Australia, leaves of *Telopea speciosissima* R. Br., coll. D. Koizumi & J. Van Dersal, det. M. Palm, Oct. 1997, US 1108820 (BPI).

Phyllosticta telopeae is distinguished from *P. owaniana* by its larger conidia and much longer appendages. It was originally described by Yip (1989) from *T. speciosissima* leaves collected in Tasmania. Our material correlates well with the description given by Yip, with the only difference being the length of the mucoid appendages. The fact that appendages were observed to vary in length depending on the medium on which they were cultured, once again stresses the importance of standardising conditions and media when comparing species and descriptions of *Phyllosticta*. As far as we are aware, this is the first description of *P. telopeae* from culture, and the first record of its microconidial state.

***Septoria protearum* L. Viljoen & Crous sp. nov., Figures 12, 20.**

Conidiomata pycnidialia, globosa ad subglobosa, 65-200 µm diam. Conidiophorae hyalinae, laeves, subcylindraceae, nonramosae vel superne ramosae, 0-5-septatae, 8-30 × 1.5-3.5 µm. Conidiogenae cellulae terminales et laterales, hyalinae, subcylindraceae, non ramulosae, ad apices rotundatas planes contractae, 4-12 × 1.5-3 µm diam.; sympodialiter proliferantes. Conidia holoblastica, solitaria hyalina laevia guttulata, (0-)1-3(-4)-septata, subcylindracea ad anguste obclavata, apice subobtusum, base angusta, obconico-truncata ad truncata, recta ad curvata (6-)15-22(-30) × 1.5-2 µm *in vitro*.

Conidiomata pycnidial, associated with leaf spots, amphigenous, black on surface, subepidermal, becoming erumpent. Pycnidia globose to subglobose, 65-200 µm diam.; wall consisting of 3-4 layers of brown cells of *textura angularis*; ostioles slightly papillate, up to 60 µm wide. Conidiophores hyaline, smooth, subcylindrical, unbranched or branched above, 0-5-septate, 8-30 × 1.5-3.5 µm. Conidiogenous cells terminal and lateral, hyaline, subcylindrical, unbranched, tapering to rounded or flattened apices, 4-12 × 1.5-3 µm diam., proliferating sympodially. Conidia holoblastic, solitary, hyaline, smooth, guttulate, (0-)1-3(-4)-septate, subcylindrical to narrowly obclavate, apex subobtusum, base narrow obconically truncate to truncate, straight to curved, (6-)15-22(-30) × 1.5-2 µm *in vitro*.

Cultural characteristics: Colonies with smooth margins, iron grey 23^{mm}k (bottom), with moderate whitish aerial mycelium. Colonies reaching 10.3 mm diam. on MEA after 2 weeks in the dark at 25°C.

Specimen examined: South Africa, Gauteng Province, Pretoria, leaves of *Protea cynaroides*, L. Viljoen, Sept. 1996, PREM 55353 (holotype), culture ex-type STE-U 1470, IMI 375230, ATCC 201159, CBS 778.97.

As far as we could establish, only one other species of *Septoria* has thus far been associated with leaf spots on, or described from leaves of *Protea*. *Septoria proteae* Ciccar. was described as having 1-3-septate conidia, 40-50 × 3-4 µm (Ciccarone 1951), thus being much larger than those of *S. protearum*.

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