

Batcheloromyces species occurring on Proteaceae in South Africa

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Batcheloromyces spp. are dematiaceous hyphomycetes which cause leaf spots on members of the Proteaceae in South Africa. The leaf spots are non-necrotic and composed of numerous sporodochial conidiomata. Conidiogenesis is holoblastic with percurrent, enteroblastic proliferation producing conidiogenous cells with ragged and irregular annellations. Brown, verrucose, aseptate and multiseptate conidia are produced. Conidia produced percurrently, can remain adhered to each other in fragile chains, disarticulating to produce aseptate conidia or, in some cases, they remain as multiseptate conidia where further schizolytic cleavage, at the delimiting septa, may or may not occur. This paper compares *Batcheloromyces* with the morphologically similar *Stigmina*, discusses the current members of *Batcheloromyces* and introduces the new species *B. leucospermi*.

Batcheloromyces was first described causing leaf spots on *Protea cynaroides* in South Africa (Marasas, Van Wyk & Knox-Davies, 1975). The type species is *B. proteae* Marasas, P. S. van Wyk & Knox-Dav. *B. leucadendri* P. S. van Wyk, Marasas & Knox-Dav. was described from *Leucadendron gandogeri* (Van Wyk, Marasas & Knox-Davies, 1985). To date, this genus has been recorded only from members of the Proteaceae in South Africa.

The generic concept of *Batcheloromyces* was reviewed by Sutton & Pascoe (1989) who concluded that the genus (represented by *B. proteae*) was congeneric with *Stigmina*. The basis for their decision was the similarity of their conidiomata and conidiogenesis. *Stigmina* conidiomata are effuse to sporodochial, develop from stromata formed within stomatal cavities, and have superficial or immersed mycelium associated with the sporodochia (Sutton & Pascoe, 1989). In *Stigmina* spp. holoblastic, dark brown, distoseptate conidia arise solitarily from percurrently proliferating, verruculose, ragged conidiogenous cells.

A distinguishing feature of *Batcheloromyces*, the production of conidia in chains, was considered a significant enough feature for the retention of *Batcheloromyces* as separate from *Stigmina* (Crous & Braun, 1996). The production of basipetal, catenate conidial chains (Madelin, 1979) by *Batcheloromyces*, was disputed by Sutton & Pascoe (1989), who stated that this observation was incorrect and that the conidia are solitary. It was further suggested that what appeared to be chains were in fact, '0–1 septate conidia, still attached to conidiogenous cells, displaced in preparation of the material giving the impression of disarticulating, seceding short chains of conidia because the conidiogenous cells and the conidia are of similar sizes' (Sutton & Pascoe, 1989). It is probable that Sutton &

Pascoe (1989) did not observe the chains, because Crous & Braun (1996) subsequently observed them on fresh material.

In this paper, comparisons are made between *Batcheloromyces* and *Stigmina*, *B. leucospermi* sp. nov. is described and the three species of *Batcheloromyces* are compared.

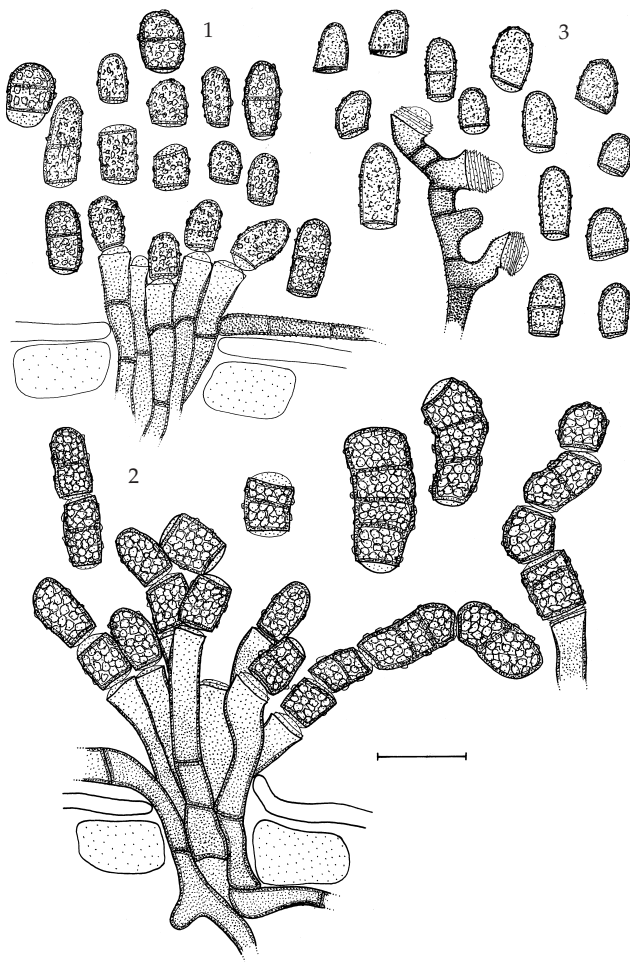
MATERIALS AND METHODS

Specimens of wild and cultivated hosts were collected and returned to the laboratory for further study. All measurements and photographs were made from specimens mounted in lactophenol. Comparisons of cultural characteristics and behaviour of single spore isolates were made on 2% malt extract agar (MEA, Biolab) at 22 °C under a 12 h fluorescent white light/12 h dark regime. Colony colours were rated according to Rayner (1970). Herbarium specimens were lodged at PREM and cultures deposited at STE-U.

TAXONOMY

Batcheloromyces Marasas, P. S. van Wyk & Knox-Dav., *J. S. Afr. Bot.* **41**: 43 (1975).

Colonies on leaves circular, radiating, causing reddish-brown leaf spots. Hyphae restricted in the leaf tissue to the stomata as stromatic mycelial plugs; forming, on the leaf surface above the stomata, pulvinate to effuse sporodochia from which radiate superficial, brown, septate, branched, reticulate hyphae. Conidiophores macronematous, mononematous, simple, brown, developing as short, erect or repent branches of the superficial hyphae, with a single terminal conidiogenous cell. Conidiogenous cells holoblastic, integrated, terminal, brown, calyciform to doliiform, percurrent with distinct ragged



Figs 1–3. Diagrammatic representation of *Batcheloromyces* spp. **Fig. 1.** *B. leucadendri*. **Fig. 2.** *B. leucospermi*. **Fig. 3.** *B. proteae*. Scale bar: 10 μ m.

annellations. Conidia arise singly, dry, brown, smooth or verrucose, thick-walled, ellipsoidal or bacilliform with a marginal frill at one or both ends, with the apex rounded in the former, aseptate or multiseptate; conidia produced by successive percurrent proliferations of the conidiogenous cell, may hang together in short fragile, false, basipetal chains.

Batcheloromyces leucadendri P. S. van Wyk, Marasas & Knox-Dav., *S. Afr. J. Bot.* **51**: 344 (1985). (Figs 1, 4–13)

Leaf spots amphigenous, circular to irregular, radiating, discrete, becoming confluent and ultimately covering large areas of the leaf, non-necrotic, brown to black, sometimes causing discolouration of the leaf, 1–5(–8) mm diam.; comprising numerous flattened, brown sporodochia (Fig. 4). *Conidiomata* sporodochial, composed of a single layer of radiating, septate, brown hyphae which are formed from a substomatal stroma, composed of thick-walled, dark brown mycelia, 25–110 μ m diam.; hyphae radiating from sporodochia and adhering closely to the host surface and following the contours of the cell borders, 2.5–4 μ m wide (Fig. 1). *Conidiophores* erect or repent lateral branches on the superficial hyphae, simple, brown, effuse but occurring mainly towards the centre of the sporodochial plate, above the stomata, sometimes proliferating

to form hyphae (Figs 1, 8). *Conidiogenous cells* doliform to calyciform, holoblastic with percurrent proliferation, forming up to four irregular, ragged annellations, (2.5–) 3.5–4(–5) μ m high \times (2.5–) 3.5–4(–5) μ m diam., narrowing to (2–) 2.5–3 μ m at the base (Figs 1, 5–7). *Conidia* arising singly from blown out ends of the conidiogenous cells, solitary, separating from the conidiogenous cell by schizolytic secession, aseptate or multiseptate, with the latter usually separating into aseptate conidia (or sometimes remaining as 1- or 2-septate conidia) by schizolytic cleavage, and often remaining attached to form fragile chains of up to three conidia; oblong or bacilliform, with thick verrucose walls, possessing a marginal frill at one or often both ends derived from conidial secession; most conidia have truncate ends, both possessing a marginal frill, but the aseptate conidia, produced singly or as the apical cells of the fragile chain, have rounded apices, and truncate bases with marginal frills; aseptate conidia, (4–) 5.5–6.5(–8) \times (3–) 4–5(–6) μ m; 1-septate conidia, (6.5–) 7.5–9(–11.5) \times (3.5–) 4.5–5.5(–7) μ m; 2-septate conidia, 11–13 \times 6–7 μ m (Figs 1, 9–13).

Cultural characteristics. Colonies moderately slow growing, up to 40 mm diam. in 60 d, iron grey 25''''K and olivaceous black 27''''K in reverse, smooth with irregular margins, aerial mycelium sparse and grey or lacking.

Host range. *Leucadendron argenteum* (L.) R. Br., *L. coniferum* (L.) Meisn., *L. coniferum* \times *floridum* R. Br. cv. Pisa, *L. discolor* E. Phillips & Hutch., *L. elimense* E. Phillips, *L. gandogeri*, *L. laureolum* (Lam.) Fourc., *L. salicifolium* (Salisb.) I. Williams, *L. salignum* P. J. Bergius, *L. uliginosum* R. Br., *L. xanthoconus* (Kuntze) K. Schum., *Leucadendron* spp. (Van Wyk *et al.*, 1985).

Teleomorph. Unknown.

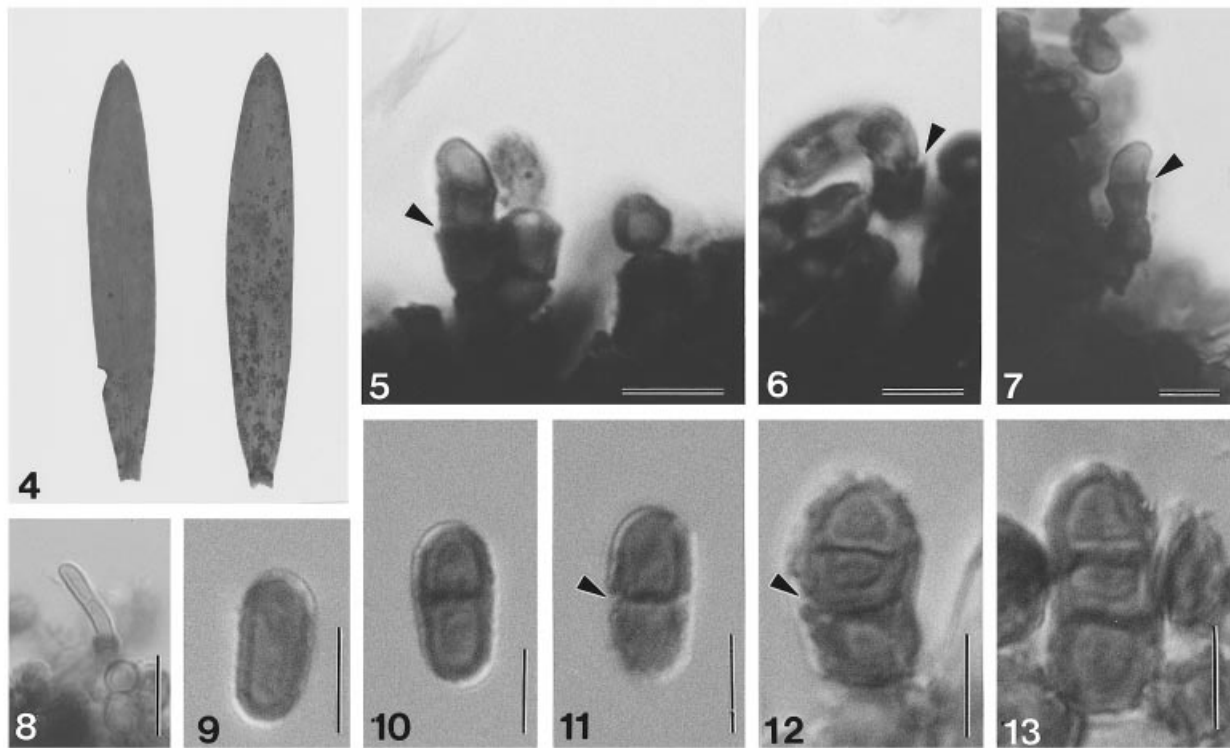
Known distribution. South Africa.

Material examined: South Africa, Western Cape, Cape Town, Kirstenbosch Botanical Gardens, on a living leaf of *L. coniferum*, 1996, L. Swart, F54, PREM 55954, STE-U 1840; Porterville, Osdam Farm, on a living leaf of *Leucadendron* sp., 15 Jan. 1998, J. E. Taylor, JT84, PREM 55940; Stellenbosch, Helderberg Nature Reserve, on a living leaf of *Leucadendron* sp., 19 Jan. 1998, J. E. Taylor, JT107, PREM 55941; Stellenbosch, Protea Heights farm, on a living leaf of *L. laureolum*, 1996, L. Swart, F48, PREM 55949, STE-U 1838; *ibid.*, *L. coniferum* \times *floridum* cv. Pisa, F49, PREM 55950; Stellenbosch, J. S. Marais Park, on a living leaf of *L. salicifolium*, 1996, L. Swart, F50, PREM 55951; *ibid.*, *Leucadendron* sp., F51, PREM 55952, STE-U 1839; *ibid.*, *L. elimense*, F52, PREM 55953.

Batcheloromyces leucadendri has been collected only from *Leucadendron* spp. in South Africa. It can be distinguished from *B. proteae* by the smaller leaf spots (1–5(–8) mm diam. *vs.* 1–25 mm for *B. proteae*) and sporodochia (25–110 μ m diam. *v.* 100–120 μ m diam. for *B. proteae*), and the rougher-walled spores.

Batcheloromyces leucospermi J. E. Taylor & Crous, sp. nov. (Figs. 2, 14–15)

Etymology: In reference to its host, *Leucospermum*.



Figs 4–13. *Batcheloromyces leucadendri*. **Fig. 4.** Leaf spot on *Leucadendron* sp. **Figs 5–7.** Calyciform conidiogeneous cells showing ragged annellations (arrows). **Fig. 8.** Conidiogeneous cell proliferating to form a hypha. **Figs 9–13.** Aseptate and multiseptate conidia, with some of the latter showing signs of schizolytic cleavage (arrowed). Scale bars: 5, 6, 9–13 = 5 μ m; 7, 8 = 10 μ m.

Coloniae epiphyllae plerumque orbiculatae ad irregulares, radiantes, atrae. *Conidiomata* sporodochia, superficialia, effusa, 55–106 μ m diam. *Hyphae* 2.5–4 μ m crassae. *Conidiophora* effusa sed creberina in centro coloniae plerumque, ex cella conidiogena una composita. *Cellae conidiogena*e doliformes vel cylindratae, usque ad 3 proliferationes successivas (annellationes) producentes, (4–) 5.5–6.5(–10) \times (2.5–) 3–3.5(–4) μ m. *Conidia* solitaria ad catenata, brunnea, crassitunicata, verrucosa, ellipsoidea vel bacillaria, continua (6–) 7–9(–14) \times (4–) 5.5–6.5(–8) μ m, vel uniseptata (5.5–) 8–9.5(–12) \times (4–) 5–5.5(–7) μ m, vel biseptata 9–15 \times 5–7 μ m, vel triseptata 12–18 \times 4–7 μ m. Habitat in foliis vivis *Leucospermum* sp. Eastern Cape, 1997, L. Swart, PREM 55955, holotypus.

Leaf spots mainly epiphyllous, circular to irregular, radiating, initially discrete, becoming confluent and sometimes covering large areas of the leaf, non-necrotic, pale to dark brown and darker in the centre, causing discolouration of the leaf; comprising sparsely scattered, effuse, flattened, brown sporodochia, 2–5 mm diam. (Fig. 14). *Conidiomata* sporodochial, effuse, spreading, composed of a single layer of radiating, branched, septate, brown hyphae which are formed from substomatal stomata, composed of thick-walled, dark brown mycelia, 55–106 μ m; hyphae radiating from the sporodochia and adhering closely to the host surface, 2.5–4 μ m diam. (Figs 2, 15). *Conidiophores* erect or repent lateral branches on the superficial hyphae, simple or branched, brown, effuse but occurring mainly towards the centre of the sporodochial plates, above the stomata, sometimes proliferating to form hyphae (Fig. 2). *Conidiogenous cells* doliform to cylindrical, holoblastic with percurrent proliferation, producing up to three irregular, ragged annellides, (4–) 5.5–6.5(–10) \times (2.5–) 3–3.5(–4) μ m (Figs 2, 16–18). *Conidia* arising singly from

blown out ends of the conidiogenous cells and separating from the conidiogenous cell by schizolytic secession, aseptate, often remaining in fragile chains of up to eight conidia or, less often, remaining as multiseptate conidia; ellipsoidal or oblong, with thick verrucose walls with the ornamentations appearing to occur in irregular rough plates. Marginal frills at one or often both ends of the conidia are remnants resulting from conidial secession; most conidia having truncate ends, both possessing marginal frills, but those produced singly, or as the apical cells of the fragile chain, have rounded apices and truncate bases with marginal frills; aseptate conidia, (6–)7–9(–14) \times (4–) 5.5–6.5(–8) μ m; 1-septate conidia (5.5–) 8–9.5(–12) \times (4–) 5–5.5(–7) μ m; 2-septate conidia, 9–15 \times 5–7 μ m; 3-septate conidia, 12–18 \times 4–7 μ m (Figs 2, 19–25).

Culture characteristics. Colonies moderately slow growing, up to 40 mm diam. in 60 d, iron grey 25[°]K and olivaceous black 27[°]K in reverse, smooth with irregular margins, aerial mycelium sparse and grey or lacking.

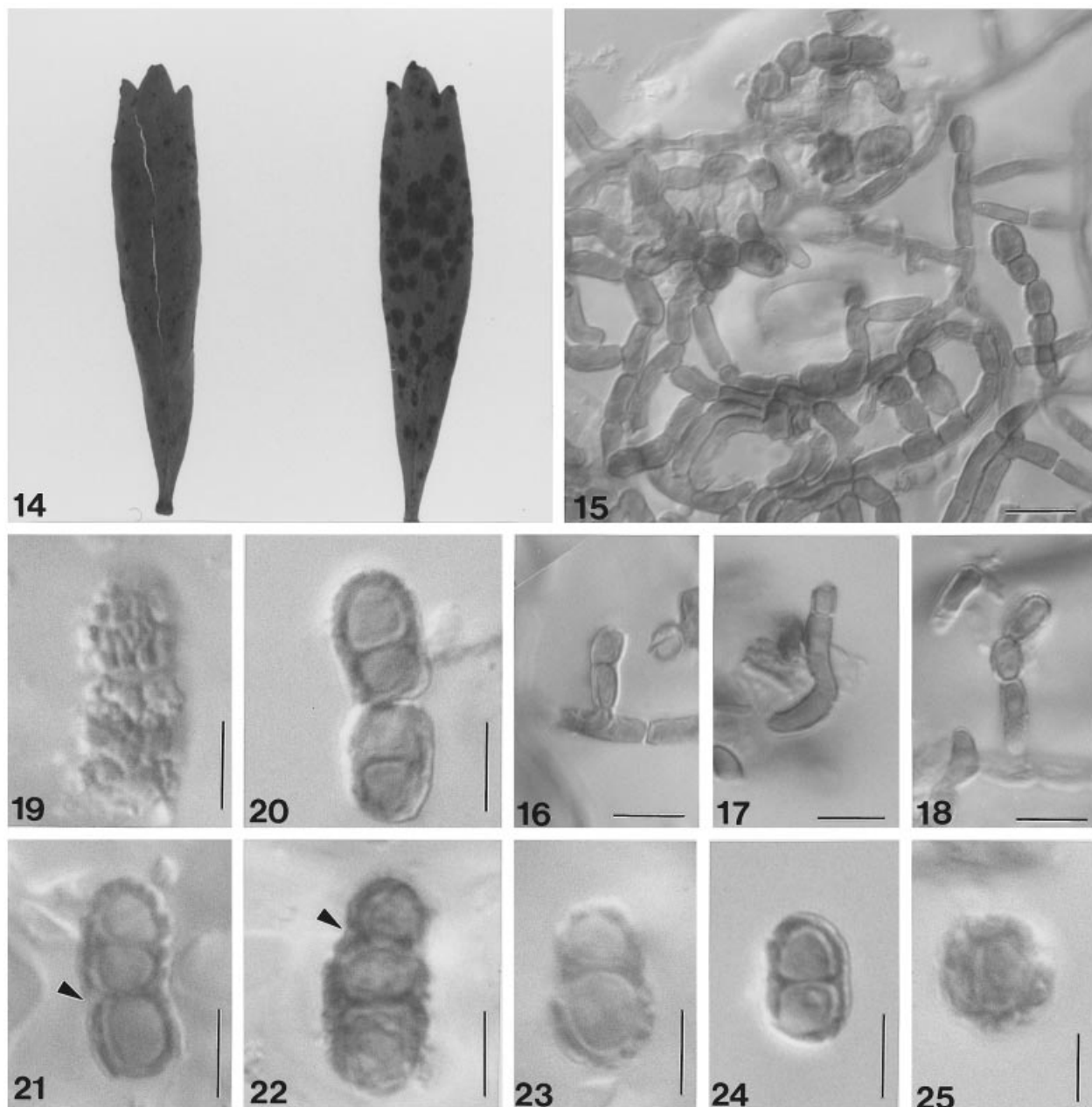
Host range. *Leucospermum* sp.

Teleomorph. Unknown.

Known distribution. South Africa.

Material examined: South Africa, Eastern Cape, Humansdorp, Thornhill Farm, on a living leaf of *Leucospermum* sp., 1997, L. Swart, PREM 55955 (holotype), culture ex-type STE-U 1841.

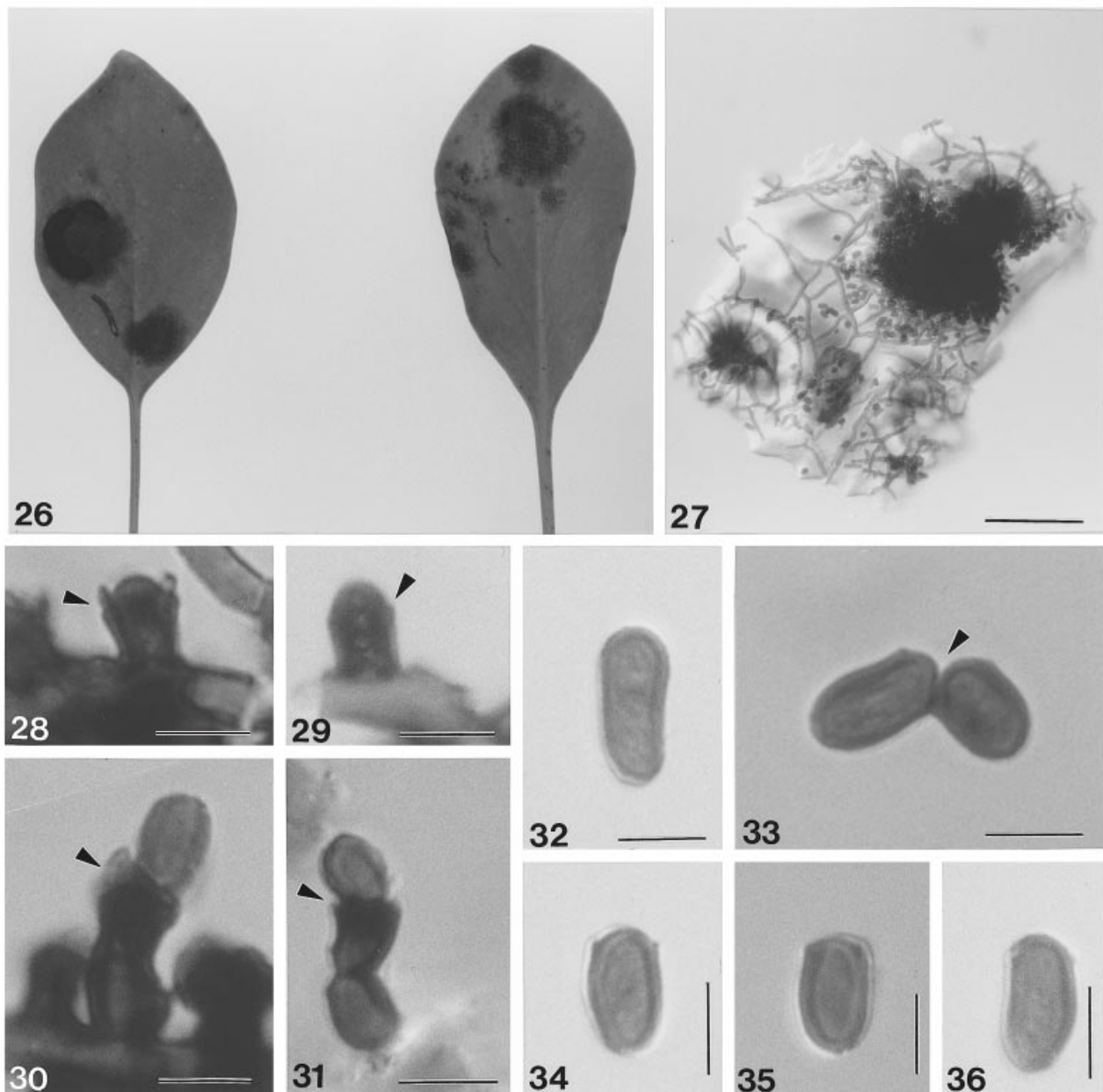
Batcheloromyces leucospermi is known only from *Leucospermum* and can be distinguished from the two previously



Figs 14–25. *Batcheloromyces leucospermi*. **Fig. 14.** Leaf spot on *Leucospermum* sp. **Fig. 15.** Effuse sporodochia and hyphae on host. Note the conidia remaining in fragile chains. **Figs 16–18.** Cylindrical conidiogenous cells. **Fig. 19.** Ornamentation of conidial wall appearing as irregular verrucose plates. **Fig. 20.** Multiseptate conidia showing signs of schizolytic cleavage. **Figs 21–25.** Aseptate and multiseptate conidia, with some of the latter showing signs of schizolytic cleavage at the delimiting septa (arrowed). Scale bars: 15, 16–18 = 10 μm ; 19–25 = 5 μm .

described *Batcheloromyces* species based on the following morphological features. *B. proteae* possesses smaller conidia ($4\text{--}9 \times 3\text{--}5 \mu\text{m}$ v. $5\text{--}18 \times 4\text{--}8 \mu\text{m}$ for *B. leucospermi*), which have smoother walls, form shorter chains (consisting of up to two conidia) and are borne on calyciform conidiogenous cells as opposed to the mainly cylindrical conidiogenous cells of *B. leucospermi*. In addition, the leaf spots formed by *B. proteae* are larger and are distinctly reddish-brown. *B. leucospermi* is most similar in morphology to *B. leucadendri*, as the dimensions and morphology of the leaf spots and sporodochia overlap, and the two species also resemble each other very closely in culture. Despite an overlap in dimensions, however, conidia

collected from *Leucospermum* are consistently larger than those of *B. leucadendri*, and the conidium walls of the former species are more verrucose with the ornamentations appearing to occur in irregular, rough plates. In addition, the conidia consistently occur in chains of up to eight, both on the host material and in culture. Significantly more aerial mycelium forms in cultures of *B. leucadendri* than in *B. leucospermi*, and the conidial chains, that are characteristic of cultures of the *B. leucospermi*, are not observed. Furthermore, the conidiophores and conidiogenous cells of the latter species are more cylindrical. The combination of these consistent differences leads us to conclude that these are different species.



Figs 26–36. *Batcheloromyces proteae*. **Fig. 26.** Leaf spot on *Protea cynaroides*. **Fig. 27.** Dense sporodochia developing from sub-stomatal stomata. Note the hyphae radiating across the host surface. **Figs 28–31.** Calyciform conidiogenous cells showing ragged annellations (arrowed). **Figs 32–36.** Aseptate conidia, occurring singly or in the process of disarticulating (arrowed). Scale bars: 27 = 50 μm ; 28–36 = 5 μm .

Batcheloromyces proteae Marasas, P. S. van Wyk, & Knox-Dav., *J. S. Afr. Bot.* **41**: 43 (1975). (Figs. 3, 26–36)
 ≡ *Stigmina proteae* (Marasas, P. S. van Wyk, & Knox-Dav.)
 B. Sutton & Pascoe, *Mycol. Res.* **92**: 214 (1989).

Leaf spots amphigenous, circular, radiating, discrete, at times becoming confluent and covering large areas of the leaf surface, non-necrotic, causing reddish-brown discolouration and at times visible on corresponding areas of the opposite leaf surface, comprising numerous black sporodochia, 1–25 mm (Fig. 26). *Conidiomata* sporodochial, composed of a single layer of radiating, septate, branched, light to dark brown, thick-walled hyphae which are formed from a sub-stomatal stroma, composed of thick-walled, dark brown mycelia, 100–120 μm diam.; hyphae radiating from the

sporodochia and adhering closely to the host surface, 2.5–4 μm diam. (Figs 3, 27). *Conidiophores* erect or repent, short lateral branches on the superficial hyphae, simple, brown, effuse but occurring mainly towards the centre of the sporodochia, above the stomata, terminating to produce conidiogenous cells mainly calyciform but occasionally doliiform, holoblastic with percurrent proliferation, forming up to three irregular, ragged annellides, (3–) 3.5–4(–5) μm high \times (3–) 3.5–4(–5) μm diam. narrowing to (2–) 2.5–3(–3.5) μm at the base (or 3.5–4 \times 2.5–3.5 μm when doliiform) (Figs 3, 28–31). *Conidia* arising singly from blown out ends of the conidiogenous cells, solitary, separating from the conidiogenous cell by schizolytic secession, aseptate but often

remaining in fragile chains of up to two conidia or occasionally remaining as 1-septate conidia where further schizolytic cleavage may or may not occur, ellipsoidal, oblong or bacilliform, with thick, smooth to verrucose walls, possessing a marginal frill at one or often both ends derived from conidial secession from the conidiogenous cell or from the chain of conidia, (4–) 6.5–7(–9) × (3–) 3.5–4(–5) μm (Figs 32–36).

Cultural characteristics. Colonies slow growing, approx. 10–18 mm diam. after 60 d, olivaceous 19°K and the same in reverse, smooth with irregular margins, segmented, with aerial mycelium lacking.

Host range. *Protea cynaroides* (L.) L., *P. grandiceps* Tratt, *P. magnifica* Link, *P. neriifolia* R. Br., *P. punctata* Meisn., *P. repens* (L.) L., (Marasas *et al.*, 1975; Smit, Engelbrecht & Knox-Davies, 1983; Van Wyk *et al.*, 1985; Knox-Davies, Van Wyk & Marasas, 1986).

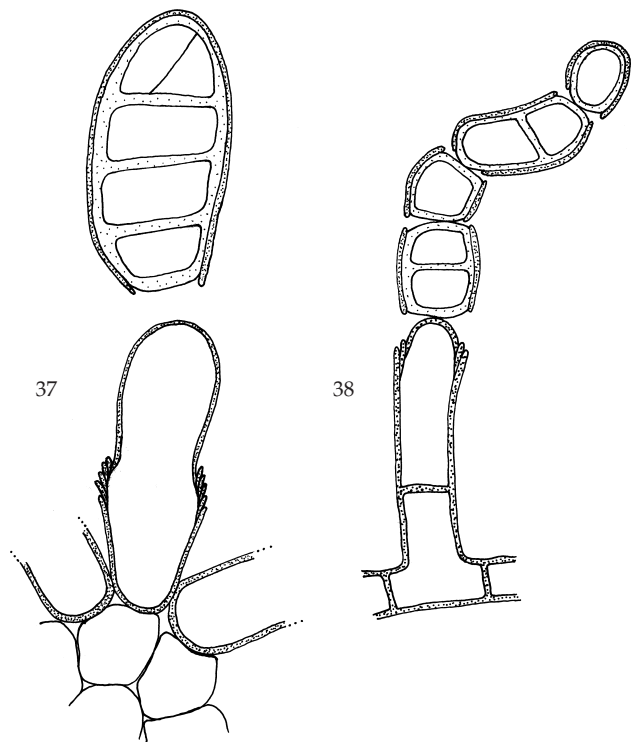
Teleomorph. Unknown.

Material examined: South Africa, Western Cape, Betty's Bay, Harold Porter Botanical Garden, on a living leaf of *P. magnifica*, 1997, L. Swart, F44, PREM 55947; *ibid*, *P. neriifolia*, F45, PREM 55948, STE-U 1836; Stellenbosch, J. S. Marais Park, on a living leaf of *P. cynaroides*, 28 Feb. 1998, J. E. Taylor, JT119, PREM 55942; *ibid*, L. Swart, 1996, F42, PREM 55946; *ibid*, L. Swart, 26 Aug. 1996, F25, PREM 55943, STE-U 1833; *ibid*, *P. repens*, F40, PREM 55944, STE-U 1834; *ibid*, *Protea* sp., F41, PREM 55945, STE-U 1835.

Batcheloromyces proteae has been reported only from *Protea* spp. in South Africa. It differs from *B. leucadendri* for the reasons discussed previously. Smit *et al.* (1983) found that *B. proteae* showed varying symptoms on *P. cynaroides*, which appeared to depend on host plant variety, and were thus due to differences in host genotype.

DISCUSSION

Conidiogenesis in *Stigmina* and *Batcheloromyces* are quite distinct. In Figs 37, 38 diagrammatic representations are compared of the type species of *Stigmina*, *S. platani* (Fuckel) Sacc. (BPI428004), and the type of *B. leucospermi* (PREM 55955). In both species conidiogenesis is holoblastic with percurrent, enteroblastic proliferation producing conidiogenous cells with ragged and irregular annellations. In *Stigmina* (Fig. 37), however, complete secession of the conidia takes place before another conidium is formed. The conidia have rounded apices and truncate bases with marginal frills. In *Batcheloromyces* (Fig. 38) single conidia are produced, which occur singly or adhered to each other in fragile chains, and sometimes fail to disarticulate and remain as multiseptate



Figs 37, 38. Diagrammatic representations of conidiogenesis. **Fig. 37.** *Stigmina platani* (type, BPI428004). **Fig. 38.** *Batcheloromyces leucospermi* (PREM 55955).

conidia. Separation of the conidia is by means of schizolytic secession at the delimiting septa. The majority of the conidia produced possess two truncate ends both possessing marginal frills. Aseptate conidia produced singly or the apical cells of a loose chain of conidia have rounded apices and truncate bases with marginal frills. Consideration of the above characteristics of both genera have led us to conclude that they are distinct. As a consequence, the new collection from a *Leucospermum* sp. is placed in *Batcheloromyces*.

The frills at one or both ends of the conidia in *Batcheloromyces*, as Sutton & Pascoe (1989) point out, are products of conidial secession (Fig. 38). This involves non-simultaneous separation of the wall layers with the outer walls breaking first, leaving the conidia still attached by the inner walls. The separation of the inner wall can be concurrent with, or independent of, the outer wall separating and the resulting conidia, therefore, possess frills of outer wall material surrounding the truncate bases (and often apices) of the conidium, which are composed only of the inner wall (Sutton & Pascoe, 1989).

Batcheloromyces is one of many interesting and unique genera of fungi that are closely associated with, or cause

Key to *Batcheloromyces* species associated with members of the Proteaceae

- 1. Conidia under 10 μm long, mainly aseptate and produced from calyciform conidiogenous cells. Large leaf spots, up to 25 mm diam., usually with a reddish tinge *Batcheloromyces proteae*
- 1. Conidia often more than 10 μm long, sometimes in fragile chains composed of aseptate and multiseptate conidia; occurring on small leaf spots of up to 8 mm diam. 2
 - 2. Conidial outer wall layers very roughly verrucose and often appearing as uneven plates; conidia often remaining in chains of up to eight; conidiogenous cells cylindrical *Batcheloromyces leucospermi*
 - 2. Conidial outer wall layers roughly verrucose; conidia remaining in chains of up to three; conidiogenous cells doliiiform to calyciform *Batcheloromyces leucadendri*

diseases of Proteaceae in South Africa. These fungi are significant components of a unique flora and an important asset in terms of biodiversity. They also cause damage to plants that are grown for the cut flower market, and they often lead to quarantine difficulties in exported flowers, which are a crucial component of this market. Despite many studies on the fungi that cause diseases of Proteaceae in South Africa (Van Wyk, Marasas & Knox-Davies, 1975; Knox-Davies *et al.*, 1986, 1987), we expect that large numbers of pathogens have yet to be discovered.

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