Mycoscience 41: 233–237, 2000

A new species of *Leptosphaerulina* from decaying mangrove wood from Hong Kong

Patrik Inderbitzin[1], E. B. Gareth Jones[2] and Lilian L. P. Vrijmoed[2]

[1] Department of Botany, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada
[2] Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong Special Administrative Region, People's Republic of China

Accepted for publication 10 April 2000

Leptosphaerulina mangrovei **sp. nov. (Pleosporaceae, Ascomycota) from decaying mangrove wood of *Kandelia candel* from subtropical Hong Kong is described and compared to related taxa.**

Key Words——mangrove fungi; marine mycology; pyrenomycetes; taxonomy; tropical mycology.

The genus *Leptosphaerulina* McAlpine (Pleosporaceae, Ascomycota) comprises 23 mainly temperate to arctic-alpine, mostly foliicolous and caulicolous species (Graham and Luttrell, 1961; Barr, 1972; Crivelli, 1983). According to Barr (1972), the genus *Leptosphaerulina* is characterized by small, immersed-erumpent ascomata which are globose to conical, and comprise one to three wall-layers of brown, glabrous cells in *textura angularis*. An apical pore opens widely at maturity. Asci are few, they are saccate or oblong, bitunicate, and the central plectenchyma is often entirely absent at maturity. The ascospores are overlapping biseriate or crowded in the ascus, hyaline, yellowish or brown, obovate to elliptic or clavate, phragmo- or dictyospore, their wall is smooth or roughened at maturity, sometimes surrounded by a thin gelatinous sheath.

Species of *Leptosphaerulina* are parasites or saprobes of dicotyledonous and monocotyledonous plants (Barr, 1972). Some are economically important pathogens on species of *Fabaceae*, e.g., *Arachis hypogaea* L. (Graham and Luttrell, 1961).

On decaying mangrove wood in subtropical Hong Kong, we found a fungus which matches the morphological characteristics of the genus *Leptosphaerulina*.

Material and Methods

Rarely submerged, still attached decaying branches of *Kandelia candel* (L.) Druce were collected, and examined upon return to the laboratory.

Ascomata were taken from the substrate, briefly submerged into 70% ethanol and placed on sea water CMA plates. The plates were incubated in the dark at 25°C. Hyphae were transferred to both sea water and fresh water CMA plates and placed on a laboratory bench at ca. 20°C for teleomorph formation.

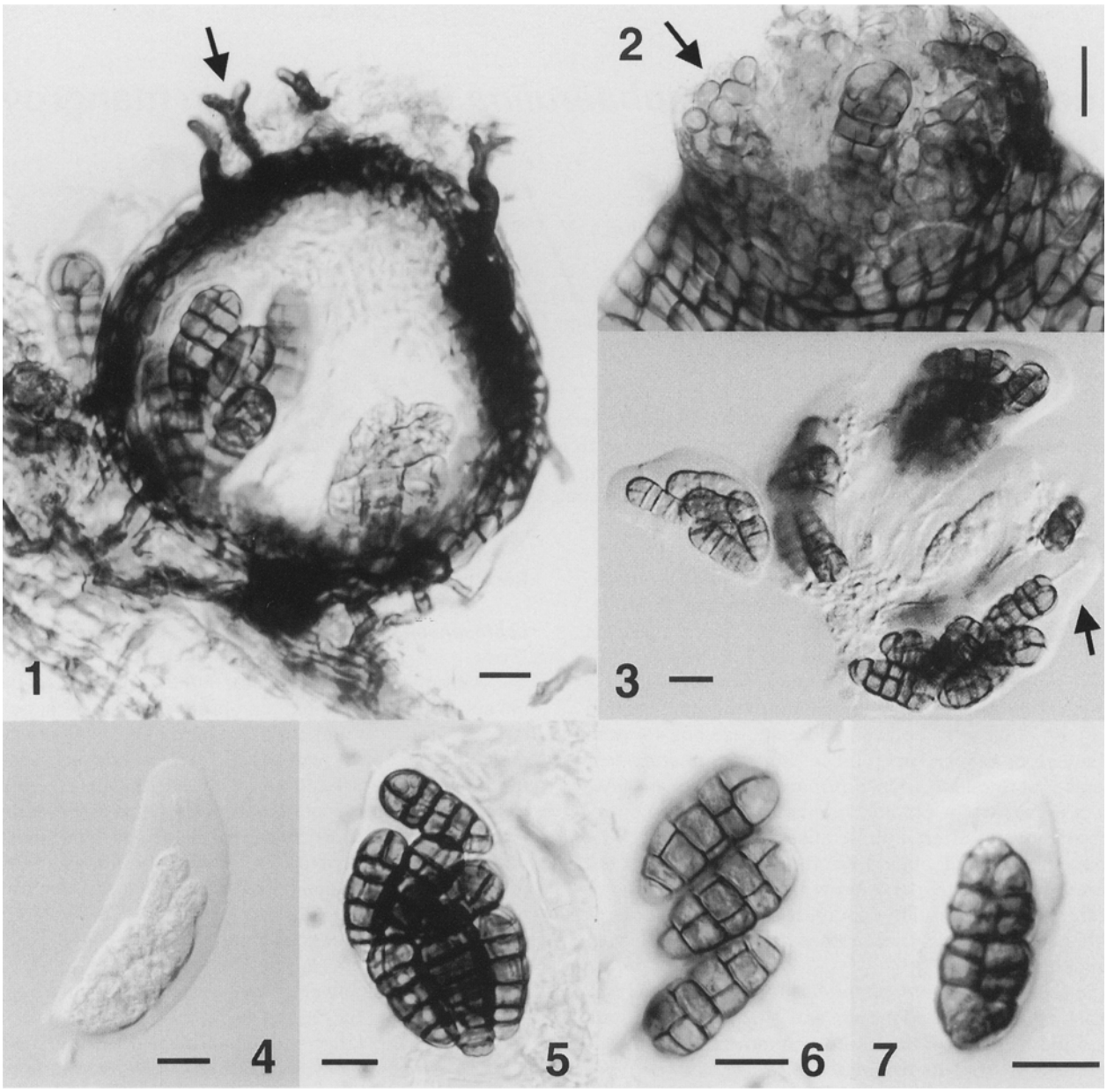
Results

Leptosphaerulina mangrovei Inderb. et E. B. G. Jones, sp. nov. Figs. 1–7

Ascomata 72–90 μm alta, 67–90 μm lata, globosa vel subglobosa, brunnea, membranacea, papillata, ostiolata, solitaria, erumpentia, superne setis hyphoideis paucibus aseptatis usque ad 3 μm crassis ad apicem furcatis praedita. Asci 50–72.5 × 21–35 μm, clavati vel saccati, octospori, bitunicati, cum apice incrassato. Ascosporae 20–28.5 × 8–11 μm, ellipsoideae vel ovales, muriformes, constrictae ad septa, brunneae. Paraphyses absentes, vestigia plectenchymatis super ascos persistentia. Holotypus: HC2.3 (UBC F13873) in ligno emortuo *Kandeliae candel*, Hong Kong.

Etymology: referring to its mangrove habitat.

Ascomata singly erumpent on wood, ascomal venter 72–90 μm high, 67–90 μm wide, dark brown, globose, sometimes with a flattened base, papillate and ostiolate (Fig. 1). Ascomal wall 2–3.2 μm thick, membranous, pseudoparenchymatous, comprising one layer of brown, thin-walled (0.8 μm), smooth cells, ca. 8 μm in diam and forming a *textura angularis* in surface view (Fig. 2). A few frequently septate brown anchoring hyphae from the substratum are attached to the lower portion of the ascoma. Dark brown hyphal setae, non-septate, up to 3 μm wide, thick-walled (1 μm), with forked apices present in upper part (Fig. 1). Papillae 8–22 × 30–40 μm (Fig. 2). Ostiole formed by the decomposition of the ascomal wall, lined at the apex by elongate, thin-walled cells from the ruptured wall (Fig. 2). Interascal tissue very sparse, remnants of centrum plectenchyma persisting above the asci (Fig. 1). Asci 8-spored, 50–72.5 × 21–35 μm (59 × 273 μm on average, n = 22), bitunicate, elongate to saccate, (3–)6–10 in number, maturating sequentially (Figs. 3–5), readily deliquescent in culture, but persistent in material collected from natural habitat. Immature asci elongate, side walls gradually thickening from the base



Figs. 1–7. Micrographs of *Leptosphaerulina mangrovei*. Figs. 1, 3–7. Differential interference contrast. Fig. 2. Bright field. 1. Vertical cryosection of ascoma. Note the forked, non-septate, apical hyphal setae (arrow) and basal anchoring hyphae, the persisting centrum plectenchyma above the asci which maturate sequentially. 2. Polar view of ascoma apex. The ostiole is surrounded by elongate cells (arrow) possibly resulting from the rupture of the apical wall of the ascoma, and topped with an exuded ascospore. Note the one-layered ascomal wall composed of thin-walled cells in *textura angularis*. 3. Squash mount of centrum tissue. Note the asci at different stages of development, the nearly absence of plectenchyma, and the apical channel which reappeared in the ascus after basal rupture (arrow). 4. Ascus containing immature ascospores. Note the thickened apex. 5. Mature ascus almost completely filled by ascospores. Note the compressed, now relatively thin-walled ascus apex. 6. Superficial view of mature ascospores. Note the hyaline basal cell. 7. Old sheathed ascospore. Scale bar = 10 μm.

towards the apex, above the ascospores abruptly extending inwards, and forming a narrow subapical channel (Figs. 3, 4). Mature asci saccate, relatively thin-walled (Fig. 5), the apical channel reappears when the ascus wall ruptures basally, indicating an elastic ascus wall (Fig. 3). Ascospores light brown, muriform, thin-walled, ellipsoidal to oval in outline, 20–28.5 × 8–11 μm (23.5 × 9.5 μm on average, n = 30) (Figs. 5–7). The upper hemispore is shorter and slightly wider than the lower hemispore (Figs. 3, 5). Mature ascospores with 4–5(–7) transsepta, 0–2 longisepta per segment, and often 1–2 angular septa in terminal segment of the upper hemispore, constricted at the septa (Figs. 5, 6), light brown already before septation completed, somewhat darker

when mature, except terminal segment of the lower hemispore which remains nearly hyaline (Fig. 6). No forcible ascospore discharge observed. A sheath-like structure around the ascospores was observed twice (Fig. 7).

Specimens examined: HC2.3 (UBC F13873), holotype, on decaying, rarely submerged attached branches of *K. candel*, Ho Chung Mangrove, New Territories, Hong Kong SAR, P. R. China, leg. M. A. Abdel-Wahab 28 May 1998. Habitat and distribution: Decaying attached branches of *K. candel* at Ho Chung mangrove, Hong Kong.

Cultural characteristics: After 3 wk in the dark at 25°C on sea water CMA plates, a single hypha originating from the concolorous ascomal wall was transferred onto both fresh and sea water CMA plates. Mature ascomata were observed 7 wk after incubation on both fresh and sea water CMA plates placed on a laboratory bench. By then, colonies measured ca. 2.5 cm in diam, the mycelium was mostly immersed in the agar, dark brown with a sharply defined margin. Upright to horizontal ascomata, 98–110 × 80–98 μm, formed either superficially in sparse, light brown colored mycelial patches or immersed-erumpent in the agar. Papillae 26–40 × 24–60 μm. Asci 64–96 × 22–45 μm (n = 5). Ascospores (23–)24–29.5 × 8.8–11.5 μm (26.5 × 10 μm on average, n = 30).

Discussion

Leptosphaerulina mangrovei matches well the generic concept of *Leptosphaerulina*. It differs from described species by its light brown, middle-sized, 4–5 transseptate ascospores with 1–2 longitudinal septa per segment, combined with the presence of forked, apical hyphal setae on ascoma and its marine habitat.

In the following, morphology, cultural characteristics and the habitat of *L. mangrovei* are discussed and compared to other members of the genus.

The ascomata of *L. mangrovei* are average in size within the genus, immersed-erumpent, delimited by a one-layered wall of brown, thin-walled, smooth cells in *textura angularis*. The apex may bear thick-walled, forked hyphae (Fig. 1). Hyphal setae from ascomal wall are also reported from a number of other species in the genus (Barr, 1959; Crivelli, 1983). Rounded, thin-walled and light-colored cells line the apex of the ostiole (Fig. 2). Similar cells associated with the ostiole have been reported in *Leptosphaerulina alpina* Crivelli by Crivelli (1983). The latter species differs morphologically in having larger spores containing 7 transverse septa and 1–3 longitudinal septa per segment.

Ascomata contain only a few asci. They maturate successively (Fig. 3), as pointed out by Graham and Luttrell (1961) for a number of other species in the genus. The apical channel which is absent in mature asci could reappear when the ascus bursts in the basal part (Fig. 3).

In cultures of *Leptosphaerulina crassiasca* (Sechet) C. R. Jackson & D. K. Bell forcible ascospore discharge has been observed (Wu and Hanlin, 1992). The exotunica ruptures, and the endotunica elongates and protrudes through the ostiole to discharge the ascospores up to 4 cm away from the ascoma. This was not observed in *L. mangrovei*. Mature asci containing mature ascospores were difficult to find in culture, they seemed to deliquesce early and release the ascospores into the venter. Ascus deliquescence was not observed in ascomata collected in the mangrove. Hence it remains unclear if ascospore discharge happens actively or passivly in natural habitat.

The ascospore morphology of *L. mangrovei* is most similar to *Leptosphaerulina pulchra* (G. Winter) M. E. Barr, with which it keys out in Crivelli (1983) (Table 1). *L. pulchra* is only known from arctic and alpine regions of the northern hemisphere, and the ascospores are not constricted at the septa (Barr, 1959). Another possibility in Crivelli's key (Table 1) is *Leptosphaerulina gei-reptantis* (Carestia) Crivelli which is of similar size, but differs in ascospore morphology, the glabrous ascomata, and different ecology: *L. gei-reptantis* is confined to arctic-alpine habitats where it forms brown-black spots on leaves of *Geum reptans* L. and *Alchemilla* spp. (Crivelli, 1983). No such symptoms have been observed in the habitat of *L. mangrovei* during collection of the material and in subsequent months.

Crivelli (1983) adds cultural aspects to the generic characteristics. They include a black, compact mycelium comprising thick-walled hyphae, and the dependency on light for teleomorph formation. The mycelium of *L. mangrovei* in culture is compact and dark brown. Fruiting was not observed in cultures growing in darkness.

As several other species described by Crivelli (1983), *L. mangrovei* is rather slow-growing, forming a teleomorph after several weeks of incubation only. In other species, e.g., *L. crassiasca*, the teleomorph is formed after 3 d in culture (Wu and Hanlin, 1991). Ascomata of *L. mangrovei* from culture present larger morphological features than the ones from the natural habitat. This has been observed for other members of the genus (Graham and Luttrell, 1961).

The subtropical occurrence and the substratum of *L. mangrovei* are rare within the genus. Only *L. crassiasca* is found in warmer, peanut growing regions such as Taiwan (Graham and Luttrell, 1961). *Leptosphaerulina hyperborea* (Fuckel) M. E. Barr is the only species of the genus reported from a woody, dicotyledoneous host (Barr, 1959).

The fact that ascoma formation and growth was not significantly different on fresh and sea water CMA reflects the high degree of tolerance of this fungus towards the wide seasonal change in salinity of the mangroves in the Pearl River Estuary, Hong Kong (Sadaba et al., 1995).

Acknowledgements——We thank Profs. M. E. Barr and C. A. Shearer for their comments on this fungus, and M. A. Abdel-Wahab for the collection of the holotype material. P. Inderbitzin acknowledges an NSERC operating grant (principal investigator M. L. Berbee) for partial support.

Table 1. Key to species of *Leptosphaerulina*. Translated and adapted from Crivelli (1983).

1. Ascospores with primary transverse septum distinctly above middle, and upper hemispore broader than lower hemispore. 2
1. Ascospores not so. 13
2. Mature ascospores always with 1–2 transverse septa. 3
2. Ascospores usually with 3 transverse septa, or more than 3 transverse septa. 4
3. Ascospores with 1 transverse septum, brown, 20–24.5 × 8–9.5 μm, on *Potentilla valderia* L. Maritime Alps, France. ..... *L. potentillae* (Müller) Crivelli[a]
3. Ascospores with 2 transverse septa, hyaline, thick-walled, 19–23 × 10–12 μm, on *Primula* spp. Swiss Alps. ..... *L. primulaecola* (Winter) Crivelli[a]
4. Ascospores with (1)–3 or 3 or 3–4 transverse septa. 5
4. Ascospores with 5 or more transverse septa. 8
5. Ascospores with both transverse and longitudinal septa. 6
5. Ascospores usually with transverse septa only. 7
6. Ascospores distinctly constricted at the primary septum. ..... *L. mangrovei*[b]
6. Ascospores not constricted at the septa, with 4 transverse septa, 16–21 × 5–7.5 μm, light brown, on *Potentilla caulescens* L. and other hosts. Subarctic-subalpine Europe and North America. ..... *L. pulchra* (Winter) Barr[a,c]
7a. Ascospores with (1)–3 transverse septa, thick-walled, dark brown, 13.5–32 × 5.5–9(–10) μm, on *Cassiope tetragona* (L.) D. Don., and other subarctic hosts. Subarctic North America. ..... *L. hyperborea* (Fuckel) Barr[d]
7b. Ascospores with 3 transverse septa, thin-walled, light brown, upper part only slightly wider than lower part, 15–27 × 4.5–7.5 μm, on monocotyledons. Temperate Europe and North America. ..... *L. personata* (Niessl) Barr[c]
7c. Ascospores with 3–4 transverse septa, upper part distinctly wider than lower part, light brown, 11–18.5 × 4.5–6.5 μm, on *Senecio carniulicus* Willd. Swiss Alps. ..... *L. senecii* Crivelli[a]
8. Ascospores when young hyaline or light yellow, later light brown. 9
8. Ascospores light to dark brown already when young. 11
9a. Ascospores with 5–6 transverse septa. 10
9b. Ascospores with 8–12 transverse septa, 32–43 × 11–16 μm, on abaxial side of leaves of *Dryas octopetala* L. Arctic-alpine Europe, East Siberia. ..... *L. dryadis* (Starb.) Holm[a,e]
9c. Ascospores with 10–13 transverse septa, 31–45 × 13.5–17 μm, on *Alchemilla nitida* Buser. Swiss Alps. ..... *L. nitida* Crivelli[a]
10a. Ascospores in upper part with several angular septa, 21–32 × 9.5–14.5 μm, on *Potentilla rupestris* L. Alpine and temperate Switzerland. ..... *L. rupestris* Crivelli[a]
10b. Ascospores in upper part with transverse and longitudinal septa only, 19–28 × 9.5 μm, on *Potentilla* spp. and *Alchemilla* spp. Arctic-alpine Europe and North America. ..... *L. vitrea* (Rostr.) Wehm.[a]
10c. Ascospores 32–43 × 11–16 μm, on *Dryas octopetala* L. See point 9b. ..... *L. dryadis* (Starb.) Holm
11. Ascospores generally with 5 or fewer transverse septa. 12
11. Ascospores with 7 transverse septa, with 1 to 3 longitudinal septa per segment, 29–38 × 10–16 μm, on leaves of *Arctostaphylos alpina* (L.) Spreng. European Alps. ..... *L. alpina* Crivelli[a]
12. Ascospores with 5 transverse septa, and 0–1 longitudinal septum per segment, 20–31 × 8–11 μm, on leaves of *Geum reptans* L. European Alps and Arctic. ..... *L. gei-reptantis* (Carestia) Crivelli[a]
12. Ascospores generally with 4–5 transverse septa, 0–2 longitudinal septa per segment, and 0–2 angular septa in terminal segment of upper hemispore, 20–28.5 × 8–11 μm, on decaying wood of a mangrove tree. ..... *L. mangrovei*[b]
13. Ascospores usually with 3 transverse septa, rarely 4, hyaline. 14
13. Ascospores with 4 or more transverse septa, hyaline or colored. 15
14. Central segments of ascospores distinctly shorter than terminal segments, longitudinal septa lacking, 35–45 × 15–18 μm, on leaves of *Vaccinium myrtillus* L. causing leasons. Europe. ..... *L. myrtillina* (Fautr. et Sacc.) Petrak[a]
14. Ascospore segments of similar length, some with a longitudinal septum, 38–62 × 17–26 μm, on *Trifolium* spp. Europe, U.S.A. ..... *L. trifolii* (Rost.) Petrak[f]
15. Ascospores usually with 4 transverse septa. 16
15. Ascospores with more than 4 transverse septa. 18
16. Ascospores generally shorter than 40 μm. 17
16. Ascospores up to 50 μm long and 11–19 μm wide, generally on *Medicago* spp. Europe, U.S.A., possibly Brazil. ..... *L. briosiana* (Poll.) Graham et Luttrell[f]
17. Ascospores always with longitudinal septa 25–40 × 10–15 μm, on hosts of the Poaceae, Violaceae, Brassicaceae. Australia, U.S.A, Europe, possible Japan. ..... *L. australis* McAlp.[f]
17. Ascospores dimorphic, either ellipsoidal with longitudinal septa, or cylindrical without longitudinal septa, on *Arachis* spp. Taiwan, U.S.A. ..... *L. arachidicola* Yen et al.[f]
18. Ascospores with 5–6 transverse septa. 19
18. Ascospores with more than 6 transverse septa. 20
19. Ascospores usually with 5 transverse septa and up to 3 longitudinal septa, 24–43 × 12–17 μm, saprobic on various plants. Argentina, U.S.A. ..... *L. argentiniensis* (Speg.) Graham et Luttrell[f]
19. Ascospores usually with 6 transverse septa and up to 4 longitudinal septa, 35–60 × 13–21 μm, saprobic on various plants. North America. ..... *L. americana* (Ell. et Ev.) Graham et Luttrell[f]
20. Ascospores brown, with 8 transverse septa, the two central segments lentil shaped, without longitudinal septa, the remaining segments with 1 to 2 longitudinal septa, 48–57 × 17–22 μm, on *Geum reptans* L., European Alps. ... *L. sieversiae* (Müller) Crivelli[a]
20. Ascospores with 9 or more transverse septa. 21
21. Ascospores light yellow to light brown, ellipsoidal, with 9–11 transverse septa and several longitudinal septa, 45–55 × 20–25 μm, on *Potentilla caulescens* L., Swiss Alps. ..... *L. albulae* Crivelli[a]
21. Ascospores hyaline, light brown in age, oval, with 12–13 transverse septa and 0–2 longitudinal septa per segment, 55–75 × 14–17 μm on *Ranunculus alpestris*. L., European Alps. ..... *L. carinthiaca* (Petrak) Crivelli[a,g]

Information not contained in Crivelli's (1983) key was taken from:
a) Crivelli (1983).
b) This paper.
c) Barr (1972).
d) Barr (1959).
e) Holm (1979).
f) Graham and Luttrell (1961).
g) Petrak (1955).

Literature cited

Barr, M. E. 1959. Northern Pyrenomycetes I. Canadian Eastern Arctic. Contrib. Inst. Bot. Univ. Montréal **73**: 1–101.

Barr, M. E. 1972. Preliminary studies on the Dothideales in temperate North America. Cont. Univ. Michigan Herb. **9**: 523–638.

Crivelli, P. G. 1983. Über die heterogene Ascomycetengattung *Pleospora* RABH.; Vorschlag für eine Aufteilung, Diss. ETH Nr. 7318. ADAG Administration & Druck AG, Zürich.

Graham, J. H. and Luttrell, E. S. 1961. Species of *Leptosphaerulina* on forage plants. Phytopathology **51**: 680–693.

Holm, L. 1979. Microfungi on *Dryas*. Bot. Notiser **132**: 77–92.

Petrak, F. 1955. Neue Askomyzeten und Fungi imperfecti der österreichischen Flora. Sydowia **9**: 574–584.

Sadaba, R. B., Vrijmoed, L. L. P., Jones, E. B. G. and Hodgkiss, I. J. 1995. Observations on vertical distribution of fungi associated with standing senescent *Acanthus ilicifolius* stems at Mai Po mangrove, Hong Kong. Hydrobiology **295**: 119–126.

Wu, M.-L. and Hanlin, R. T. 1991. Neotypification of *Leptosphaerulina crassiasca*. Mycotaxon **41**: 27–41.

Wu, M.-L. and Hanlin, R. T. 1992. Ascomal development in *Leptosphaerulina crassiasca*. Mycologia **84**: 241–252.