Three new species of *Leptographium* from pine

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Leptographium species are common inhabitants of fresh conifer logs and lumber that are known for their ability to cause blue-stain and, in some cases, their association with disease. *L. procerum* has been associated with a root disease although controversy surrounds its role in tree death. During the course of the past two decades, a relatively large number of isolates tentatively identified as *L. procerum* have been collected in various parts of the world. Some of these display morphological characters unlike those of *L. procerum s. str.* and this has prompted us to re-examine them. Four groups of morphologically distinct isolates were identified, of which *L. procerum s. str.* represented one. The remaining isolates of *Leptographium* are newly described as *L. alethinum*, *L. pityophilum* and *L. euphyes* spp. nov.

INTRODUCTION

Species of *Leptographium* are anamorphs of *Ophiostoma* (*Ascomycota*). Species of this group are well known for their association with insects and particularly bark beetles (*Coleoptera*: *Scolytidae*) that infest conifers (Münch 1907, Rennerfelt 1950, Mathiesen-Käärik 1953). Condiophores of the *Leptographium* states are mononematous, erect and terminate in a series of branches, which give rise to slimy masses of hyaline single-celled conidia (Kendrick 1962, Wingfield 1993). These commonly occur in galleries of bark beetles and are thus well suited to be transferred from one tree to another by the bark beetles as well as any other insects that visit these galleries (Harrington 1988, 1993, Wingfield 1993).

Leptographium procerum is well known in Europe and North America where it has been associated with the disease, white pine root decline on *Pinus strobus* (Kendrick 1962, Alexander *et al*. 1988, Wingfield *et al*. 1988). The fungus, however, occurs on a wide range of conifers (Kendrick 1962, Mackenzie & Dick 1984, Alexander *et al*. 1988) and its role in tree death has been a matter of some considerable debate (Wingfield 1983a, Alexander *et al*. 1988, Harrington 1988). *L. procerum* is closely associated with a number of root and root collar infesting insects and is, thus, commonly found in this niche (Kendrick 1962, Wingfield 1983b, Harrington 1988, Alexander *et al*. 1988). Pathogenicity tests with the fungus have yield contradictory results (Prey 1975, Lackner & Alexander 1982, Harrington & Cobb 1983, Wingfield 1982, 1983a, b) and have not resolved its role as plant pathogen.

Leptographium procerum is characterized by long conidiophores with two or three primary branches on the stipe (Kendrick 1962). A conidiogenous apparatus of three to five series of branches terminate in the conidiogenous cells that produce obovoid conidia with truncate ends. This species is further characterized by the presence of rhizoids at the base of the conidiophores (Kendrick 1962). *L. procerum* can also be recognised by its colonies in which conidiophores are arranged to form dark concentric rings on the surface of the agar.

In recent years, we have accumulated a large number of cultures from many parts of the world, that have tentatively been identified as *L. procerum*. Although these isolates superficially resemble *L. procerum*, significant differences in their morphology and physiology have been noted. The aim of this investigation was to undertake a detailed study of these cultures and to determine whether they can justifiably be retained in a single taxon. This will enable pathologists to accurately identify *L. procerum* isolates and determine its role in causing disease in pines.

MATERIALS AND METHODS

Isolates examined in this study were obtained from a wide range of hosts and geographic locations (Table 1). Comparisons with herbarium specimens, including the holotype of *L. procerum* was also made. These included: **Canada**: *Quebec*: St Paul, *Pinus banksiana*, 4 Sept. 1959, *W. B. Kendrick* (DAOM 63700 – holotype); **USA**: *New York*: Montgomery County, *Pinus resinosa* (interior of roots with resinous lesions), Feb. 1959, *D. S. Welch* (DAOM 62093); *New York*: Newfield,

Table 1. *Leptographium* isolates used in this study.

Number	Identification	Origin	Host	Collector
CMW 2460	*L. procerum*	Poland	*Pinus spp.*	T. Kawalski
CMW 3	*L. procerum*	USA	*P. strobus*	J. Altman
CMW 12	*L. procerum*	USA	*P. strobus*	M. J. Wingfield
CMW 1831	*L. procerum*	USA	*P. monticola*	P. Kulhavy
CMW 825	*L. procerum*	England	*Hylastes opacus*	J. N. Gibbs
CMW 828	*L. procerum*	England	*Hylurgops palliatus*	J. N. Gibbs
CMW 2172	*L. procerum*	England	*Hylobius* sp.	J. N. Gibbs
CMW 522	*L. procerum*	RSA	*Pinus* infested with *Hylastes* sp.	G. Tribe
CMW 699	*L. procerum*	Italy	*P. pinea*	P. Capretti
CMW 3797	*L. procerum*	Norway	*Picea* sp.	M. J. Wingfield
CMW 25	*L. procerum*	Yugoslavia	*P. strobus*	M. Halambek
CMW 747	*L. procerum*	France	*Picea abies*	M. Morelet
CMW 261	*L. procerum*	New Zealand	*P. strobus*	M. Dick
CMW 20	*L. procerum*	Canada	*P. strobus*	Lincar
CMW 2159	*L. alethinum*	England	Corsican pine	J. N. Gibbs
CMW 3764	*L. alethinum*	England	*Hylobius abietis*	A. Uzonovic
CMW 3765	*L. alethinum*	England	*Hylobius abietis*	A. Uzonovic
CMW 3766	*L. alethinum*	England	*Hylobius abietis*	A. Uzonovic
CMW 3767	*L. alethinum*	England	*Hylobius abietis*	A. Uzonovic
CMW 2892	*L. pityophylum*	Italy	*P. nigra*	S. Frisullo
CMW 2838	*L. pityophylum*	Italy	*P. nigra*	S. Frisullo
CMW 2840	*L. pityophylum*	Italy	*P. nigra*	S. Frisullo
CMW 2874	*L. pityophylum*	Italy	*P. nigra*	S. Frisullo
CMW 3047	*L. pityophylum*	Italy	*P. nigra*	S. Frisullo
CMW 3063	*L. pityophylum*	Italy	*P. nigra*	S. Frisullo
CMW 259	*L. euphyes*	New Zealand	*P. strobus*	M. Dick
CMW 264	*L. euphyes*	New Zealand	*P. radiata*	M. Dick
CMW 291	*L. euphyes*	New Zealand	*P. strobus*	M. Dick
CMW 301	*L. euphyes*	New Zealand	*Pinus* sp.	M. Dick

CMW: Culture collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, Republic of South Africa.

DAOM 62094); *New York*: Columbia County, Conoan, *Pinus resinosa* (interior of roots with resinous lesions), Feb. 1959, *D. S. Welch* (DAOM 62095); *New York*: Stockton, Chatauqua County, *Pinus resinosa* (interior of roots with resinous lesions), Feb. 1959, *D. S. Welch* (DAOM 62096); **Sweden:** *Södermanland*: Järna, *Pinus* sp., Aug. 1959, *A. Mathiesen-Käärik*; **Canada**: Sudbury, *Pinus strobus*, Nov. 1952, *S. N. Linszon* (DAOM 33940); DAOM represents the herbarium specimens held by the National Mycological Herbarium, Agriculture and Agri-Food Canada, Ottawa.

Fungal structures produced on 2% Malt extract agar (MEA, 20 g Biolab malt extract, 20 g Biolab agar and 1000 ml distilled water) were used for light as well as scanning electron microscopic study. For light microscopy, relevant structures from the agar cultures, as well as herbarium specimens, were mounted in lactophenol on glass slides. Fifty measurements of each relevant morphological structure were made and ranges and averages computed. Colours of structures and colonies were determined using the charts of Rayner (1970).

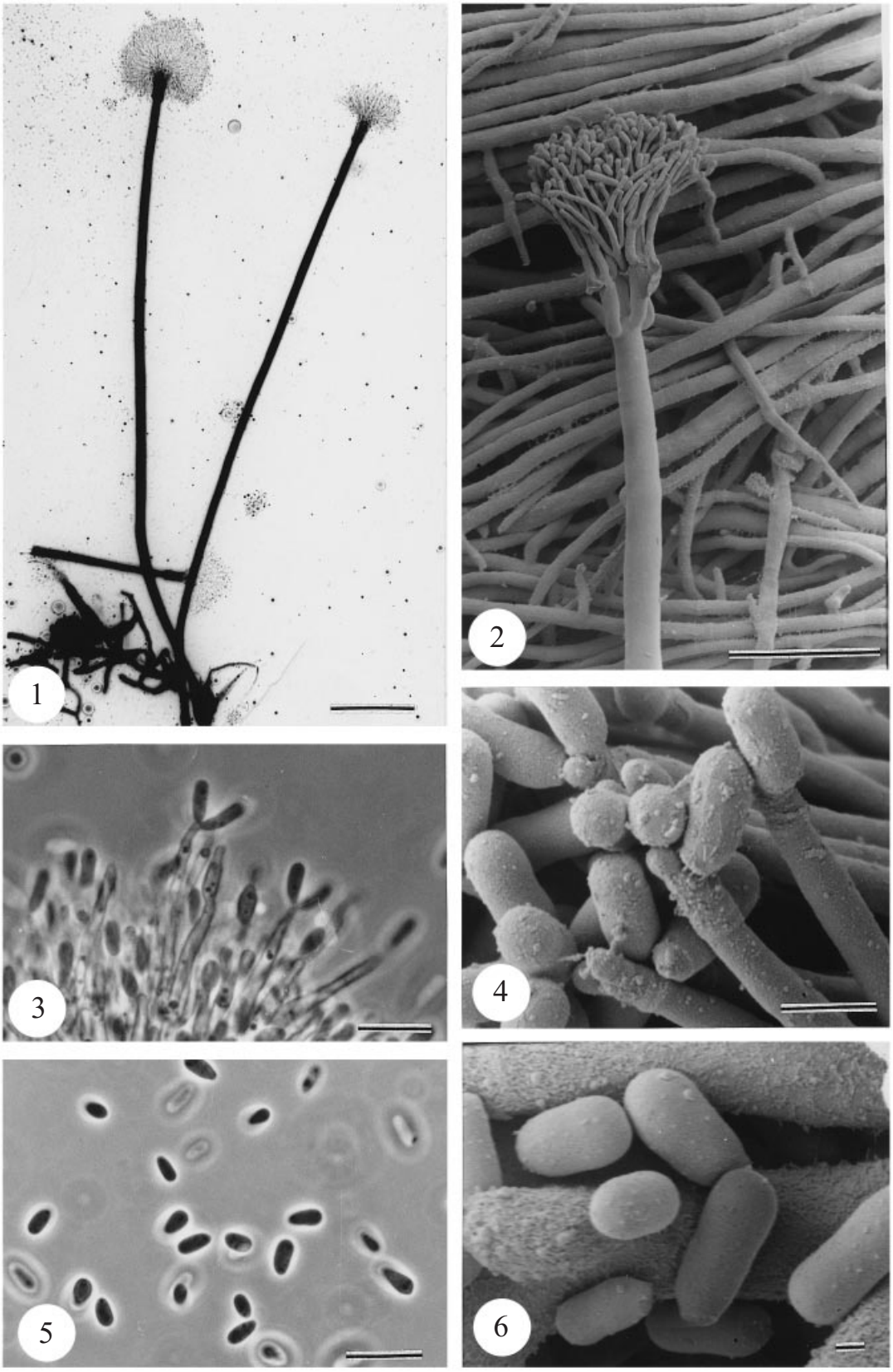
For scanning electron microscopy (SEM), small blocks of agar cut from sporulating colonies were fixed in 3% glutaraldehyde and 0.5% osmium tetroxide in a 0.1 M phosphate buffer, dehydrated in a graded acetone series and critical-point dried. Specimens were mounted and coated with gold palladium alloy and examined using a Joel JSM 840 scanning electron microscope.

Four morphological groups, including the isolates representing *L. procerum s. str.* were identified. Optimal temperatures for growth of representative isolates of these groups [PREM 56349, PREM 56350 (*L. alethinium*); PREM 5635, PREM 56367, (*L. pityophilum*); PREM 56363, PREM 45705 (*L. euphyes*); CMW 2460, CMW 12 (*L. procerum sensu stricto*)] were determined by inoculating eight MEA plates for each temperature with a 6 mm diameter agar disk taken from the actively growing margin of a fresh isolate. The plates were incubated in the dark at temperatures ranging from 5 to 35 °C at 5 ° intervals for 8 days. Colony diameters were measured in two directions perpendicular to each other on the fourth and the eighth day after commencing the experiment, and the diameters of colonies computed as an average of eight readings.

Cycloheximide tolerance of representative isolates of the four morphological groups representing *L. procerum s. lat.* was determined by growing them on 2% MEA amended with different concentrations of cycloheximide (0, 0.05, 0.1, 0.5, 1, 2.5 and 5 g l^{-1}) in Petri dishes. Dishes were incubated in the dark at 25 ° for 8 d and the colony growth was measured. Five replicate plates were inoculated for each concentration and the growth was determined based on an average of ten readings with two readings perpendicular to each other, for each plate.

TAXONOMY

Four morphologically different groups arose from our detailed comparison of the larger set of isolates that had been



Figs 1–6. *Leptographium alethinum* (PREM 56350). **Fig. 1.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (bar = 100 µm). **Fig. 2.** Complex conidiogenous apparatus (bar = 100 µm). **Fig. 3.** Conidiogenous cells showing false sympodial conidiogenesis (bar = 10 µm). **Fig. 4.** Conidiogenous cells showing false sympodial conidiogenesis (bar = 5 µm). **Fig. 5.** Conidia (bar = 10 µm). **Fig. 6.** Conidia (bar = 1 µm).

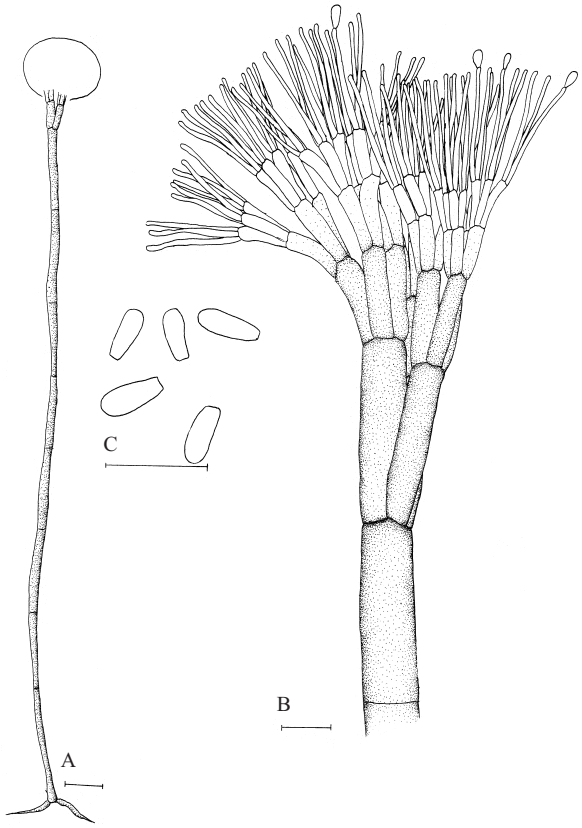


Fig. 7. *Leptographium alethinum* (PREM 56350). A, Habit sketch of the conidiophore (bar = 50 µm). B, Conidiogenous apparatus (bar = 10 µm). C, Conidia (bar = 10 µm).

designated as *L. procerum s. lat*. One of the three represents *L. procerum s. str*. which was confirmed through comparison with the herbarium holotype specimen. Isolates of *L. procerum s. str*. are characterised by long dark conidiophores with two to three primary branches and rhizoids at the bases of the conidiophores. Colonies can easily be recognised by the dark concentric rings formed by clusters of conidiophores. Conidia of *L. procerum* are small and obovoid.

The second morphological group of isolates was characterised by having long conidiophores and obovoid conidia that are considerably longer than those of *L. procerum s. str*. Conidiophores in this group also had rhizoids at their bases. However, the conidiogenous apparatuses in this group of isolates were not as darkly pigmented as those found in *L. procerum s. str*. The third sub-group of isolates was characterised by conidiophores with several short primary branches, similar to those of *L. serpens*. However, unlike *L. serpens*, the hyphae did not have a serpentine growth pattern on the surface of agar. The fourth sub-group of isolates was characterised by short robust conidiophores, unlike those observed in *L. procerum*. This species resembles the *Leptographium* anamorph of *Ophiostoma grandifoliae*. Comparison with other known species of *Leptographium* revealed that the three groups of isolates previously accommodated in *L. procerum s.lat.*, did not resemble any known *Leptographium* species. We, therefore, describe them as new species.

Leptographium alethinum K. Jacobs, M. J. Wingf. & Uzunovic, **sp. nov.** (Figs 1–7)

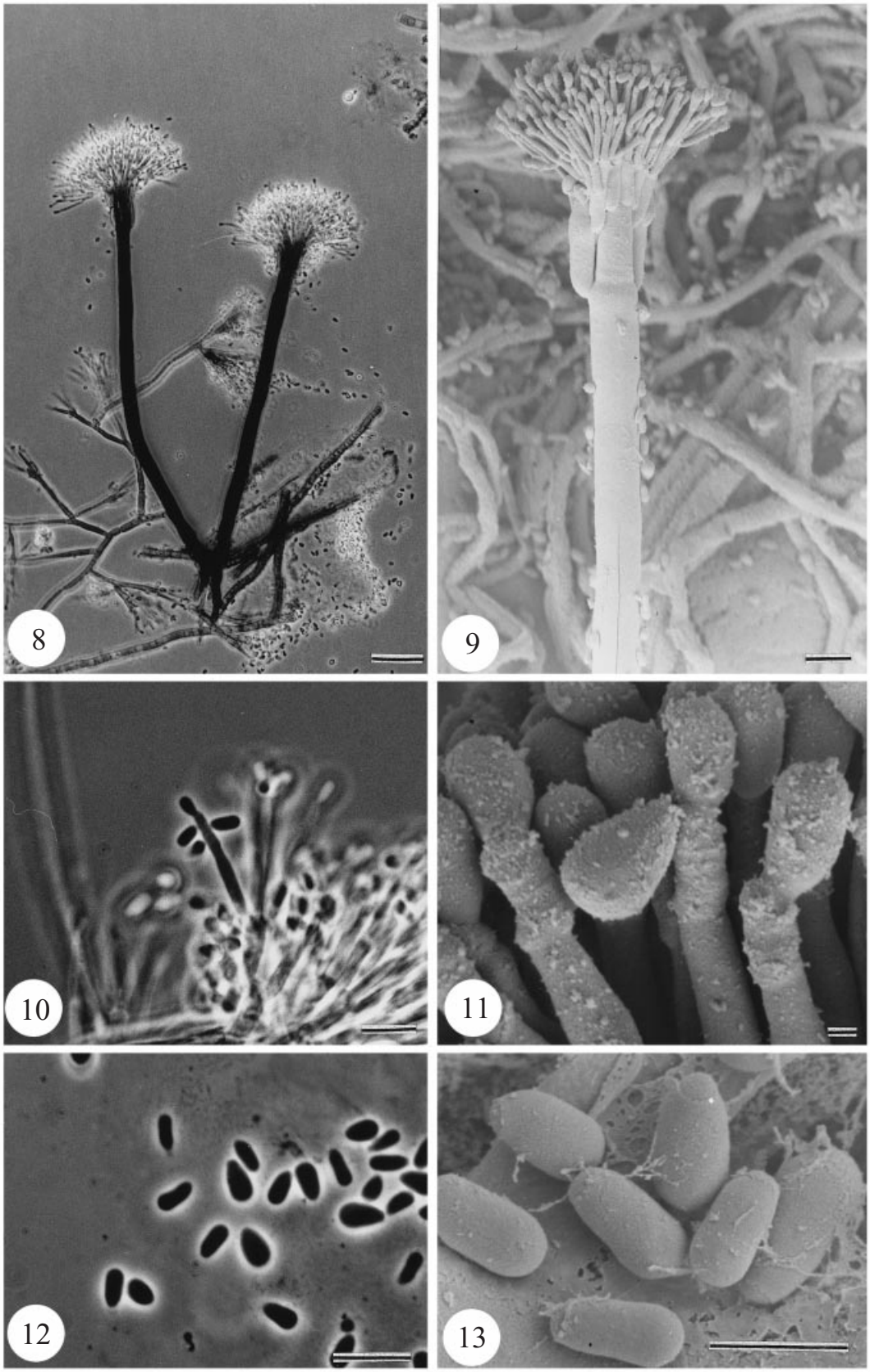
Etym.: From the Greek meaning real or genuine. The name refers to the well developed conidiophores characteristic of species in *Leptographium*.

Teleomorphosis ignota.
Coloniae optime in temperatura 20° crescentes; olivaceae; margine laevi. Hyphae immersae, sine myceliis aeriis. Conidiophores singula vel ad sena, e mycelio recta exorientia, macronematosa, mononematosa, (560–)103(–1270) µm longa, cum 3 vel 4 seriebus ramorum cylindricorum; 2–4 ramis primariis; sine structuris rhizoidiformibus. Conidia aseptata, obovoidea extremitatibus truncatis, (4–)6(–9) × 2–3 µm.

Typus: **Magna Britannia**: *Suffolk*: Thetford, *Hylobus abietis* galleries, on *Pinus nigra* var. *maritima* log, Aug. 1993, *A. Uzunovic* (PREM 56349 – holotypus).

Colonies with optimal growth at 20 °C on 2% MEA, reaching 23 mm diam in 6 d. There was a little growth below 5 ° and no growth above 30°. Able to withstand high concentrations of cycloheximide with a 12% reduction in growth on 0.1 g l^{-1} cycloheximide after 6 days at 20° in the dark. Colony olivaceous (19″). Colony margin smooth. Hyphae submerged with no aerial mycelium, olivaceous to light olivaceous (Rayner 1970), smooth, not constricted at the septa, (2.0–)6.0(–12) µm diam. *Conidiophores* occurring singly or in groups of up to six, arising directly from the mycelium, erect, macronematous, mononematous, (560–)1032(–1270) µm in length, rhizoid-like structures occasionally present. Stipe dark olivaceous, smooth, cylindrical, simple, 6–10 septate, (500–)922(–1150) µm long (from first basal septum to below primary branches), (10–)11.5(–12.5) µm wide below primary branches, apical cell not swollen, (10–)13(–15) µm wide at base, basal cell not swollen (Figs 1, 7a). *Conidiogenous apparatus* (60–)111(–170) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches, 2–4 primary branches, olivaceous, smooth, cylindrical, aseptate, (25–)37(–55) µm long and (5–)8(–13) µm wide, secondary branches olivaceous to hyaline, aseptate, (12–)20(–33) µm long (3–)5(–9) µm wide, tertiary branches hyaline, aseptate, (10–)14(–20) µm long, (2–)3(–5) µm wide, quaternary branches aseptate, hyaline, (8–)12(–17) µm long, (2–)2.5(–3) µm wide (Figs 2, 7b). *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (12–)17(–23) µm long and (1–)2(–3) µm wide. Conidium development annellidic, occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed secession giving a false impression of sympodial proliferation (Minter, Kirk & Sutton 1982, 1983, van Wyk, Wingfield & Marasas 1988) (Figs 3–4). *Conidia*, aseptate, obovoid with truncated ends, (4.0–)6.0(–9.0) × 2.0–3.0 µm (Figs 5–6, 7c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Leptographium alethinum can be distinguished from *L. procerum* by the absence of rhizoids, whereas these structures are prominent in isolates of *L. procerum*. Furthermore, the conidia of *L. alethinum* are obovoid, but slightly longer (4–9 µm) than those of *L. procerum* (3–5 µm). *L. alethinum* is



Figs 8–13. *Leptographium pityophilum* (PREM 56367). **Fig. 8.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (bar = 20 µm). **Fig. 9.** Complex conidiogenous apparatus (bar = 10 µm). **Fig. 10.** Conidiogenous cells showing false sympodial conidiogenesis (bar = 10 µm). **Fig. 11.** Conidiogenous cells showing false sympodial conidiogenesis (bar = 1 µm). **Fig. 12.** Conidia (bar = 10 µm). **Fig. 13.** Conidia (bar = 5 µm).

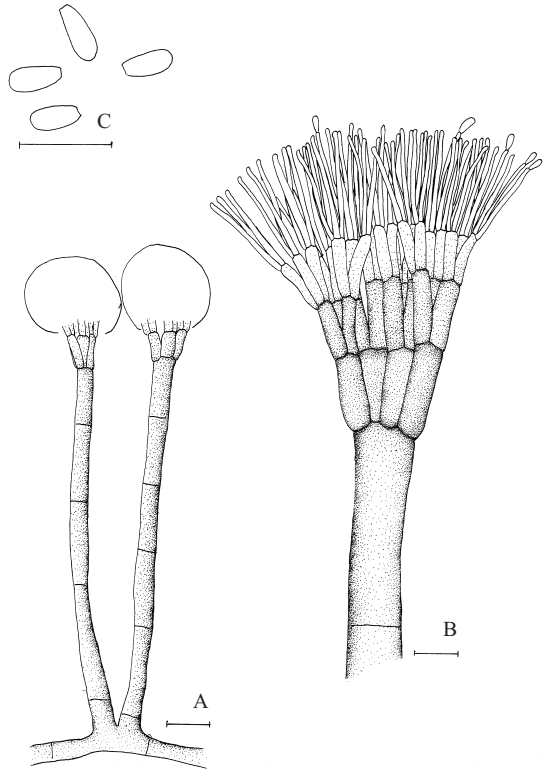


Fig. 14. *Leptographium pityophilum* (PREM 56367). A, Habit sketch of the conidiophore (bar = 20 µm). B, Conidiogenous apparatus (bar = 10 µm). C, Conidia (bar = 10 µm).

morphologically similar to *L. douglasii* (Wingfield, Harrington & Crous 1994). *L. douglasii* occurs on Douglas-fir in the western United States, where it has been associated with the feeding activities of the root feeding weevil (*Coleoptera*: *Curculionidae*) *Hylobus nigrinus*. In contrast, *L. alethinum* was isolated from the galleries of the bark beetle *Hylobius abietis* in England. *L. alethinum* can be distinguished from *L. douglasii* based on its considerably longer conidiophores (560–1270 µm) than those found in cultures of *L. douglasii* (57–512 µm). *L. alethinum* is also characterized by primary branches that are almost twice as long as those of *L. douglasii* and the absence of rhizoids, which are present in *L. douglasii*.

Additional specimens examined: **British Isles**: *Scotland*: from *Hylobius abietis* galleries on stained *Pinus sylvestris* stumps, Nov. 1994, *A. Uzunovic* (PREM 56350, PREM 56348, PREM 3764); [unlocalized], from *P. nigra* var. *maritina J. N. Gibbs* (PREM 56397).

Leptographium pityophilum K. Jacobs, M. J. Wingf. & S. Frisullo, **sp. nov.** (Figs 8–14)

Etym.: From the Greek word meaning pine-loving and refers to the host of this species, namely pine.

Teleomorphosis ignota

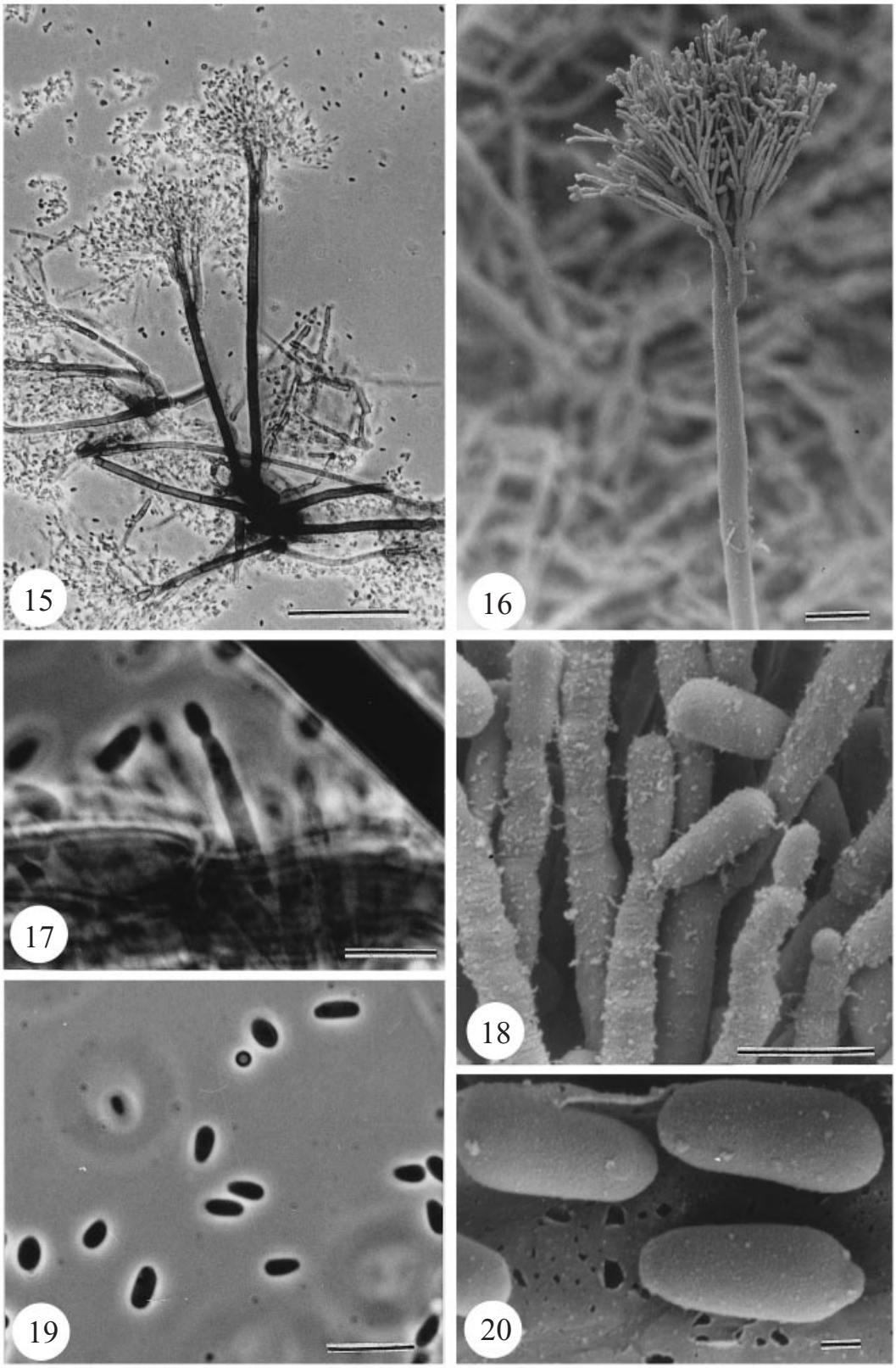
Coloniae optime in temperatura 20°C crescentes; atro-olivaceae; margine laciniato. Hyphae immersae vel emersae in medio solido, sine myceliis aeriis. Conidiphora singulata, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (142–)381(–626) µm longa, cum 3 vel 4 seriebus ramorum cylindricorum; 2–5 tramis primariis; sine structuris rhizoidiformibus. Conidia aseptata, obovoidea extremitatibus truncatis (4–)5(–6) × 2–3 µm.

Typus: **Italia**: Potenza (Rossellino area), isolated from *Pinus nigra*, May 1993, *S. Frisullo* (PREM 56365 – holotypus).

Colonies with optimal growth at 20° on 2% MEA, reaching 25 mm diam in 6 days. No growth below 5° or above 30°. Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.1 g $^{-1}$ cycloheximide after 6 days at 25° in the dark. Colony dark olivaceous (19″f). Colony margin laciniate. Hyphae submerged or on top of solid medium with no aerial mycelium, light olivaceous to dark olivaceous, surrounded by rough granular layer, not constricted at the septa, 2.0–3.0 µm diam. *Conidiophores* occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (142–)381(–626) µm in length, rhizoid-like structures absent. Stipe dark olivaceous, smooth, cylindrical, simple, 3–9 septate, (105.5–)317(–564) µm long (from first basal septum to below primary branches), (7.5–)10(–12.5) µm wide below primary branches, apical cell not swollen, (7.5–)11(–12.5) µm wide at base, basal cell not swollen (Figs 8, 14a). *Conidiogenous apparatus* (37–)66(–99) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches, 2–5 primary branches, olivaceous, smooth, cylindrical to barrel-shaped, aseptate, (11–)17(–25) µm long and (5.0)7(–11) µm wide, secondary branches light olivaceous to hyaline, aseptate, (8–)12(–17) µm long, (3–)5(–8) µm wide; tertiary branches hyaline, aseptate, (7–)10.5(–16) µm long, (2–)3(–5) µm wide, quaternary branches aseptate, hyaline, (8–)10(–12) µm long, (2–)3(–4) µm wide (Figs 9, 14b). *Conidiogenous cells* discrete, 2–3 per branch, tapering slightly at the apex, (14–)16.5(–21) µm long and (1.5–)2(–3) µm wide. Conidium development annellidic, occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed session giving a false impression of sympodial proliferation (Minter *et al.* 1982, 1983 van Wyk *et al.* 1987) (Figs 10–11). *Conidia*, aseptate, obovoid with truncated ends, (4–)5(–6) × 2–3 µm (Figs 12–13, 14c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Leptographium pityophilum can be distinguished from *L. procerum* by the absence of rhizoids as well as by the distinct arrangement of its primary branches. *L. procerum* is characterized by 2 to 3 primary branches of almost equal size. In contrast, *L. pityophilum* is characterized by 2 to 5 primary branches with one central branch that is almost twice the size of the others. In this respect, *L. pityophilum* is more similar to species such as *L. serpens* and *L. wageneri* than to *L. procerum*.

L. pityophilum can be distinguished from *L. wageneri* based on its optimal growth temperature at 20 °, compared with 15 ° for *L. wageneri*. *Leptographium pityophilum* can be distinguished from *L. serpens* based on its straight uncurved hyphae, compared to the distinctly serpentine hyphae of *L. serpens*. *Leptographium serpens* is further characterized by longer (250–1270 µm) conidiophores with rhizoids (Kendrick 1962), compared to the shorter conidiophores (142–626 µm) without rhizoids in *L. pityophilum*. *L. pityophilum* and *L. serpens* share a



Figs 15–20. *Leptographium euphyes* (PREM 56571). **Fig. 15.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (bar = 50 µm). **Fig. 16.** Complex conidiogenous apparatus (bar = 20 µm). **Fig. 17.** Conidiogenous cells showing false sympodial conidiogenesis (bar = 10 µm). **Fig. 18.** Conidiogenous cells showing false sympodial conidiogenesis (bar = 10 µm). **Fig. 19.** Conidia (bar = 10 µm). **Fig. 20.** Conidia (bar = 1 µm).

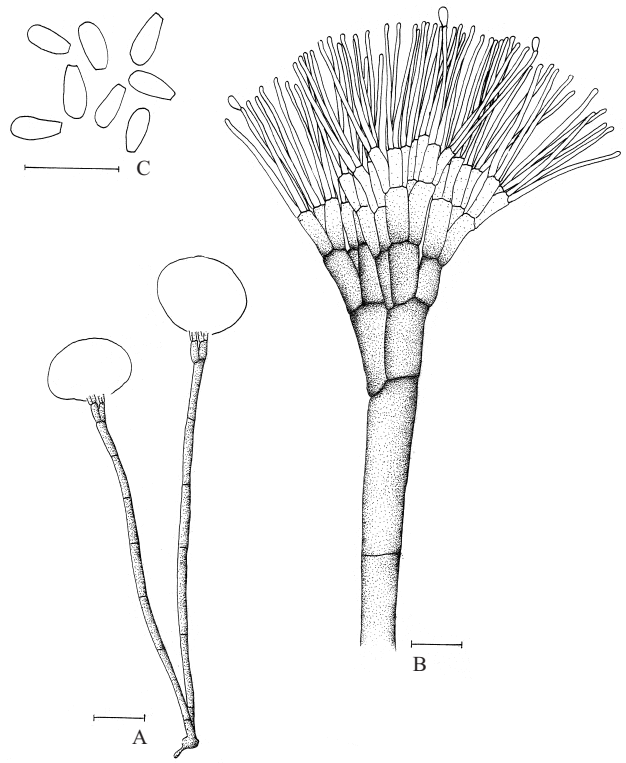


Fig. 21. *Leptographium euphyes* (PREM 56571). A, Habit sketch of the conidiophore (bar = 50 μm). B, Conidiogenous apparatus (bar = 10 μm). C, Conidia (bar = 10 μm).

similar habitat as both have been isolated from *Pinus nigra* in Europe. Because of their morphological similarity, they might have mistakenly been treated as a single species. No insects are known to be associated with *L. pityophilum* although these are most likely to exist.

Additional specimens examined: **Italy**: Potenza (Avigliano area), isolated from *Pinus nigra*, May 1993, *S. Frisullo* (PREM 56367, PREM 56366, PREM 56395).

Leptographium euphyes K. Jacobs & M. J. Wingf., **sp. nov.** (Figs 15–21)

Etym.: From the Greek word for shapely. It refers to the small but shapely conidiophores of this species.

Teleomorphosis ignota.
Coloniae optime in temperatura 25 ° crescentes; olivaceae; marginae laevi. Hyphae immersae vel emerse in medio solido, sine myceliis aeriis. Conidiophora singula, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (204–)300(–315) μm longa, cum 3 vel 4 seriebus ramorum cylindricorum; 2–3 ramis primariis; structurae rhizoidiformes adsunt. Conidia aseptata, obovoidea extremitatibus truncatis, aliquando oblonga, (4–)5(–6) × 2–3 μm.

Typus: **Novae Zelandiae**: Gwavas State Forest, *Pinus strobus*, May 1979. *M. Dick* (PREM 45703 – holotypus).

Colonies with optimal growth at 25 ° on 2% MEA, reaching 19 mm diam in 6 d. No growth below 5 ° or above 30 °. Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.1 g l^{-1} cycloheximide after days at 25 ° in the dark. Colony olivaceous (19″f). Colony margin smooth. Hyphae submerged or on top of solid medium with no aerial mycelia, light olivaceous to hyaline, smooth, occasionally constricted at the septa, (2–)3(–5) μm diam. *Conidiophores* occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (204–)300(–315) μm in length, rhizoid-like structures present. Stipe olivaceous, smooth, cylindrical, simple, 3–9 septate, (142.5–)224(–353.5) μm long (from first basal septum to below primary branches), (6–)7(–9) μm wide below primary branches, apical cell not swollen, (6–)7(–12.5) μm) wide at base, basal cell not swollen (Figs 15, 21a). *Conidiogenous apparatus* (31–)73(–93) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches, 2–3 primary branches, light olivaceous, smooth, cylindrical, aseptate, (11–)18.5(–47) μm long and (5–)6(–8) μm wide, secondary branches light olivaceous to hyaline, aseptate, (8–)12(–18) μm long, (3–)4(–6) μm wide, tertiary branches hyaline, aseptate, (8–)12(–18) μm long, (3–)4(–6) μm wide, tertiary branches hyaline, aseptate, (8–)10.5(–13) μm long, (2–)3(–5) μm wide, quaternary aseptate, hyaline, (7–)10(–12) μm long, 2–3 μm wide (Figs 16, 21b). *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (10–)14.5(–20) μm long and 1–2 μm wide. Conidium development annellidic, occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed secession giving a false impression of sympodial proliferation (Minter *et al*. 1982, 1983, van Wyk *et al*. 1988) (Figs 17–18). *Conidia*, aseptate, obovoid with truncated ends, occasionally oblong (4–)5(–6) × 2–3 μm (Figs 19–20, 21c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Leptographium alethinum, *L. pityophilum* and *L. euphyes* can easily be distinguished from *L. procerum* on a number of morphological differences. The most obvious distinguishing character in these species is the absence of the characteristic concentric rings typically formed in agar colonies of *L. procerum*. *L. euphyes* can be distinguished from *L. procerum* based on its short robust conidiophores, which were unlike the long conidiophores described for *L. procerum*. Both these species have rhizoids and conidia of similar shape and size (Kendrick 1962). Of the three new taxa described here. *L. euphyes* is most unlike *L. procerum*. Comparison with other *Leptographium* species revealed that it is morphologically most similar to the *Leptographium* anamorph of *Ophiostoma grandifoliae*. These species could, however, be distinguished based on the presence of a teleomorph in the latter species (Davidson 1976) and its absence in the former species. In the absence of a teleomorph, *L. euphyes* can be distinguished from *O. grandifoliae* based on more complex conidiogenous apparatusses as well as larger conidia (4–6 μm) compared to *O. grandifoliae* (2.5–4 μm). Furthermore, *O. grandifoliae* occurs on *Fagus grandifolia* in the USA, whereas *L. euphyes* originates from the roots of pine, an introduced tree in New Zealand.

L. euphyes is commonly isolated together with *L. procerum* in New Zealand. The fungus originates from a collection of isolates that were linked to a report of a root disease of *Pinus strobus* (Shaw & Dick 1980). Later Wingfield & Marasas (1983)

Key to the* Leptographium *species treated

1(1) Dark concentric rings visible in colonies on 2% MEA . . . . . **procerum**
Colonies on 2% MEA without concentric rings 2

2(1) Conidiophore length 500–1000 µm . . . . . **alethinum**
Conidiophore length less than 600 µm 3

3(2) 2 or 3 primary branches at the apex of the stipe . . . . . **euphyes**
2 to 5 primary branches at the apex of the stipe . . . . . **pityophilum**

studied this collection of isolates and noted that it represented fungi having two distinct morphological forms. These included one group that was typical of *L. procerum* and another which was different. The latter group represents *L. euphyes*.

Additional specimens examined: **New Zealand**: *Pinus strobus*, 1 May 1979, *M. Dick*.

DISCUSSION

The three species described in this paper have mistakenly been treated as *Leptographium procerum* in the past. This is understandable as they have a morphology superficially similar to *L. procerum* and they occur on *Pinus* roots. This is similar to the habitat of *L. procerum*. *L. procerum* is one of the best known *Leptographium* species, and, in the absence of a comprehensive taxonomic treatment, it is not surprising that other *Leptographium* species have been mistaken for it. This emphasises the need for the clear delineation of *Leptographium* species and the evaluation of morphological characters to correctly identify these fungi (Wingfield 1993).

This study has included a relatively large set of isolates of *L. procerum s. str.*, that have been defined through careful comparison with type specimens of this species. These will be useful in taxonomic studies based on DNA sequence data that are planned for the future. They have also somewhat expanded the known geographic distribution of *L. procerum*. One of the interesting records includes that from South Africa, where the fungus has previously not been known. Various *Leptographium* species occur in that country where they are associated with roots and the introduced pine root feeding bark beetles. *Hylastes angustatus* and *Hylurgus ligniperda* (Wingfield & Marasas 1980, 1983). These insects are native to Europe and we assume that *L. procerum* was introduced into South Africa with one or both of them.

L. procerum is a common pine root and root collar infecting fungus in North America, east of the Rocky Mountains and in Europe. It is most commonly associated with conifer root and root collar infesting weevils (*Coleoptera*: *Curculionidae*) (Wingfield 1983b). In our view, its association with the disease known as white pine root decline (Anderson & Alexander 1979) is linked to the fact that it is carried by insects, that infest the roots and root collars of stressed pines including *Pinus strobus* (white pine). This is consistent with the results of pathogenicity tests by a variety of authors that have failed to demonstrate a high degree of virulence in the fungus (Wingfield 1982, 1983, Harrington & Cobb 1983). Nothing is known regarding the pathogenicity of *L. alethinum*, *L. pityophilum* and *L. euphyes* although we expect that they are also mildly pathogenic or saprotrophic associates of the insects with which they are associated.

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REFERENCES

Alexander, S. A., Horner, W. E. & Lewis, K. J. (1988) *Leptographium procerum* as a pathogen of pines. In *Leptographium Root Diseases on Conifers* (T. C. Harrington & F. W. Cobb, jr, eds): 97–112. American Phytopathological Society Press, St Paul, MN.

Anderson, R. L. & Alexander, S. A. (1979) *How to Identify and Control White Pine Root Decline*. [Forestry Bulletin No. SA-FR/P6.] USDA Forest Service.

Davidson, R. W. (1976) Sapwood staining fungi from two tree species. *Memoirs of the New York Botanical Garden* **28**: 45–49.

Harrington, T. C. (1988) *Leptographium* species, their distributions, hosts and insect vectors. In *Leptographium Root Diseases on Conifers*: (T. C. Harrington & F. W. Cobb jr, eds): 1–39. American Phytopathological Society Press, St Paul MN.

Harrington, T. C. (1993) Diseases of conifers caused by species of *Ophiostoma* and *Leptographium*. In *Ceratocystis* and *Ophiostoma*: *taxonomy, ecology and pathogenicity* (M. J. Wingfield, K. A. Seifert & J. F. Webber, eds): 161–172. American Phytopathological Society Press, St Paul MN.

Harrington, T. C. & Cobb, F. W. (Jr.) (1983) Pathogenicity of *Leptographium* and *Verticicladiella* spp. isolated from roots of Western North American conifers. *Phytopathology* **73**: 596–599.

Kendrick, W. B. (1962) The *Leptographium* complex. *Verticicladiella* Hughes. *Canadian Journal of Botany* **40**: 771–797.

Lackner, A. L. & Alexander, S. A. (1982) Occurrence and pathogenicity of *Verticicladiella procera* in Christmas tree plantations in Virginia. *Plant Disease* **66**: 211–212.

Mackenzie, M. & Dick, M. (1984) *Verticicladiella* root disease. *Forest Pathology in New Zealand* **8**: 1–4.

Mathiesen-Käärik, A. (1953) Eine Übersicht über die gewöhnlichsten mit Borkenkärfen assoziierten Bläuepilze in Schweden und einige für Schweden neue Bläuepilze. *Meddelanden Från Statens Skogforsknigsintitut* **43**: 3–74.

Minter, D. W., Kirk, P. M. & Sutton, B. C. (1982) Holoblastic phialides. *Transactions of the British Mycological Society* **79**: 75–93.

Minter, D. W., Kirk, P. M. & Sutton, B. C. (1983) Thallic phialides. *Transactions of the British Mycological Society* **80**: 39–66.

Münch, E. (1907) Die Blaufaule des nadelhoizes. *Naturwissenschaftliche Zeitschrift für Forest* **5**: 531–573.

Prey, A. J. (1975) Forest pest conditions in Wisconsin. *Annual Report, Department of Natural Resources, Madison, Wisconsin*: 2. Department of Natural Resources, Madison.

Rayner, R. W. (1970) *A Mycological Color Chart*. Commonwealth Mycological Institute Kew.

Rennerfelt, E. (1950) Über den zusammehang zwishchen dem verblauen des holzes und den insekten. *Oikos* **2**: 120–137.

Shaw, C. G. III & Dick, M. (1980) *Verticicladiella* root disease of *Pinus strobus* in New Zealand. *Plant Disease* **64**: 96–98.

van Wyk, P., Wingfield, M. J. & Marasas, W. F. O. (1988) Differences in

synchronisation of stages of conidial development in *Leptographium* species. *Transactions of the British Mycological Society* **90**: 451–456.

Wingfield, M. J. (1982) *Verticicladiella procera* associated with root weevil damage. *Phytopathology* **72**: 141.

Wingfield, M. J. (1983a) Pathogenicity of *Verticicladiella procera* and *Leptographium terebrantis* in greenhouse and field inoculations. *Phytopathology* **73**: 838–839.

Wingfield, M. J. (1983b) Association of *Verticicladiella procera* and *Leptographium terebrantis* with insects in the Lake states. *Canadian Journal of Forest Research* **13**: 1238–1245.

Wingfield, M. J. (1993) *Leptographium* species as anamorphs of *Ophiostoma*: progress in establishing acceptable generic and species concepts. In *Ceratocystis* and *Ophiostoma*. *Taxonomy, ecology and pathogenicity* (M. J. Wingfield, K. A. Seifert & J. F. Webber, eds): 43–51). American Phytopathological Press, St Paul: MN.

Wingfield, M. J. & Marasas, W. F. O. (1980) *Ceratocystis ips* associated with *Orthotomicus erosus* (*Coleoptera*: *Scolytidae*) on *Pinus* spp., in the Cape province of South Africa. *Phytophylactica* **12**: 65–69.

Wingfield, M. J. & Maraas, W. F. O. (1983) Some *Verticicladiella* species, including *V. truncata* sp. nov., associated with root diseases of pine in New Zealand and South Africa. *Transactions of the British Mycological Society* **80**: 231–236.

Wingfield, M. J., Capretti, P. & Mackenzie, M. (1988) *Leptographium* spp. as root pathogens on conifers. An international perspective. In *Leptographium Root Diseases on Conifers* (T. C. Harrington & F. W. Cobb jr, eds): 113–128.

Wingfield, M. J., Harrington, T. C. and Crous, P. W. (1994) Three new *Leptographium* species associated with conifer roots in the United States. *Canadian Journal of Botany* **72**: 227–238.

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