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New *Leptographium* species from Indonesia and Eastern North America

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***Leptographium* species have predominantly been described from North America, Canada and Europe. These fungi generally occur on conifers and many cause blue-stain of lumber. Most *Leptographium* species are also associated with insects and in particular, bark beetles (Coleoptera: Scolytidae). Recently, an unknown species of *Leptographium* was isolated from pine infested with an *Ips* sp. in Indonesia. In addition, two unknown species have been collected from red spruce (*Picea rubra*) and balsam fir (*Abies balsamea*) roots from high elevation sites in Eastern North America. The latter isolates are unusual in that they are associated with the feeding wounds made by the conifer swift moth *Korscheltellus gracilus* (Lepidoptera: Hepialidae), which is a habitat unique for species of *Leptographium*. Comparison with known *Leptographium* species has revealed that the isolates from Indonesia and those from Eastern North America represent three previously undescribed taxa. They are, therefore, described in this study as *L. pineti* sp. nov, *L. abieticolens* sp. nov. and *L. peucophilum* sp. nov.**

Key Words——conifer swift moth; conifers; *Ips*; *Leptographium*.

Species of *Leptographium* Lagerb. & Melin are generally characterised by dark mononematous conidiophores with complex conidiogenous apparatuses (Kendrick, 1962; 1964; Wingfield, 1993). Numerous conidia are produced from conidiogenous cells through percurrent proliferation (Kendrick, 1962). Delayed secession of the conidia can lead to the false appearance of sympodial development (Van Wyk et al., 1988). *Leptographium* species are generally associated with conifers (Lagerberg et al., 1927; Kendrick, 1962; Harrington, 1988), with only a few exceptions described (Davidson, 1942; 1958; 1971; 1976; Jooste, 1978; Weber et al., 1996). Most species of *Leptographium* are carried by insects, especially, bark beetles (Coleoptera: Scolytidae) and they sporulate profusely in galleries of these insects (Lagerberg et al., 1927; Leach et al., 1934; Harrington, 1988).

Species of *Leptographium* have been recorded from many parts of the world and many species have been accidentally introduced into new areas along with bark beetles (Wingfield and Marasas, 1980). However, the majority of species are native to the Northern hemisphere and especially North America and Europe, where most conifers and their bark beetle pests originate (Lagerberg et al., 1927; Rumbold, 1936; Goidanich, 1936; Parker, 1957; Kendrick, 1962; Robinson-Jeffrey and Grinchenko, 1964; Kendrick and Molnar, 1965; Robinson-Jeffrey and Davidson, 1968; Griffin, 1968; Davidson, 1971; Morelet, 1988; Jacobs et al., 1997). Among the North American species, the three varieties of *L. wageneri* (W. B. Kendrick) M. J. Wingf. are probably best known due to their role in causing black-stain root disease on pine (*Pinus* spp.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Kendrick, 1962; Harrington and Cobb, 1987; Harrington, 1988).

Most of the *Leptographium* species described from North America have been associated with insects. Exceptions include *L. antibioticum* (W. B. Kendrick) M. J. Wingf., *L. brachiatum* (W. B. Kendrick) M. J. Wingf. and *O. trinacriforme* (*Leptographium* anamorph) (A. K. Parker) T. C. Harr. (Parker, 1957; Kendrick, 1962). The association of *Leptographium* species with bark beetles is well recognised, and various hypotheses exist regarding the relationships between the fungi and these insects (Craighead, 1928; Harrington, 1988, Six and Paine, 1996). A common view is that most species are accidental contaminants of bark beetles and that they are generally saprophytic (Harrington, 1988). In some cases, they might serve as a source of nutrition for the insect larvae (Six and Paine, 1996) and their role as pathogens has been extensively recorded, although in some cases this is also disputed (Wingfield et al., 1995; Krokene and Solheim, 1996, 1998).

In Europe and Asia, many *Leptographium* species have been associated with bark beetles and particularly species of *Ips* (Solheim, 1986; Van der Westhuizen et al., 1995; Yamaoka et al., 1997; Jacobs et al, 1998). From studies conducted on conifers infested with *Ips* species in Japan, two new *Leptographium* species were recently described. However, these species were not found to be associated with similar insects in Europe (Wingfield et

al., 1994; Van der Westhuizen et al., 1995; Jacobs et al., 1998). Apart from *O. penicillatum* (Grosmann) Siemaszko and *O. piceaperdum* (Rumbold) Arx, that are associated with *I. typographus* in Europe (Solheim, 1986), various *Leptographium* species from east Asia have not been recorded elsewhere in the world (Yamaoka et al., 1997). Two interesting examples include, *L. laricis* K. van der Westh., M. J. Wingf. & Yamaoka and *L. aenigmaticum* K. Jacobs, M. J. Wingf. & Yamaoka, from Larch (*Larix* spp.), associated with *I. cembrae* and *I. typographus*, respectively (Van der Westhuizen et al, 1995; Jacobs et al., 1998).

In recent years, a collection of isolates of *Leptographium* species has emerged from *Pinus merkusii* infested with *Ips* sp. in Indonesia as well as balsam fir (*Abies balsamea* (L.) Mill.) and red spruce (*Picea rubens* Sarg.) in North America associated with damage to roots by the conifer swift moth, *Korscheltellus gracillus* (Lepidoptera: Hepialidae). The main objective of this study was to examine these isolates and to provide appropriate names for them.

Materials and Methods

Galleries of an *Ips* sp., commonly infesting *P. merkusii* trees in Northern Sumatra, Indonesia, were examined and the dominant fungus in these galleries was a *Leptographium* species. Red spruce and balsam fir roots wounded by the conifer swift moth, *Korcheltellus gracillus*, collected on White Face Mountain, New York, USA were also found to be infested with *Leptographium* species. Conidial masses from these fungi were transferred from the apices of conidiophores to 2% malt extract agar (MEA) (20 g Biolab malt extract, 20 g Biolab agar and 1000 ml distilled water) plates amended with 0.05 g/l cycloheximide. Resulting colonies were transferred to clean 2% MEA plates and incubated at 25°C until the onset of sporulation. Fungal structures for microscopic examination were mounted on glass slides in lactophenol. Fifty measurements of each relevant morphological structure were made and ranges and averages computed. Colours of the colonies and fungal structures were determined using the colour charts of Rayner (1970). Cultures have been stored in the collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (CMW), as well as in the culture collection of the National Collection of Fungi, Pretoria, South Africa (PPRI).

The optimal temperatures for growth of isolates representing three distinct *Leptographium* species (CMW 3831; PPRI 6911 and CMW 3832; PPRI 6910 from Indonesia, CMW 2865; PPRI 6917 and CMW 2866; PPRI 6918 from balsam fir, CMW 2875; PPRI 6920 and CMW 2876; PPRI 6922 from red spruce) were determined by inoculating eight MEA plates for each temperature with a 0.6 mm diameter agar disk taken from the actively growing margins of a fresh isolate. Plates were incubated at temperatures ranging from 5 to 35°C at 5°C intervals. Colony diameters were measured on the fourth and the eighth day after commencement of the trail, and the diameters of colonies were computed as an average of eight readings.

Cycloheximide tolerance of the same isolates representing the three species that were used in the temperature studies, 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) in Petri dishes. Dishes were incubated in the dark at 25°C for 8 d and the colony diameters determined from two measurements taken at 90° to each other. Five replicates for each cycloheximide concentration were included, and growth was determined based on averages of ten diameter measurements.

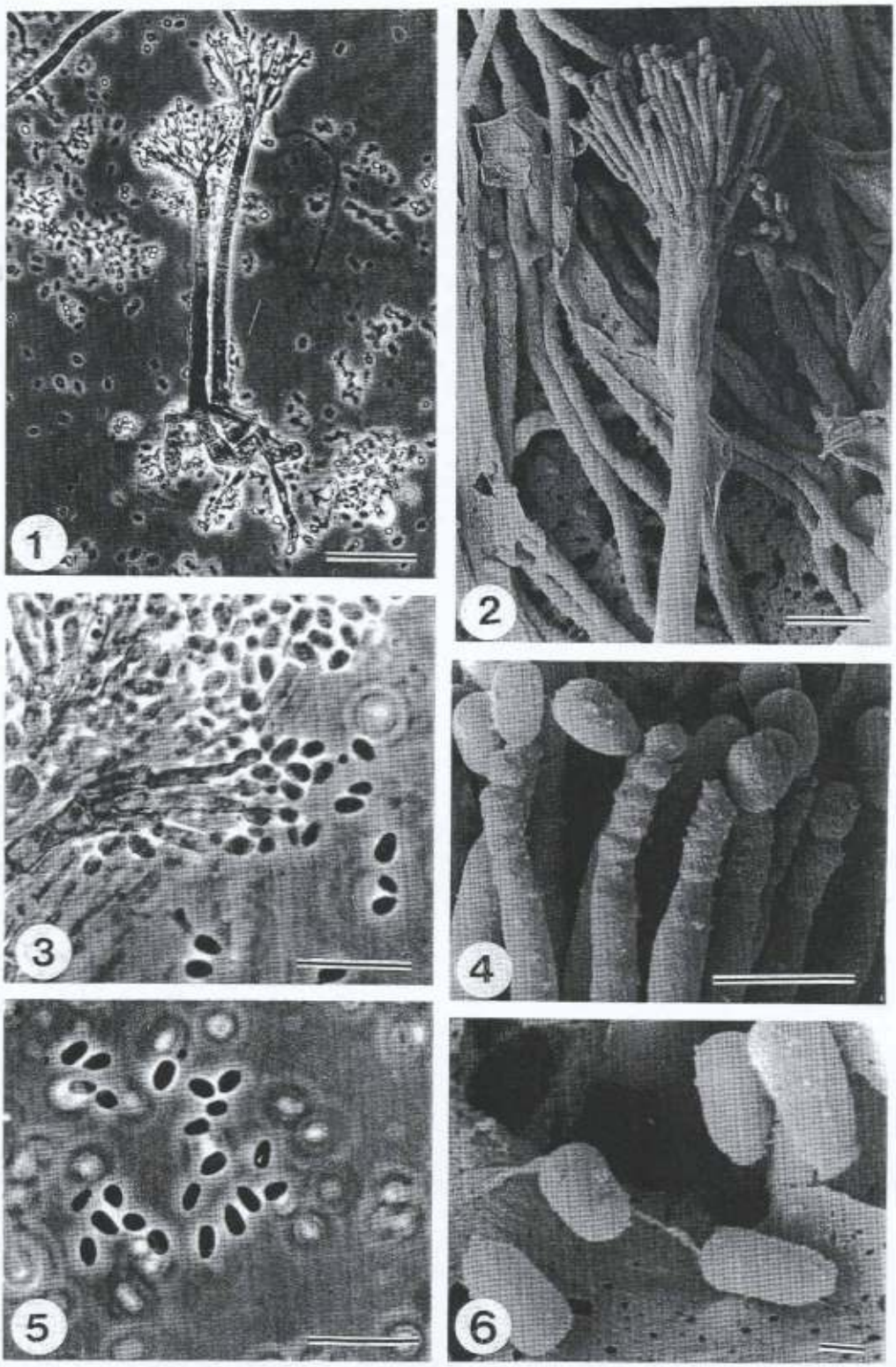
Results

The *Leptographium* species from *P. merkusii* infested with *Ips* sp. in Sumatra is characterised by short robust conidiophores with dark stipes and short conidiogenous apparatus made up of two to three series of branches. These isolates are, furthermore, characterised by their short conidiophores that produce small, obovoid conidia. Comparison with all other *Leptographium* species revealed that these isolates do not resemble any known taxon, and we conclude that they represent a previously undescribed species, which is described as follows:

Leptographium pineti K. Jacobs & M. J. Wingf., sp. nov. Figs. 1–7

Coloniae in 2% MEA ad 25°C optime crescents, et post 6 dies 15 mm diam. attingentes atro-olivaceae, margine integro, sub 5°C vel supra 30°C non crescents, ex mycelio vegetativo immerso vel emerso pallide olivaceo vel hyalino laevi non constricto (2.0–)2.5–4.0(–6.0) µm diam. constantes, sine hyphis aeriis. Conidiophora singula, e mycelio rectangulatim exorientia, erecta, macronematosa, mononematosa, 100–202(–210) µm longa; stipites cylindracei, simplices, olivacei, leaves, 2-4-septati, (50–)70–125(–150) µm longi, infra ramos primaries 5.0–7.5 µm lati, ad basim (5.0–)6.0–10 µm lati et haud tumidi. Apparatus conidiogeni 17–74 µm longi ex ramis cylindricis, 2–3 seriatis compositi; rami primarii 2–3, pallide olivacei cel hyaline, aseptati, (10–)13–17(–20) × 3.0–5.0(–6.0) µm; rami secundarii hyaline, aseptati, (7.0–)9.0–12(–15) × 2.0–4.0 µm; rami tertiatoo hyaline, aseptati, (5.0–)7.0–9.0(–15) × 2.0–3.0; structura rhizoideiformi absens. Cellulae conidiogenae discretae, 2–3 per ranum, cylindricae, sursum leviter attenuatae, holoblasticae, 6.0–16 × 2.0 µm. Conidia aseptata, oblonga vel obovoidea, ad apicem apparatus conidiogeni in massa guttulata mucilagiosa accumulata, 2.0–3.0 × 1.0 µm.

Colonies with optimal growth at 25°C on 2% MEA, reaching 15 mm in diam in 6 d. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 12% reduction in growth on 0.1 g/l cycloheximide after 6 d at 25°C in the dark. Colony dark olivaceous (19″f). Colony margin smooth. Hyphae submerged or on top of solid medium with no aerial mycelium, light olivaceous to hyaline, smooth, not constricted at the septa, (2.0–)3.0(–6.0) µm in diam.



Figs. 1–6. *Leptographium pineti* (CMW 3831). 1. Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 μm). 2. Complex conidiogenous apparatus (Bar = 10 μm). 3. Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 μm). 4. Conidiogenous cells showing false sympodial conidiogenesis (Bar = 5 μm). 5. Conidia (Bar = 10 μm). 6. Conidia (Bar = 1 μm).

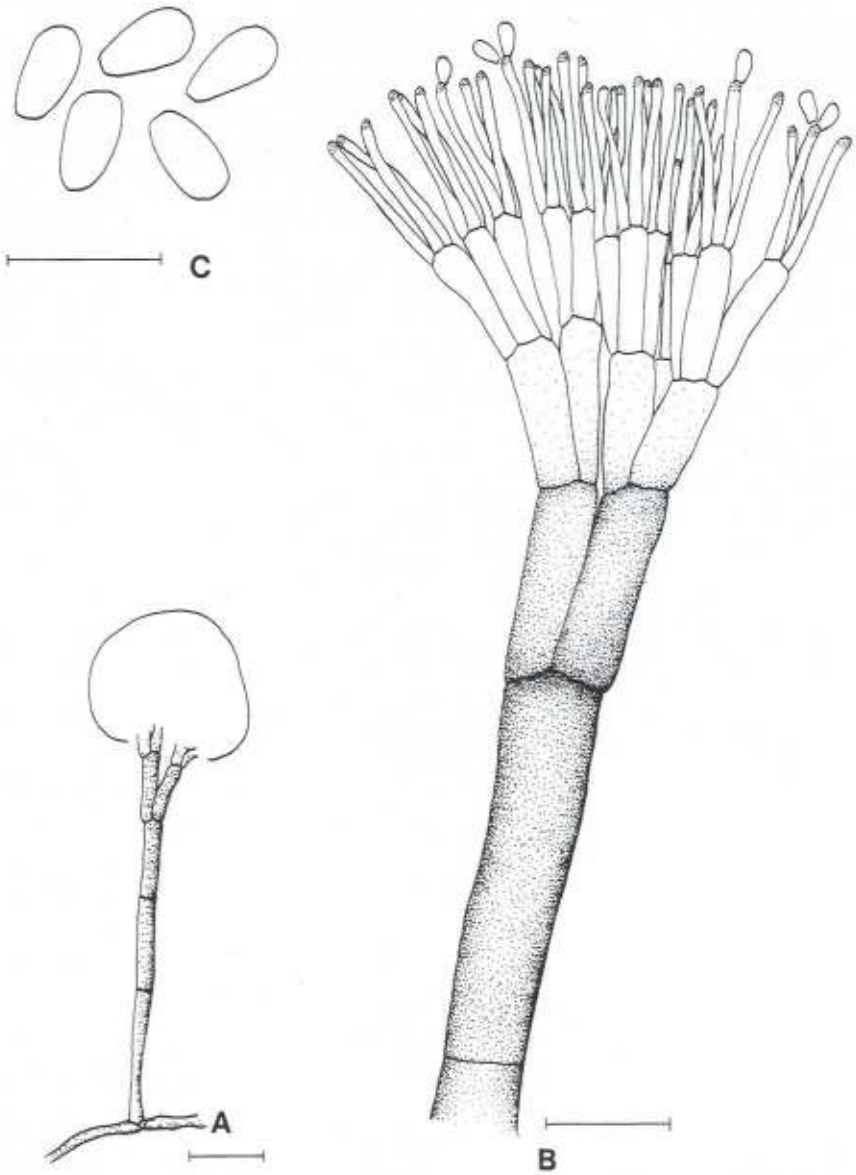


Fig. 7. *Leptographium pineti* (CMW 3831). A. Habit sketch of the conidiophore (Bar = 50 μm). B. Conidiogenous apparatus (Bar = 10 μm). C. Conidia (Bar = 10 μm).

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, 100–202(–210) μm in length, rhizoid-like structures occasionally present. Stipe olivaceous, smooth, cylindrical, simple, 2-4-septate, (50–)99(–150) μm long (from first basal septum to below primary branches), (4.0–)5.0(–7.5) μm wide below primary branches, apical cell not swollen, (5.0–)7.5(–10) μm wide at base, basal cell not swollen (Figs. 1, 7a). Conidiogenous apparatus 30–70 long, excluding the conidial mass, with 2 to 3 series of cylindrical branches, 2–3 primary branches, light olivaceous to hyaline, smooth, cylindrical, aseptate, (10–)15(–20) μm long and 3.0–5.0(–6.0) μm wide, secondary branches hyaline, aseptate, (7.0–)9.0–12(–15) μm long, 2.0–4.0 μm wide, tertiary branches hyaline, aseptate, (5.0–)7.0–9.0(–15) μm long, 2.0–3.0 μm wide (Figs. 2, 7b). Conidiogenous cells discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (6.0–)10(–16) μm long and 2 μm wide. Conidium development occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed secession giving a false impression of sympodial proliferation (Minter, Kirk and Sutton, 1982; 1983; Van Wyk et al., 1988) (Figs. 3, 4). Conidia, aseptate, obovoid, 2.0–3.0 × 1.0 μm (Figs. 5, 6, 7c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Teleomorph state: not known

Holotype: PREM 56391 (dried specimen), from galleries of *Ips* sp. under the bark of *P. merkusii*, collected by M. J. Wingfield, Samosir Island, Sumatra, Indonesia, March 1996.

Additional specimens: PREM 56351, PREM 56354, PREM 56392, PREM 56355, PREM 56353, PREM 56352, PREM 56351 (dried specimens), from galleries of *Ips* sp. under the bark of *P. merkusii*, collected by M. J. Wingfield, Samosir Island, Sumatra, Indonesia, March 1996.

Etymology: From the Latin noun *pinetum*: a pine stand. This specific epithet refers to the habitat of the fungus on *Pinus* spp.

The isolates from both *A. balsamea* and *P. rubra*, were characterised by dark conidiophores and a high degree of tolerance to cycloheximide, which is similar to other *Leptographium* species. The isolates from *A. balsamea*, are characterised by optimal growth at low temperatures and slow growing colonies. These isolates are further characterised by dark, medium-length conidiophores with rhizoids at their bases. The conidia of these isolates are broadly ellipsoidal to obovoid. The *Leptographium* sp. from *A. balsamea*, superficially resembles certain *Leptographium* species. It could, however, be distinguished from these and is thus described as follows:

Leptographium abieticolens K. Jacobs & M. J. Wingf., sp. nov. Figs. 8–14

Coloniae in 2% MEA 15°C optime crescents et post 14 dies 18 mm diam. attingentes, atro-olivaceae (19″f); margine integro, sub 5°C vel supra, 25°C non crescents, ex mycelio vegetativo immerso vel emerso pallide olivaceo vel hyalino laevi non constricto, 1.0–6.0 μm diam, constantes hyphis aeriis abundantibus formantes. Conidiophora singula vel usque ad sex gregaria, e mycelio rectangulatim exorientia, erecta, macronematosa, mononematosa, (120–)160–195(–360) μm longa; stipites cylindricei, leaves, 2-11-septati, (72–)92–239(–264) μm longi, infra ramos primaries 3.0–6.0 μm lati, ad basim 4.5–7.5 μm lati, non tumidi. Apparatus conidiogeni (32–)55–68(–104) μm longi, ex ramis cylindricis 3-4-seriatis compositi; rami primarii, 2–3, olivacei vel pallide olivacei, aseptato, (8.0–)12–14(–31) × 3.0–5.0 μm; rami secundarii pallide olivacei vel hyaline, aseptati, 6.0–12(–15) × 2.0–3.0 μm; rami tertiarii hyaline, aseptati, (6.0–)7.5–10(–12) × 2.0–4.0 μm; rami quartii hyaline, aseptati, (5.0–)7.0–9.0(–10) × 2.0–4.0 μm; structura rhizoideiformes adest. Cellulae conidiogenae discretae, 2–3 per ranum, cylindricae, sursum, leviter, attenuatae, holobalsticae (8.0–)11–15(–23) × 2.0–3.0 μm. Conidia aseptata, late ellipsoidea vel obovoidea, ad apicem apparatus conidiogeni in massa guttulata mucilaginosa accumulata (4.0–)5.0(–7.0) × 2.0–3.0 μm.

Colonies with optimal growth at 15°C on 2% MEA, reaching 18 mm in diam in 14 d. No growth below 5°C or above 25°C. Able to withstand high concentrations of cycloheximide with a 17% reduction in growth on 0.1 g/l cycloheximide after 6 d at 15°C in the dark. Colony dark olivaceous (19″f). Colony margin smooth. Hyphae submerged or on top of solid medium with abundant aerial mycelium, light olivaceous to hyaline, smooth, not constricted at the septa, (1.0–)3.0(–6.0) μm diameter.

Conidiophores occurring singly or in groups of up to six, arising directly from the mycelium, erect, macronematous, mononematous, (120–)160–195(–360) μm in length, rhizoid-like structures present. Stipe dark olivaceous, smooth, cylindrical, simple, 2-11-septate, (72–)92–239(–264) μm long (from first basal septum to below primary branches), 3.0–6.0 μm wide below primary branches, apical cell not swollen, 4.0–7.0 μm wide at base, basal cell not swollen (Figs. 8, 14a). Conidiogenous apparatus (32–)55–68(–104) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches, 2–3 primary branches, olivaceous to light olivaceous, smooth, cylindrical, aseptate, (8.0–)12–15(–31) μm long and (3.0–)3.5(–5.0) μm wide, secondary branches light olivaceous to hyaline, aseptate, 7.0–12(–15) μm long, 2.0–4.0 μm wide, tertiary branches hyaline, aseptate, (6.0–)7.0–10(–12) μm long, 2.0–4.0 μm wide, quaternary branches aseptate, hyaline, (5.0–)7.0–9.0(–10) μm long, 2.0–4.0 μm wide (Figs. 9, 14b). Conidiogenous cells discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (8.0–)11–15(–23) μm long and 2.0–3.0 μm wide. Conidium development 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. 10, 11). Conidia, aseptate, broadly ellipsoidal to obovoid, (4.0–)5.0–7.0 × 2.0–3.0 μm (Figs. 12, 13, 14c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Teleomorph state: not known

Holotype: PREM 56336 (dried specimen), from *A. balsamea* roots wounded by *K. gracilus*, collected D. R. Bergdahl, White Face Mountain, New York, USA, August 1990.

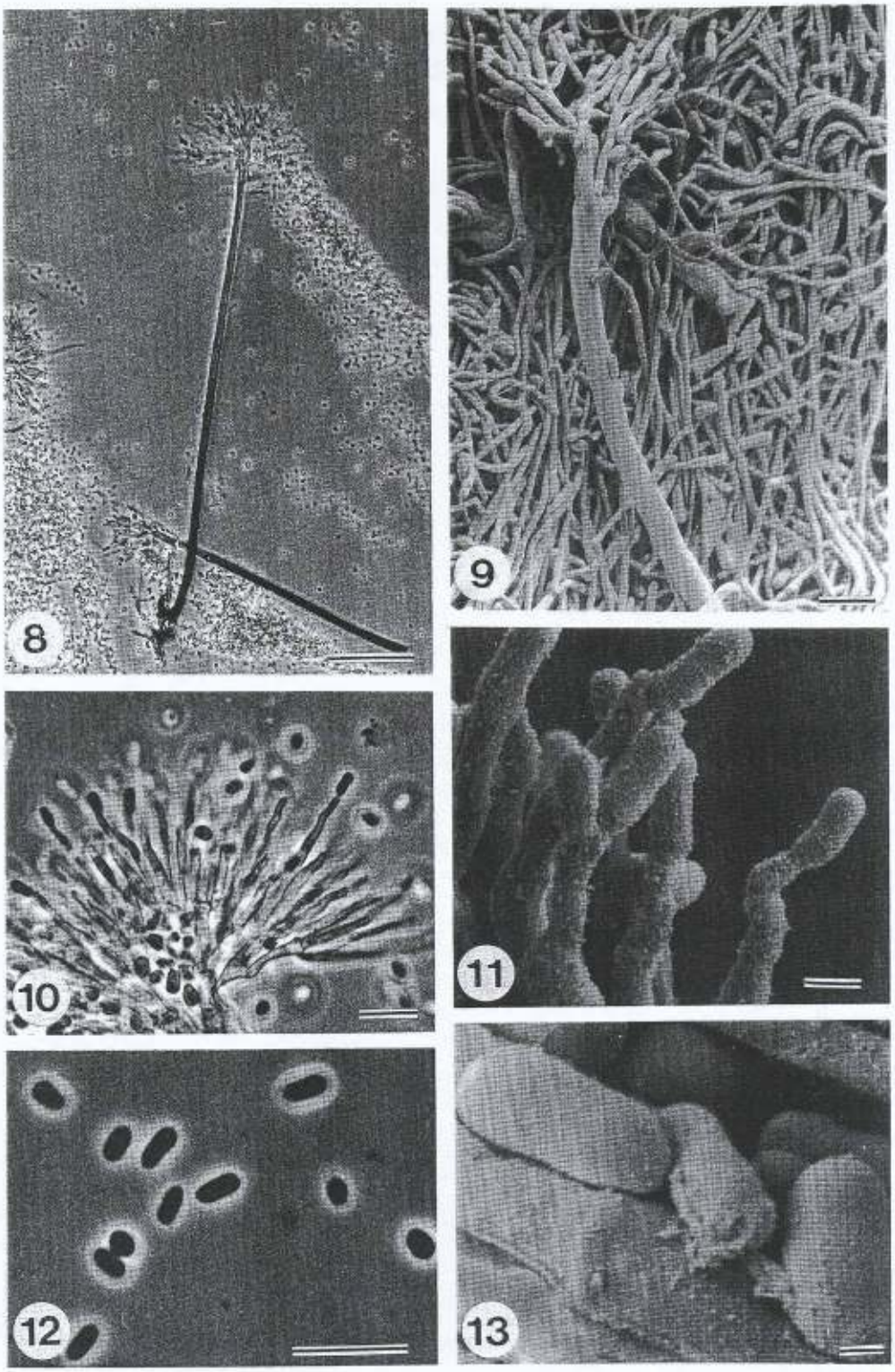
Additional specimens: CMW 2875 (live isolate), PREM 56337 (dried specimen), from *A. balsamea* roots wounded by *K. gracilus*, collected D. R. Bergdahl, White Face Mountain, New York, USA, August 1990.

Etymology: From the Latin noun *abies*: fir and Latin verb *incolere*: to inhabit. This specific epithet refers to *Abies* which is the only known host of this species.

The *Leptographium* species from red spruce has long conidiophores with 3 to 4 series of cylindrical branches. Isolates were also found to display slow growth in culture and low optimal growth temperature similar to *L. abieticolens*. Comparison with other *Leptographium* species has shown that this fungus resembles *L. procerum*, but could be distinguished based on longer conidiophores, lower optimal growth temperature and differences in colony morphology. It is thus described as follows:

Leptographium peucophilum K. Jacobs & M. J. Wingf., sp. nov. Figs. 15–21

Coloniae in 2% MEA 15°C optime crescents et post 10 dies 10 mm diam. attingentes, atro-olivaceae (19″f); margine laciniato, sub 10°C vel supra, 30°C non



Figs. 8–13. *Leptographium abieticolens* (CMW 2865). 8. Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 μm). 9. Complex conidiogenous apparatus (Bar = 10 μm). 10. Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 μm). 11. Conidiogenous cells showing false sympodial conidiogenesis (Bar = 5 μm). 12 Conidia (Bar = 10 μm). 13. Conidia (Bar = 1 μm).

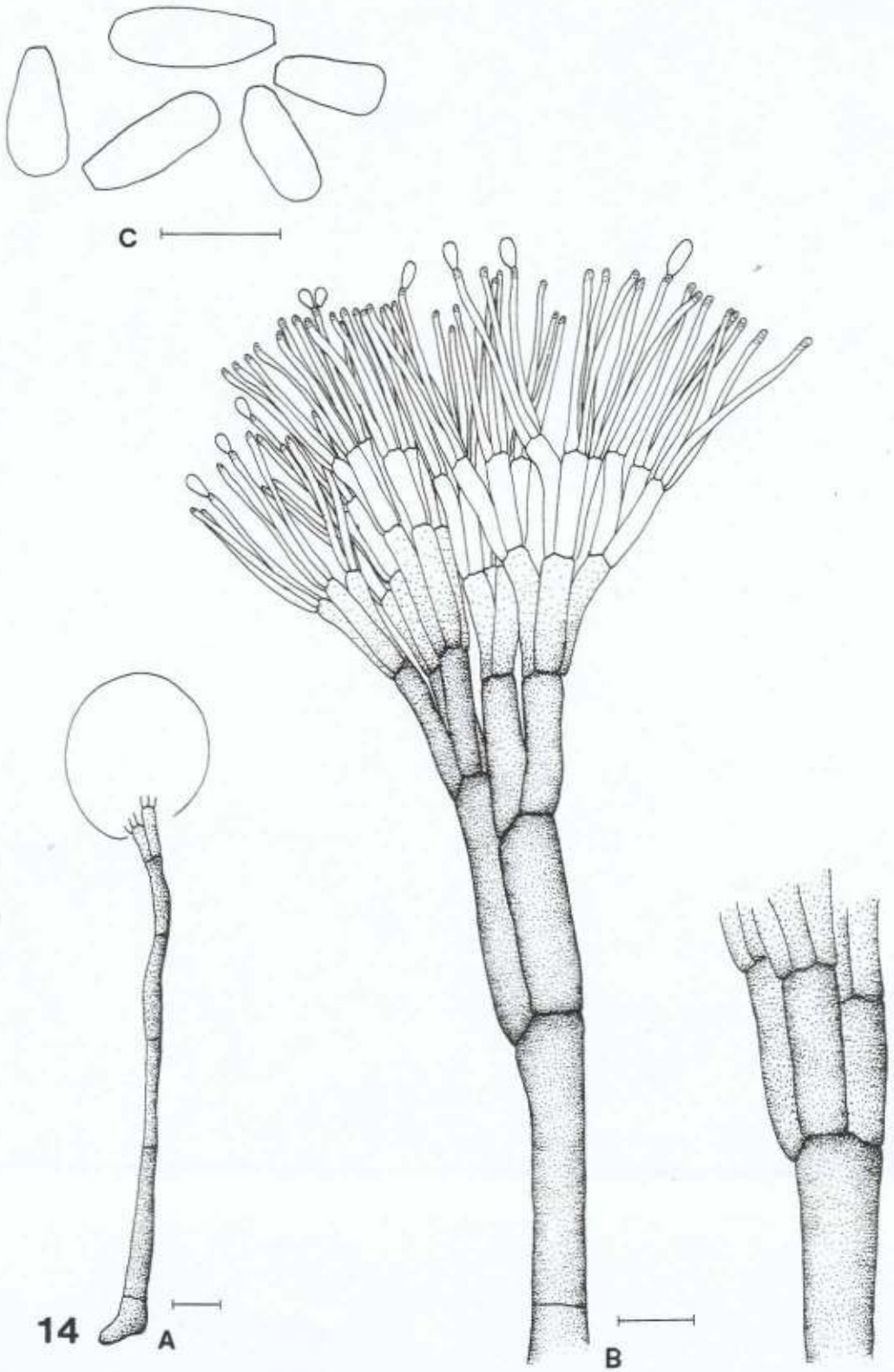


Fig. 14. *Leptographium abieticolens* (CMW 2865). A. Habit sketch of the conidiophore (Bar = 20 μm). B. Conidiogenous apparatus (Bar = 10 μm). C. Conidia (Bar = 10 μm).

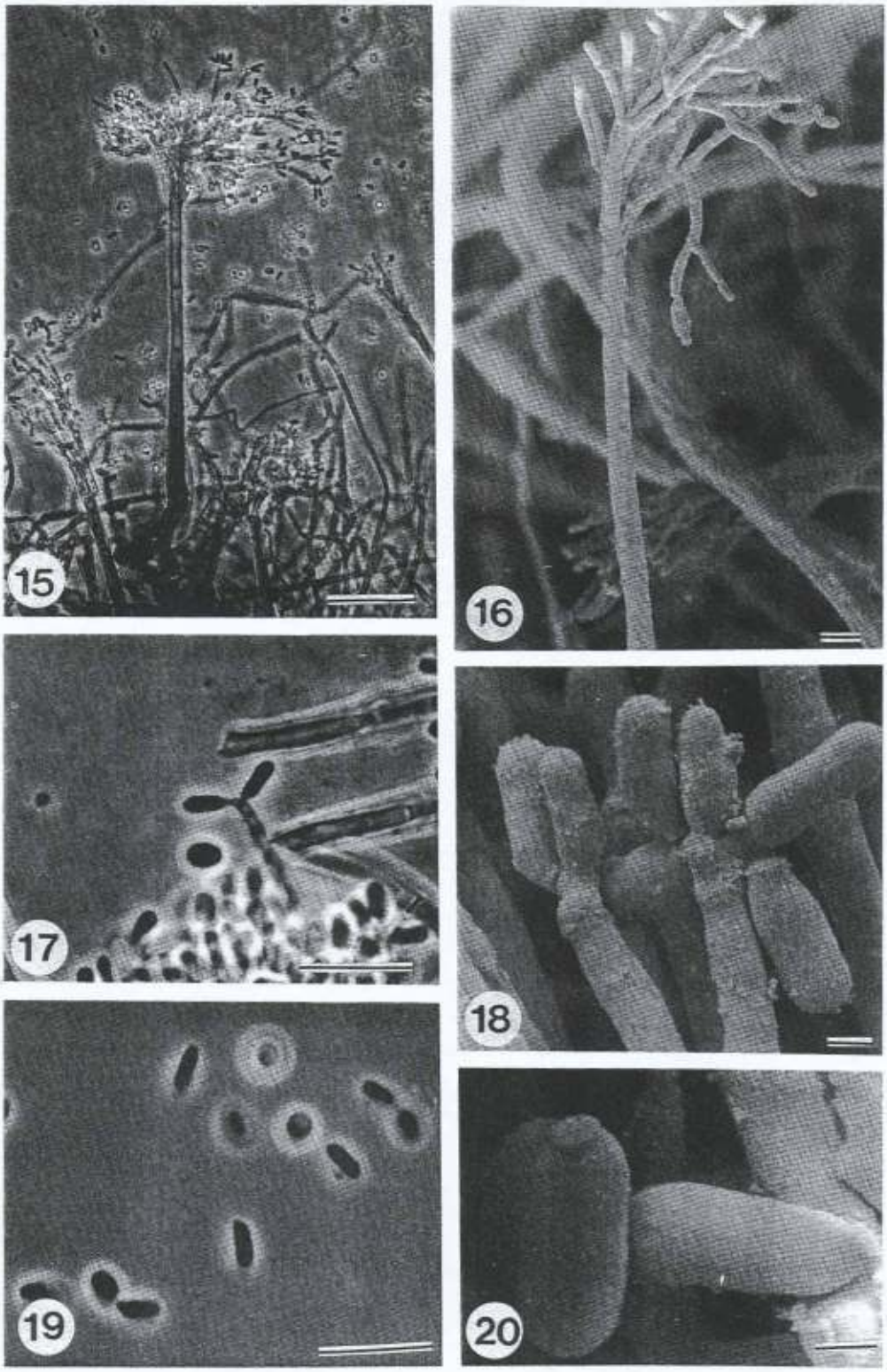


Fig. 15–20. *Leptographium peucophilum* (CMW 2876). 15. Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 μm). 16. Complex conidiogenous apparatus (Bar = 10 μm). 17. Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 μm). 18. Conidiogenous cells showing false sympodial conidiogenesis (Bar = 1 μm). 19. Conidia (Bar = 10 μm). 20. Conidia (Bar = 1 μm).

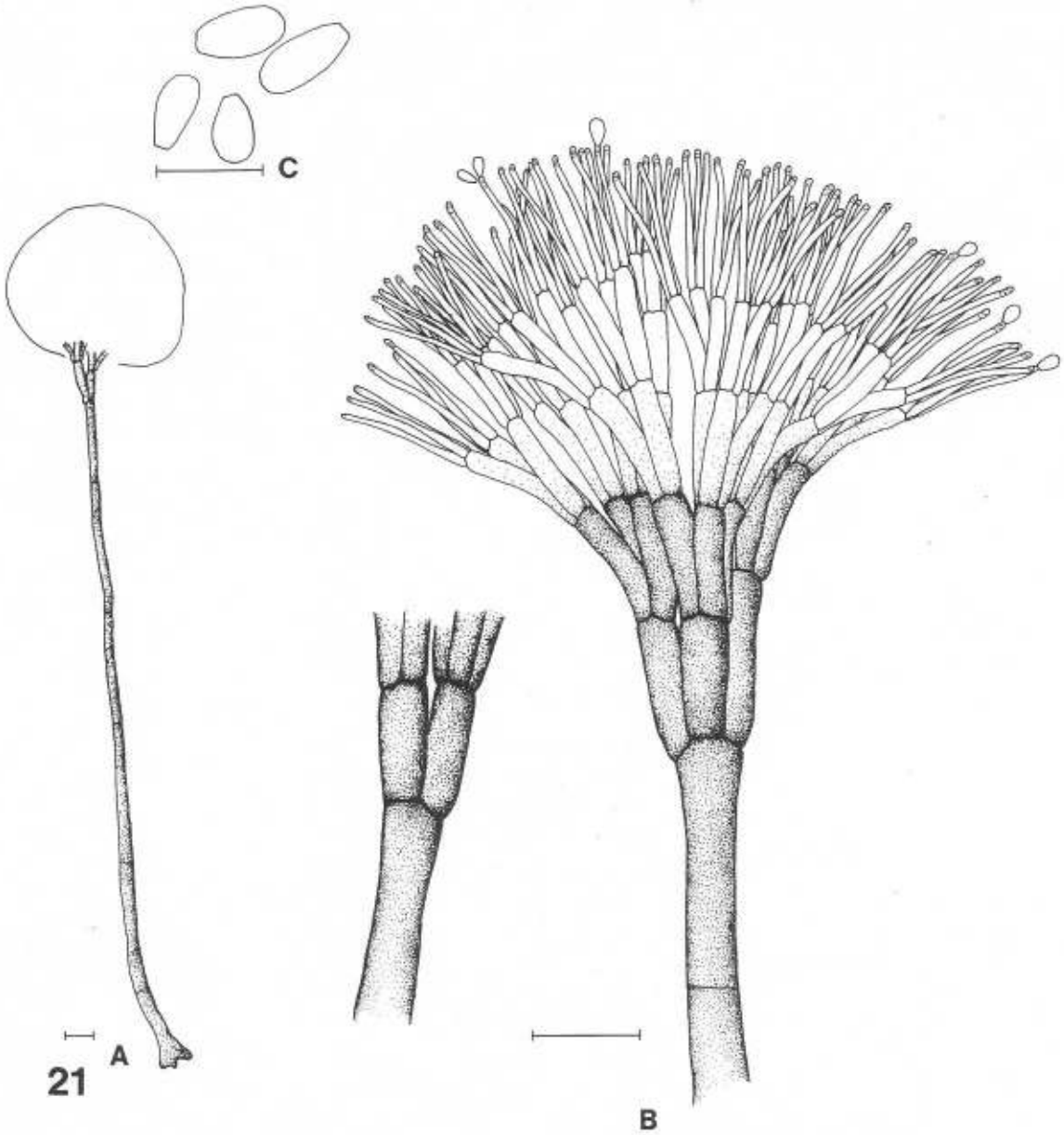


Fig. 21. *Leptographium peucophila* (CMW 2876). A. Habit sketch of the conidiophore (Bar = 10 μm). B. Conidiogenous apparatus (Bar = 10 μm). C. Conidia (Bar = 10 μm).

crescents,ex mycelio vegetativo immerso vel emerso olivaceo vel hyalino laevi non constricto, 2.0–3.0 μm diam, sine hyphis aeriis. Conidiophora singula vel bina, e mycelio rectangulatim exorientia, erecta, macronematosa, mononematosa, (230–)310–352(–520) μm longa; stipites cylindricei, simplices, atro-olivacei, leaves. 3-7-septati, 170–255(–420) μm longi, infra ramos primaries 3.0–8.0 μm lati, ad basim 4.5–11 μm lati, non tumidi. Apparatus conidiogeni 40–96(–120) μm longi, ex ramis cylindricis 3-4-seriatis compositi; rami primarii, 2–3, olivacei, cylindrici, aseptati, 10–25 × 3.0–8.0 μm; rami secundarii pallide olivacei vel hyaline, aseptati, (7.0–)8.0–17 × 2.0–5.0 μm; rami tertiarii hyaline, aseptati, (7.0–)8.0–13(–15) × 2.0–4.0 μm; rami quartii hyaline, aseptati, (7.0–)8.0–10(–13) × 2.0–3.0 μm; structura rhizoideiformes adest. Cellulae conidiogenae discretae, 2–3 per ranum, cylindricae, sursum, leviter, attenuatae, holoblasticae (8.0–)9.0–17(–20) × 2.0 μm. Conidia aseptata, hyaline, obovoidea, ad apicem apparatus conidiogeni in massa guttulata mucilaginosa accumulata 3.0–6.0 × 2.0–3.0 μm.

Colonies with optimal growth at 20°C on 2% MEA, reaching 10 mm in diam in 10 d. No growth below 10°C or above 30°C Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.1 g/l cycloheximide after 6 days at 25°C in the dark. Colony dark olivaceous (19"f). Colony margin laciniate. Hyphae submerged or on top of solid medium with no

aerial mycelia, olivaceous to hyaline, smooth, not constricted at the septa, 2.0–3.0 μm in diam.

Conidiophores occurring singly or in pairs, arising directly from the mycelium, erect, macronematous, mononematous, (230–)310–352(–520) μm in length, rhizoid-like structures present. Stipe dark olivaceous, smooth, cylindrical, simple, 3-7-septate, (170–)255–270(–420) μm long (from first basal septum to below primary branches), (3.0–)5.5(–8.0) μm wide below primary branches, apical cell not swollen; (4.5–)7.0(–11.0) μm wide at base, basal cell not swollen (Figs. 15, 21a). Conidiogenous apparatus 40–96(–120) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches; 2–3 primary branches, olivaceous, smooth, cylindrical, aseptate, 9.0–25.0 μm long and (3.0–)4.0–6.0(–8.0) μm wide, secondary branches light olivaceous to hyaline, aseptate, (7.0–)8.0–13(–17) μm long, 2.0–5.0 μm wide, tertiary branches hyaline, aseptate, (7.0–)8.0–11(–15) μm long, 2.0–4.0 μm wide, quaternary branches aseptate, hyaline, (7.0–)8.0–10(–13) μm long, 2.0–3.0 μm wide (Figs. 16, 21b). Conidiogenous cells discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (8.0–)13.0(–20.0) μm long and 2.0 μm wide. Conidium development 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, 3.0–6.0 × 2.0–3.0 μm (Figs. 19, 20, 21c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Teleomorph state: not known.

Holotype: PREM 56591, from *P. rubra* roots wounded by *K. gracillus*, collected: D. R. Bergdahl, White Face Mountain, New York, USA, August 1990.

Additional specimens: PREM 56390 (herbarium specimen) CMW 2875, CMW 2839 (live specimen), from *P. rubra* roots wounded by *Korscheltellus gracilus*, collected: D. R. Bergdahl, White Face Mountain, New York, USA, August 1990

Etymology: From the Greek noun *peuce*: spruce and Greek adjective *philos*: loving. This species refers to *Picea*, which is the host of this fungus.

Discussion

Leptographium pineti most closely resembles the *Leptographium* anamorph of *O. robustum* (Rob.-Jeffr. & R. W. Davids.) T. C. Harr. Although *O. robustum* is similar to *L. pineti* and also originated from *Pinus* spp., it has been described only from Canada and is associated with the bark beetles in the genus *Dendroctonus* (Robinson-Jeffrey and Davidson, 1968). This is in contrast to *L. pineti*, which is associated with *Ips* sp. in a very distinct geographical area. The two species can also be distinguished by the presence of a teleomorph in *O. robustum* and no evidence of perithecia associated with *L. pineti*. The *Leptographium* anamorph of *O. robustum* can be distinguished from *L. pineti* by the considerably shorter (31–116 μm) conidiophores in the former species, compared with the relatively longer conidiophores of the latter species (100–210 μm). *Leptographium pineti* is also characterised by small obovoid conidia (2–3 μm long) compared to the large (8–17 μm) oblong conidia of *O. robustum* (Robinson-Jeffrey and Davidson, 1968).

Leptographium calophylli J. F. Webber, K. Jacobs & M. J. Wingf. is another *Leptographium* species that is morphologically similar to *L. pineti* (Webber et al., 1999). The most striking difference between these fungi lies in their very different hosts. *Leptographium calophylli* is known only from the non-coniferous *Calophyllum inophyllum* L. (Takamaka), which is a native of various tropical islands. *Leptographium calophylli* is also characterised by optimum growth temperature of 30°C, compared with the optimum of 25°C of *L. pineti*. Morphologically, these species can also be distinguished by the short (41–100 μm) and long (100–210 μm) conidiophores of *L. calophylli* and *L. pineti*, respectively. Furthermore, *L. calophylli* also has considerably larger conidia (3–7 μm) (Webber et al., 1999) than those of *L. pineti* (2–3 μm).

Several *Leptographium* species are found in association with *Ips* spp. on spruce, larch and pine in Europe and Japan. These are *L. penicillatum* Grosm., *L. piceaperdum* K. Jacobs, M. J. Wingf. & Crous, *L. laricis* and *L. aenigmaticum*, respectively (Solheim, 1986; Van der Westhuizen et al., 1995; Jacobs et al., 1998). *Leptographium pineti* can easily be distinguished from these species by its small obovoid conidia and short robust conidiophores. This is in contrast to the large allantoid conidia and long conidiophores associated with *L. penicillatum* (Grosmann, 1931). This is also different from the larger obovoid conidia and considerably longer conidiophores associated with *L. piceaperdum*, *L. laricis* and *L. aenigmaticum* (Rumbold, 1936; Van der Westhuizen et al., 1995; Jacobs et al., 1998). Of the three species, only *L. piceaperdum* has been associated with *Pinus* spp. (Griffin, 1968; Hutchison and Reid, 1988).

Leptographium abieticolens morphologically resembles *L. antibioticum* (W. B. Kendrick) M. J. Wingf. *Leptographium antibioticum* was described by Kendrick (1962) and is characterised by its ability to produce antibiotic substances in culture. *Leptographium abieticolens* can easily be distinguished from *L. antibioticum* based on its darker stipes and considerably more complex conidiogenous apparatus. These species can further be distinguished based on their different optimal growth temperatures. *Leptographium abieticolens* grows optimally at 15°C, in contrast to *L. antibioticum*, which grows optimally at 25–30°C. *Leptographium abieticolens* is characterised by its 2 to 3 primary branches, whereas up to 5 primary branches have been observed in isolates of *L. antibioticum*. *Leptographium abieticolens* also can be distinguished from *L. antibioticum* by its larger, broad ellipsoidal conidia (4–7 μm), in contrast to the smaller obovoid to oblong conidia (2.5–5 μm) in the latter species (Kendrick, 1962).

Leptographium peucophilum morphologically resembles *L. procerum* (W. B. Kendrick) M. J. Wingf. These two species can, however, easily be distinguished by

colony appearance. Isolates of *L. procerum* are characterised by concentric rings of conidiophores on 2% MEA in Petri dishes at 25°C. This character is not observed in *L. peucophilum* which is also considerably slower growing than *L. procerum*. Furthermore, the conidiophores of *L. procerum* are slightly longer (average = 408 μm) than those of *L. peucophilum* (average = 330 μm). Both of these species are characterised by the presence of rhizoids and 2 to 3 primary branches in the conidiogenous apparatus. Comparison of the conidia revealed that both species have obovoid conidia that are between 3 and 6 μm long.

The occurrence of *L. abieticolens* and *L. peucophilum* on conifers is not unusual although their association with moth damage is unique for *Leptographium*. The larval stage of this moth feeds on the roots of many plant species, including spruce and fir. The fungi appear to enter through the wounds caused by the feeding habits of the swift moth. It is not known whether *L. abieticolens* or *L. peucophilum*, are pathogenic, although large areas of discoloration are usually associated with the feeding wounds caused by moth larvae.

Almost nothing is known of the ecology of *L. abieticolens* and *L. peucophila*. It seems unlikely that the conifer swift moth would be able to carry these fungi directly, as the adult insects never enter roots directly. It is possible that these are soil inhabiting *Leptographium* species that are able to colonise wounds made by the moth larvae. They may also be endophytes of spruce and fir, respectively, that are adapted to sporulate and grow in wounded tissue. Another hypothesis is that they are carried by phoretic mites vectored by the conifer swift moth. This hypothesis would be supported by the fact that a close association is known to exist between phoretic mites on bark beetles and a *Pyxidiophora* fungus (Blackwell et al., 1986). Such secondary vectorship is thought to play a role in the association of *Ophiostoma* species and long horn beetles (Coleoptera: Cerambycidae). The adult insects never enter wood, but *Ophiostoma* spp. are commonly found sporulating in the galleries of their larvae (Wingfield, 1987).

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