Chapter 7

Jacobs, K.; Wingfield, M.J and Bergdahl, B.D. (1999). New *Leptographium* species from Indonesia and Eastern North America. Mycoscience (submitted).

New *Leptographium* species from Indonesia and Eastern North America

Leptographium spp. 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* spp. 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, several 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* spp. 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. abicolens* sp. nov. and *L. peucophilum* sp. nov.

Keywords: conifer swift moth, conifers, *Ips, Leptographium.*

Introduction

Leptographium spp. are generally characterized 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, Wingfield and Marasas, 1988). *Leptographium* spp. are generally associated with conifers (Lagerberg, Lundberg and Melin, 1927; Kendrick, 1962; Harrington, 1988), with only a few exceptions described (Davidson, 1942; 1958; 1971; 1976; Jooste, 1978; Weber, Spaaij and Wingfield, 1996). Most *Leptographium* spp. are carried by insects, especially, bark beetles (Coleoptera: Scolytidae) and they sporulate profusely in galleries of these insects (Lagerberg *et al.,* 1927; Leach, Orr and Christensen, 1934; Harrington, 1988).

Leptographium spp. have been recorded from many parts of the world and many species have been accidentally introduced into new areas along with bark beetles (Wingfield & Marasas, 1980). However, most species are native to the Northern hemisphere and especially North America and Europe, where most conifers and their bark beetle pests originate (Lundberg *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, Wingfield and Bergdahl, 1997). Among the North American species, the three varieties of *L. wageneri* (Kendrick) Wingfield are probably best known due to their role in causing black-stain root disease on pine (*Pinus* spp.) and douglas-fir (*Pseudotsuga menziesii*) (Kendrick, 1962; Harrington and Cobb, 1987; Harrington, 1988).

Most of the *Leptographium* spp. described from North America have been associated with insects. Exceptions include *L. antibioticum, L. brachiatum* and

O. trinacriforme (*Leptographium* anamorph) (Parker, 1957; Kendrick, 1962). The association of *Leptographium* spp. with bark beetles is well recognized, 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, Harrington & Solheim, 1995; Krokene & Solheim, 1996, 1998).

In Europe and Asia, many *Leptographium* spp. 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* spp. in Japan, two new *Leptographium* spp. were recently described. However, these species were not found to be present in Europe associated with similar insects (Wingfield, Crous and Tzean, 1994; Van der Westhuizen *et al.,* 1995; Jacobs *et al.,* 1998). Apart from *O. penicillatum* (Grosmann) Siemaszko and *O. piceaperdum* (Rumbold) Von Arx, that are associated with *I. typographus* in Europe (Solheim, 1986), various *Leptographium* spp. from east Asia have not been recorded elsewhere in the world (Yamaoka *et al.,* 1997). Two interesting examples include, *L. laricis* Van der Westhuizen, Wingfield & Yamaoka and *L. aenigmaticum* Jacobs, Wingfield & 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* spp. has emerged from *P. merkusii* infested with *Ips* sp. in Indonesia as well as Balsam fir (*Abies balsamea* (L.) Mill.) and Red spruce [*Picea rubens* Sarg. (*Picea rubra* (Du Roi) Link.)] in North America associated with damage to roots by the conifer swift

moth, *Korscheltellus gracillus* (Lepidoptera: Hepialidae). The main objective 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* in Northern Sumatra, Indonesia, were examined and the dominant fungus in these galleries was a *Leptographium* sp. Red spruce and balsam fir roots wounded by the conifer swift moth, *K. gracillus,* collected on White Face Mountain, New York, USA were also found to be infested with *Leptographium* spp. Conidial masses from these fungi were transferred from the apices of conidiophores to 2 % malt extract (MEA) (20g Biolab malt extract, 20g 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).

The optimal temperatures for growth of isolates representing three distinct *Leptographium* spp. (CMW 3831 and CMW 3832 from Indonesia, CMW 2865 from balsam fir, CMW 2876 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 eight days 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* sp. from *P. merkusii* infested with *Ips* sp. in Sumatra is characterized by short robust conidiophores with dark stipes and short conidiogenous apparatuses made up of two to three series of branches. These isolates are, furthermore, characterized by short conidiophores that produce small, obovoid conidia. Comparison with all other *Leptographium* spp. revealed that these isolates do not resemble any known taxon and we conclude that it represents a previously undescribed species, which is described as follows:

PLEASE NOTE THAT THE FOLLOWING SPECIES DESCRIPTION SHOULD NOT BE CITED AND ARE PRINTED HERE IN PRELIMINARY FORM. THESE WILL BE FORMALLY PRINTED ELSEWHERE.

Leptographium pineti **K. Jacobs and M.J. Wingf. sp. nov.**

Teleomorph state: not known.

Coloniae optime in temperatura 25°C crescentes; atro-olivaceae. Hyphae immersae vel emersae in medio solido, sine myceliis aeriis. Conidiophora singula, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (100-) 145 (-

210) µm longa, cum 2 vel 3 seriebus ramorum cylindricorum; 2 - 3 ramis primariis; structuris rhizoidiformibus. Conidia aseptata, oblonga vel obovoidea, 2.0 - 3.0 x 1.0 µm.

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 15 mm in diameter in 6 days. 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 days 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 diameter.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (100-) 145 (-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 (Fig. 1, 7a). Conidiogenous apparatus (30-) 46 (-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-) 4.0 (-6.0) µm wide, secondary branches hyaline, aseptate, (7.0-) 10.5 (-15) µm long, (2.0-) 3.0 (-4.0) µm wide; tertiary branches hyaline, aseptate, (5.0-) 8.5 (-15) µm long, 2.0 - 3.0 µm wide (Fig. 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) (Fig. 3, 4). Conidia, aseptate, obovoid, 2.0 - 3.0 x 1.0 µm (Fig. 5, 6, 7c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

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

The isolates from both *A. balsamea* and *P. rubra,* were characterized by dark conidiophores and a high degree of tolerance to cycloheximide, which is similar to other *Leptographium* spp. The isolates from *A. balsamea,* are characterized by optimal growth at low temperatures and slow growing colonies. These isolates are further characterized 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,* does not resemble any previously described species, and it is thus described as follows:

PLEASE NOTE THAT THE FOLLOWING SPECIES DESCRIPTION SHOULD NOT BE CITED AND ARE PRINTED HERE IN PRELIMINARY FORM. THESE WILL BE FORMALLY PRINTED ELSEWHERE.

***Leptographium abicolens* K. Jacobs and M.J. Wingf. sp. nov.**

Teleomorph state: not known

Coloniae optime in temperatura 15°C crescentes; atro-olivaceae (19"f); margine laevi. Hyphae immersae vel emersae in medio solido, cum myceliis aeriis abundantibus. Conidiophora singula vel ad sena, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (120-) 228 (-360) µm longa, cum 3 vel 4 seriebus ramorum cylindricorum; 2 - 3 ramis primariis; structurae rhizoidiformes

adsunt. Conidia aseptata, late ellipsoidea vel obovoidea, (4.0-) 5.0 (-7.0) x 2.0 - 3.0 µm.

Colonies with optimal growth at 15 °C on 2 % MEA, reaching 18 mm in diameter in 14 days. 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 days 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-) 228 (-360) µm in length, rhizoid-like structures present. Stipe dark olivaceous, smooth, cylindrical, simple, 2 - 11 septate, (72-) 165 (-264) µm long (from first basal septum to below primary branches), (3.0-) 4.5 (-6.0) µm wide below primary branches, apical cell not swollen; (4.5-) 6.5 (-7.5) µm wide at base, basal cell not swollen (Fig. 8, 14a). Conidiogenous apparatus (32-) 62 (-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-) 13 (-31) µm long and (3.0-) 3.5 (-5.0) µm wide, secondary branches light olivaceous to hyaline, aseptate, (7.0-) 9.0 (-15) µm long, (2.0-) 3.0 (-4.0) µm wide; tertiary branches hyaline, aseptate, (6.0-) 9.0 (-12) µm long, (2.0-) 2.5 (-4.0) µm wide, quaternary branches aseptate, hyaline, (5.0-) 8.0 (-10.0) µm long, (2.0-) 3.0 (-4.0) µm wide (Fig. 9, 14b). Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (8.0-) 13 (-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) (Fig. 10, 11). Conidia, aseptate, broadly ellipsoidal to obovoid, (4.0-) 5.0 (-7.0) x 2.0 - 3.0

µm (12, 13, 14c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Holotype: CMW 2865, from *A. balsamea* roots wounded by *K. gracilus*, collected D.R. Bergdahl, White Face Mountain, New York, USA, August 1990.

Additional specimens: CMW 2894, CMW 2866, from *A. balsamea* roots wounded by *K. gracillus*, collected D.R. Bergdahl, White Face Mountain, New York, USA, August 1990.

The *Leptographium* sp. 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. abicolens*. Comparison with other *Leptographium* spp. has shown that this fungus represents a previously undescribed taxon, and it is described as follows:

PLEASE NOTE THAT THE FOLLOWING SPECIES DESCRIPTION SHOULD NOT BE CITED AND ARE PRINTED HERE IN PRELIMINARY FORM. THESE WILL BE FORMALLY PRINTED ELSEWHERE.

***Leptographium peucophilum* K. Jacobs and M.J. Wingf. sp. nov.**

Teleomorph state: not known.

Colonia atro-olivacea; margine laciniato. Hyphae immersae vel emersae in medio solido, sine myceliis aeriis. Conidiophora singula vel bina, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (230-) 331 (-520) µm longa, cum 3 vel 4 seriebus ramorum cylindricorum; 2 - 3 ramis primariis; structurae rhizoidiformes adsunt. Conidia aseptata, obovoidea, (3.0-) 4.0 (-6.0) x 2.0 - 3.0 µm.

Colonies with optimal growth at 20 °C on 2 % MEA, reaching 10 mm in diameter in 10 days. 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 diameter.

Conidiophores occurring singly or in groups of two, arising directly from the mycelium, erect, macronematous, mononematous, (230-) 331 (-520) µm in length, rhizoid-like structures present. Stipe dark olivaceous, smooth, cylindrical, simple, 3-7 septate, (170-) 263 (-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) µm wide at base, basal cell not swollen (Fig. 15, 21a). Conidiogenous apparatus (40-) 68 (-120) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches; 2 - 3 primary branches, olivaceous, smooth, cylindrical, aseptate, (9.0-) 14.5 (-25) µm long and (3.0-) 5.0 (-8.0) µm wide, secondary branches light olivaceous to hyaline, aseptate, (7.0-) 12 (-17) µm long, (2.0-) 3.5 (-5.0) µm wide; tertiary branches hyaline, aseptate, (7.0-) 11 (-15) µm long, (2.0-) 2.5 (-4.0) µm wide, quaternary branches aseptate, hyaline, (7.0-) 9.0 (-13) µm long, 2.0 - 3.0 µm wide (Fig. 16, 21b). Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (8.0-) 13 (-20) 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) (Fig. 17, 18). Conidia, aseptate, obovoid, (3.0-) 4.0 (-6.0) x 2.0 - 3.0 µm (Fig. 19, 20, 21c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Holotype: CMW 2876, from *P. rubra* roots wounded by *K. gracillus*, collected: D.R. Bergdahl, White Face Mountain, New York, USA, August 1990.

Additional cultures: CMW 2875, CMW 2839, from *P. rubra* roots wounded by *Korscheltellus gracillus*, collected: D.R. Bergdahl, White Face Mountain, New York, USA, August 1990

DISCUSSION

Leptographium pineti most closely resembles the *Leptographium* anamorph of *O. robustum* (Robinson-Jeffrey & Davidson) Harrington. It can, however, easily be distinguished from this and other *Leptographium* spp. based on its characteristic short, robust conidiophores and small conidia. 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* that is associated with *Ips* sp. in a very distinct geographical area. The two species can also be distinguished based on 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* based on 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 characterized 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 Webber, Jacobs & Wingfield is another *Leptographium* spp. that is morphologically similar to *L. pineti* (Webber, Jacobs & Wingfield, 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* (Takamaka), which is a native of various tropical islands. *Leptographium calophylli* is also characterized by optimum growth temperature of 30 °C, compared to the optimum of 25 °C of *L. pineti.* Morphologically, these

species can also be distinguished based on 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 - 7µm) (Webber *et al.,* 1999) than those of *L. pineti* (2 - 3 µm).

Several *Leptographium* spp. are found in association with *Ips* spp. on spruce, larch and pine in Europe and Japan. These are *L. penicillatum* and *L. piceaperdum, 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 abicolens and *L. peucophilum* differ from other *Leptographium* spp. based on their unusual habitat and insect association. Various *Leptographium* spp. are associated with root infections and are associated with the feeding activities of root feeding bark beetles (Wingfield & Knox-Davies, 1980; Wingfield & Marasas, 1983; Cobb, 1988; Wingfield, Capretti & Mackenzie, 1988; Wingfield, Harrington & Crous, 1994). *Leptographium abicolens* and *L. peucophilum* have both been isolated from the roots of their respective host trees, and are associated with the feeding activities of larvae of the conifer swift moth, which is unusual. In addition, these species have been isolated from high elevation sites, which is consistent with their low optimal growth temperatures.

Leptographium abicolens most closely resembles *L. antibioticum. Leptographium antibioticum* was described by Kendrick (1962) and is

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

Leptographium peucophilum most closely resembles *L. procerum.* These two species can, however, easily be distinguished based on colony appearance. Isolates of *L. procerum* are characterized by concentric rings of conidiophores on agar in Petri dishes. This character is not observed in *L. peucophilum* and the fungus 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 = 330µm). Both of these species are characterized 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.

Leptographium abicolens and *L. peucophilum* both occur at high elevation sites and their low optimal temperatures for growth is consistent with their habitat. Their occurrence 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. abicolens* 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. abicolens* and *L. peucophilum.* 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* spp. 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 *Pyxidiophora* spp. (Blackwell *et al.,* 1986). Such secondary vectorship is thought to play a role in the association of *Ophiostoma* spp. and long horn beetles (Coleoptera: Cerambycidae) where the adult insects never enter wood but where *Ophiostoma* spp. are commonly found sporulating in the galleries of their larvae (Wingfield, 1987).

LITERATURE CITED

Blackwell, M., Bridges, J.R., Moser, J.C. and Perry, T.J. (1986). Hyperphoretic dispersal of a *Pyxidiophora* anamorph. *Science* **232,** 993-995.

Cobb, F.W. (jr.) (1988). *Leptographium wageneri,* cause of black-stain root disease: a review of its discovery , occurrence and biology with emphasis on pinyon and ponderosa pine. In: *Leptographium* root diseases on conifers. American Phytopathological Society, St Paul, Minnesota, pp. 41-62.

Craighead, F.C. (1928). Interrelation of tree-killing bark beetles (*Dendroctonus)* and blue-stain. *Journal of Forestry* **26**, 886-887.

Davidson, R.W. (1942). Some additional species of *Ceratostomella* in the United States. *Mycologia* **34**, 650-662.

Davidson, R.W. (1958). Additional species of Ophiostomataceae from Colorado. *Mycologia* **50**, 661-670.

Davidson, R.W. (1971). New species of *Ceratocystis*. *Mycologia* **63**, 5-15.

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

Goidanich, G. (1936). Il genera di Ascomiceti "Grosmann" G. Goid. *Bollettino della R. Statione di Patologia Vegetale-Roma N.S.* **16**, 26-60.

Griffin, H.D. (1968). The genus *Ceratocystis* in Ontario. *Canadian Journal of Botany* **46**, 689-718.

Grosmann, H. (1931). Contributions to the knowledge concerning the life partnership between bark beetles and fungi. *Zeitschrift für Parasitenkunde* **3**, 56-102.

Harrington, T. C. (1988). *Leptographium* species, their distributions, hosts and insect vectors. In: *Leptographium* root diseases on conifers. pp. 1-39, American Phytopathological Society, St. Paul, Minnesota.

Harrington, T.C. and Cobb, F.W. (Jr.). (1987). *Leptographium wageneri* var. *pseudotsugae*, var. nov., cause of black stain root disease on Douglas-fir. *Mycotaxon* **30**, 501-507.

Hutchison, L.J. and Reid, J. (1988). Taxonomy of some potential wood-staining fungi from New Zealand 1. Ophiostomataceae. *New Zealand Journal of Botany*, **26**, 63-81.

Jacobs, K; Wingfield, M.J. and Bergdahl, D. (1997). A new species of *Ophiostoma* from North America, similar to *Ophiostoma penicillatum*. *Canadian Journal of Botany* **75,** 1315-1322.

Jacobs, K., Wingfield, M.J, Wingfield, B.D. and Yamaoka, Y. (1998). Comparison of *Ophiostoma huntii* and *O. europhioides* and description of *O. aenigmaticum* sp. nov. *Mycological Research* **102,** 289-294.

Jooste, W.J. (1978). *Leptographium reconditum* sp. nov. and observations on conidiogenesis in *Verticicladiella*. *Transactions of the British Mycological Society* **70**, 152-155.

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

Kendrick, W.B. (1964). The *Leptographium* complex. *Scopularia Venusta* Preuss. *Canadian Journal of Botany* **42**, 1119-1122.

Kendrick, W.B. and Molnar, A.C. (1965). A new *Ceratocystis* and its *Verticicladiella* imperfect state associated with the bark beetle *Dryocoetus confusus* on *Abies lasiocarpa*. *Canadian Journal of Botany* **43**, 39-43.

Krokene, P. & Solheim, H. (1996). Fungal associates of five bark beetle species colonizing Norway spruce. *Canadian Journal of Forestry Research* **26,** 2115-2122.

Krokene, P. & Solheim, H. (1998). Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. *Phytopathology* **88,** 39-44.

Lagerberg, T., Lundberg, G and Melin, E. (1927). Biological and practical researches into blueing in pine and spruce. *Svenska Skogsvårdsföreningens Tidskrift* **25**, 145-272, 561-691.

Leach, J.G., Orr, L.W. and Christensen, C. (1934). The interrelationships of bark beetles and blue-staining fungi in felled Norway pine timber. *Journal of Agricultural Research* **49**, 315-341.

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

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

Morelet, M. (1988). Observations sur trios Deutéromycètes inféodés aux pins. *Annales de la Société des Sciences Naturelles et d'Archéologie de Toulon et du Var* **40**, 41-43.

Parker, A.K. (1957). *Europhium*, a new genus of the ascomycetes with a *Leptographium* imperfect state. *Canadian Journal of Botany* **35**, 173-179.

Rayner, R.W. (1970). A Mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey and British Mycological Society.

Robinson-Jeffrey, R.C. and Davidson, R.W. (1968). Three new *Europhium* species with *Verticicladiella* imperfect states on blue-stained pine. *Canadian Journal of Botany* **46**, 1523-1527.

Robinson-Jeffrey, R.C. and Grinchenko, A.H.H. (1964). A new fungus in the genus *Ceratocystis* occurring on blue-stained lodgepole pine attacked by bark beetles. *Canadian Journal of Botany* **42**, 527-532.

Rumbold, C.T. (1936). Three blue-staining fungi, including two new species, associated with bark beetles. *Journal of Agricultural Research* **52**, 419-437.

Six, D.L. and Paine, T.D. (1996). *Leptographium pyrinum* is a mycangial fungus of *Dendroctonus adjunctus*. *Mycologia* **88**, 739-744.

Solheim, H. (1986). Species of Ophiostomataceae isolated from *Picea abies* infested by the bark beetle *Ips typographus*. *Nordic Journal of Botany* **6**, 199-207.

Van der Westhuizen, K., Wingfield, M.J., Yamaoka, Y., Kemp, G.H.J. and Crous, P.W. (1995). A new species of *Ophiostoma* with a *Leptographium* anamorph from Larch in Japan. *Mycological Research* **99**, 1334-1338.

Van Wyk, P., Wingfield, M.J. and 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.

Weber, G., Spaaij, F. and Wingfield, M.J. (1996). *Leptographium costaricense* sp. nov., a new species from roots of *Talauma sambuensis*. *Mycological Research* **100**, 732-736.

Webber, J.F., Jacobs, K. & Wingfield M.J. (1999). A re-examination of the vascular wilt pathogen of takamaka (*Calophyllum inophyllum*). *Mycological Research* (in press).

Wingfield, M.J. (1987). Fungi associated with the pine wood nematode, *Bursaphelenchus xylophilus,* and cerambycid beetles in Wisconsin. *Mycologia* **79**, 325-328.

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. pp. 43-51. (ed. M.J. Wingfield. K.A. Seifert and J.F. Webber). American Phytopathological Press, St. Paul, Minnesota,

Wingfield, M.J. and Knox-Davies, P.S. (1980). Root disease associated with *Verticicladiella alacris,* of pines in South Africa. *Plant Disease* **64,** 569-571.

Wingfield, M.J. and 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. and Marasas, 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. and Mackenzie, M. (1988). *Leptographium* spp. as root pathogens on conifers. An international perspective. In: *Leptographium* root diseases on conifers. American Phytopathological Society, St. Paul, Minnesota, pp. 113-128.

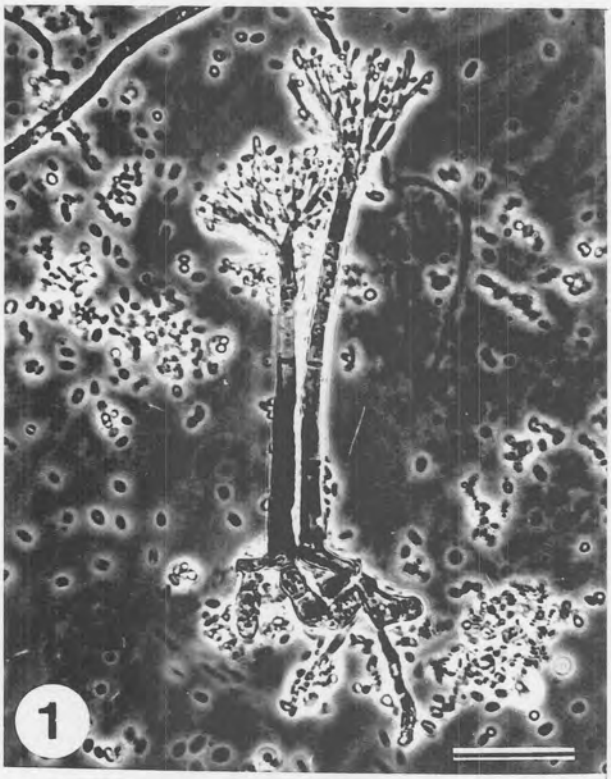
Wingfield, M. J., Crous, P.W. and Tzean, S.S. (1994). *Leptographium elegans*: a new species from Japan. *Mycological Research* **98**, 781-785.

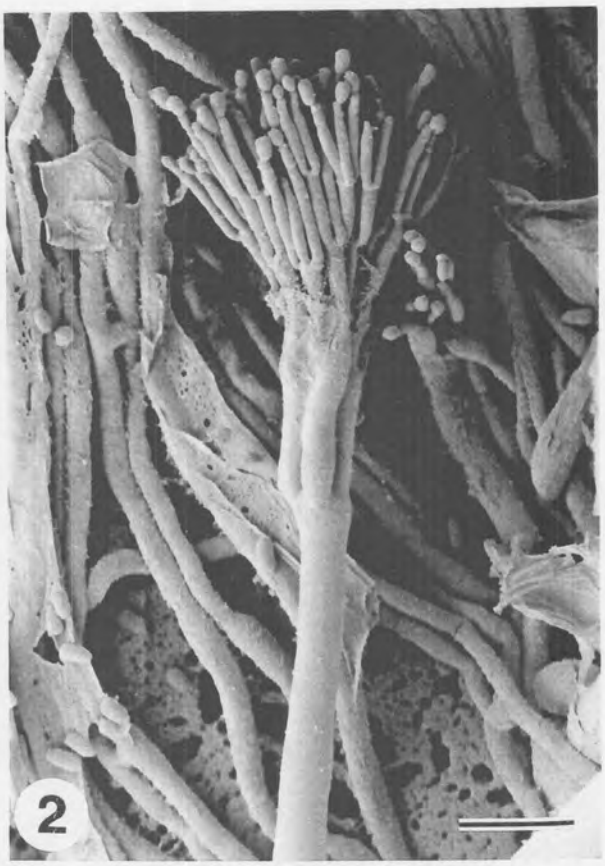
Wingfield, M.J., Harrington, T.C. and Crous, P.W. (1994). Three new *Leptographium* species associated with conifer roots in the United States. *Canadian Journal of Botany* **72**, 227-238.

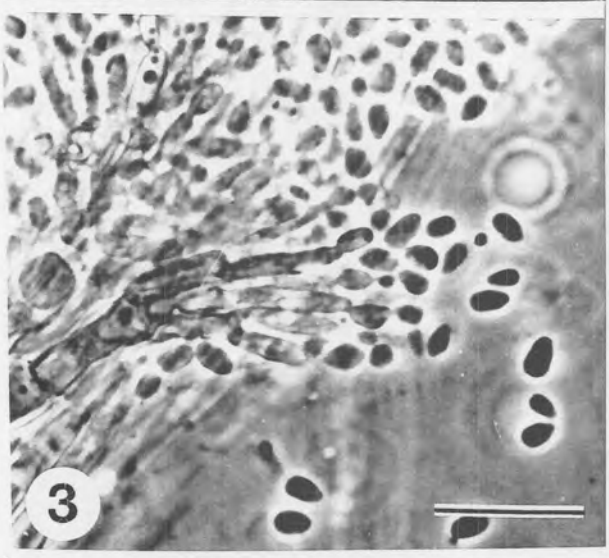
Wingfield, M.J., Harrington, T.C. and Solheim, H. (1995). Do conifer bark beetles require fungi to kill trees ? Proceedings from a symposium held at the Norwegian Forest Research Institute, Ås, Norway (ed. E. Christiansen). pp. 6

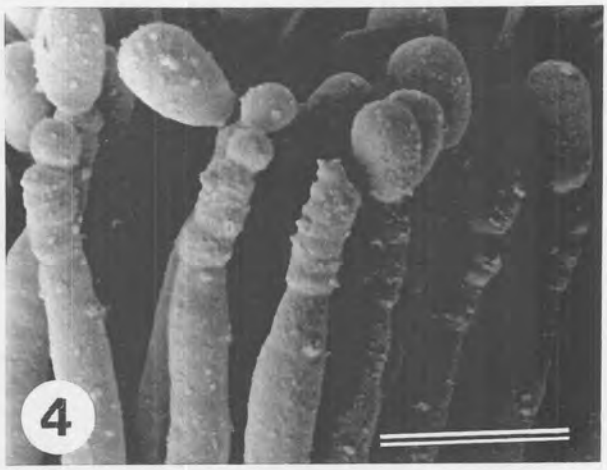
Yamaoka, Y., Wingfield, M.J., Takahashi, I. and Solheim, H. (1997). Ophiostomatoid fungi associated with the spruce bark beetle *Ips typographus japonicus* in Japan. *Mycological Research* **101,** 1215-1227.

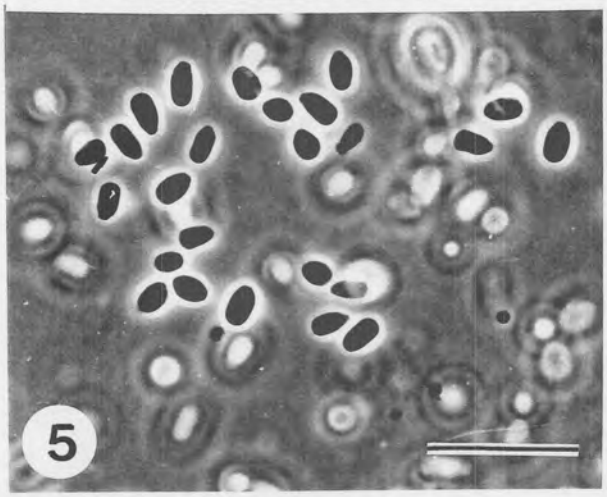
Fig. 1 - 6. *Leptographium pineti* (PREM 56391). **Fig. 1.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 µm). **Fig. 2.** Complex conidiogenous apparatus (Bar = 10 µm). **Fig. 3.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 µm). **Fig. 4.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 5 µm). **Fig. 5-** Conidia (Bar = 10 µm). **Fig. 6.** Conidia (Bar = 1 µm).











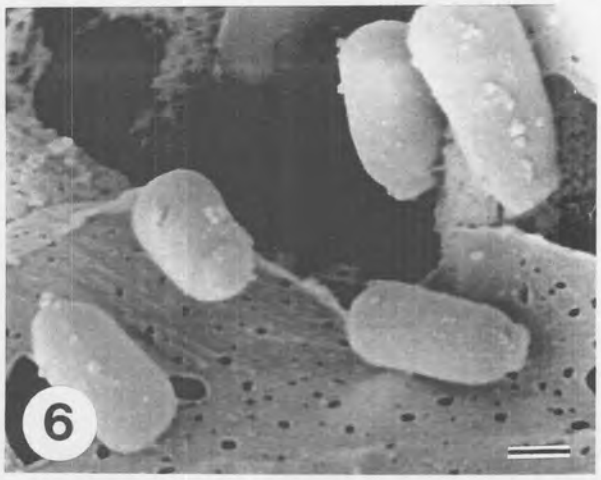
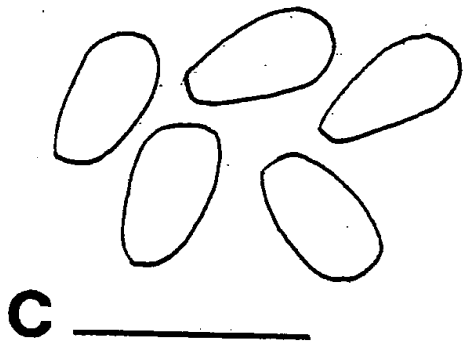
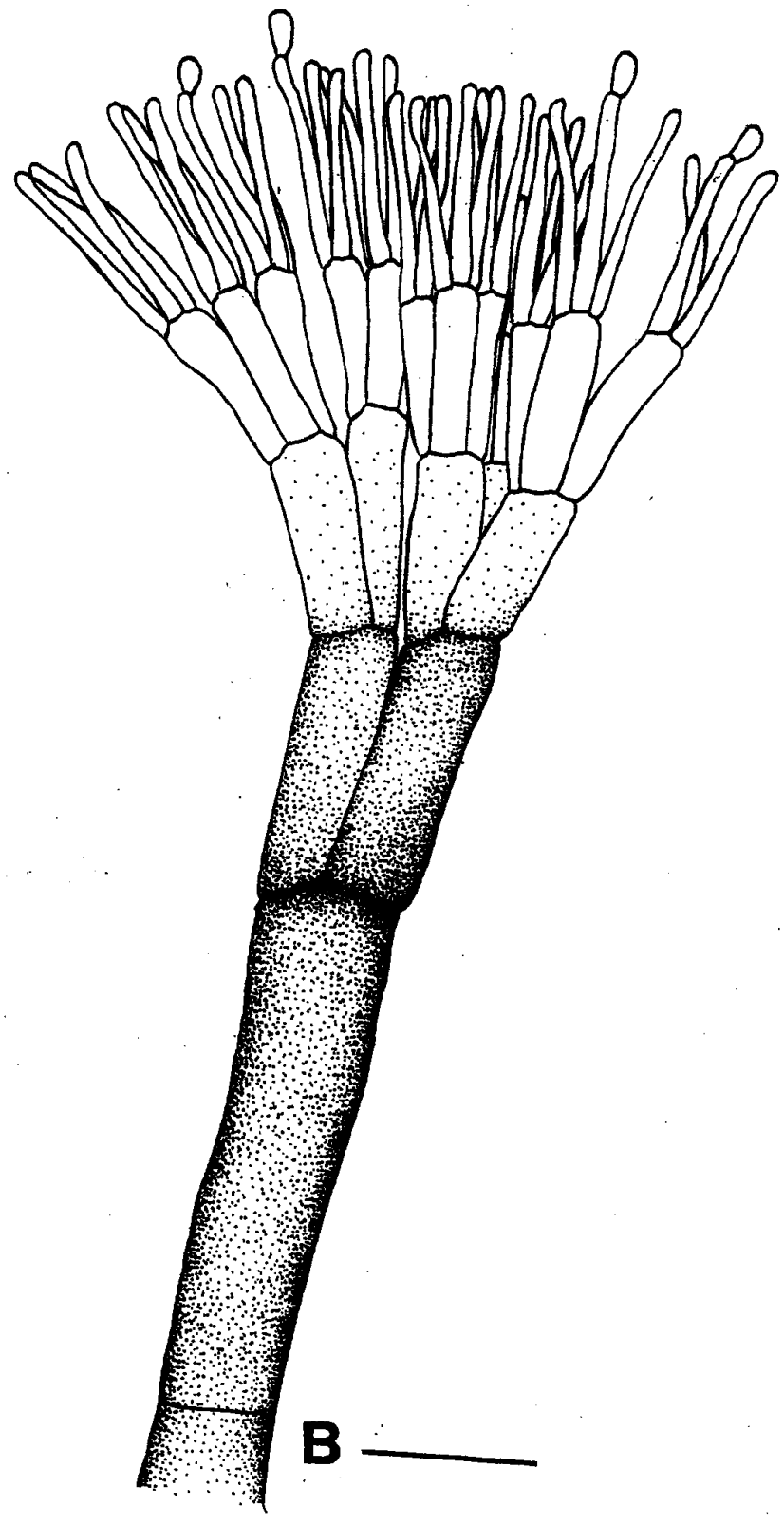
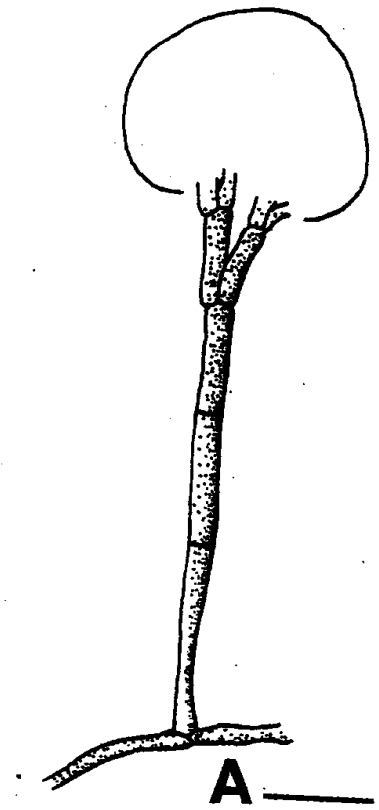


Fig. 7. *Leptographium pineti* (PREM 56391). A. Habit sketch of the conidiophore (Bar = 20 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).



C



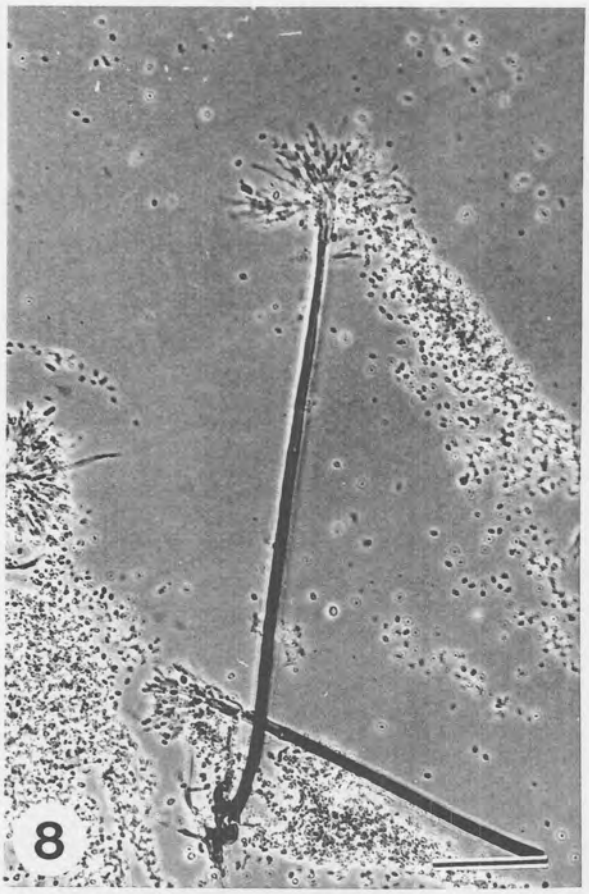


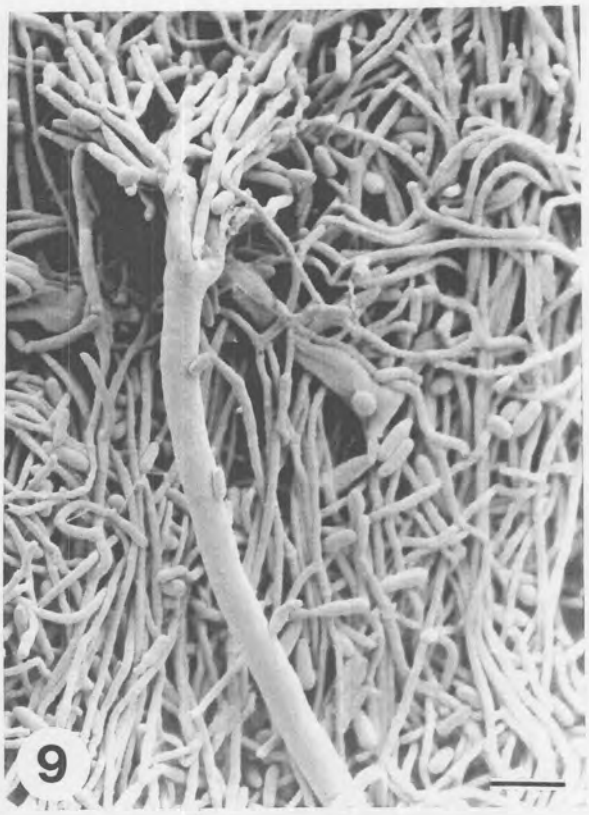
A

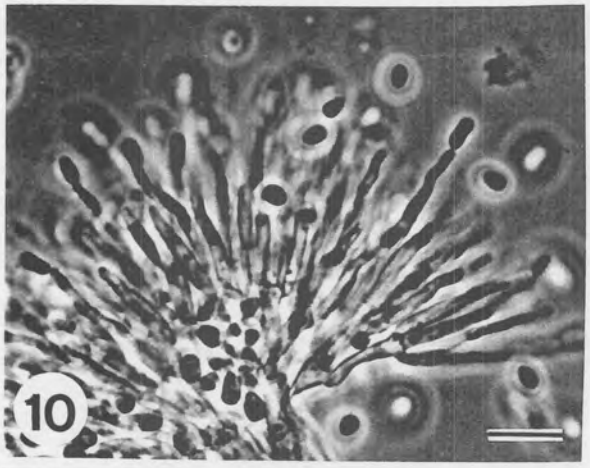
B

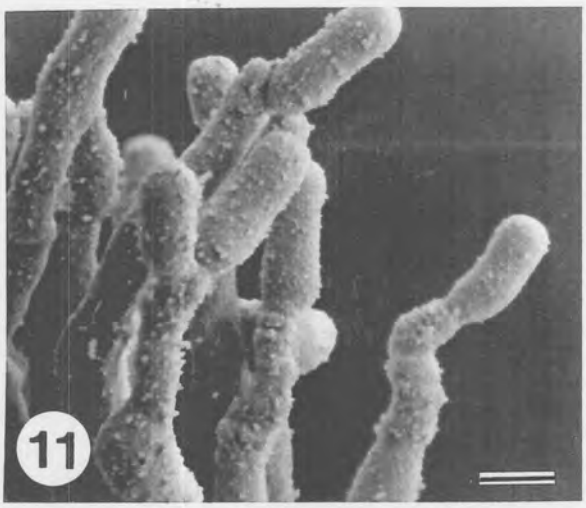
7

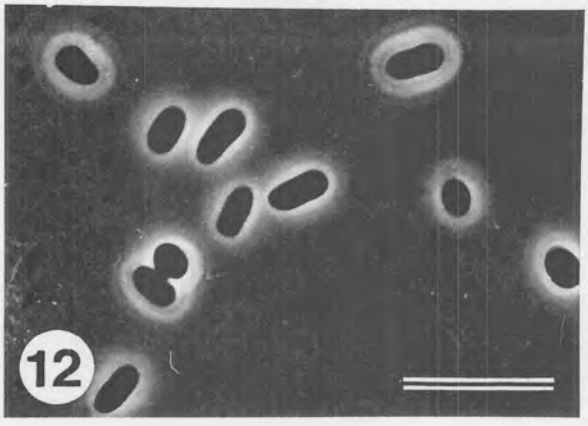
Fig. 8 - 13. *Leptographium abicolens* (CMW 2865). **Fig. 8.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 µm). **Fig. 9.** Complex conidiogenous apparatus (Bar = 10 µm). **Fig. 10.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 µm).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











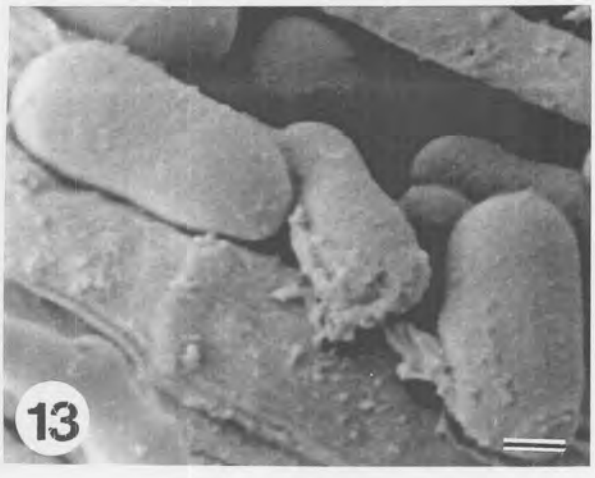
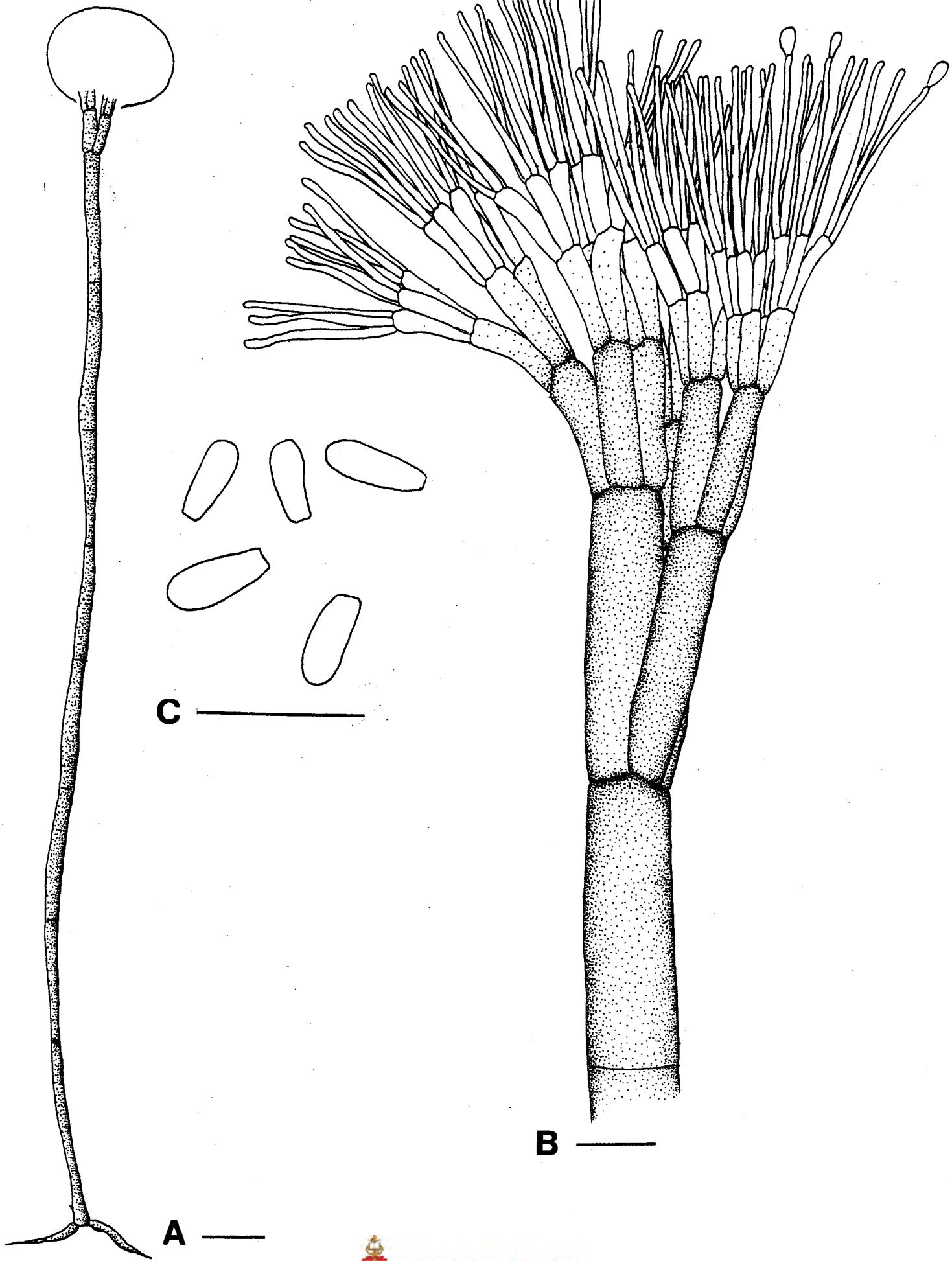


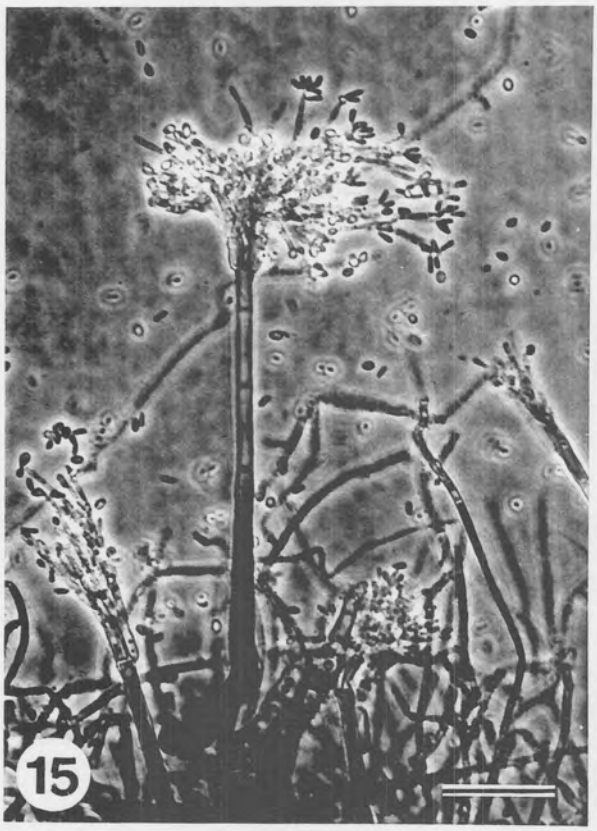
Fig. 14. *Leptographium abicolens* (CMW 2865). A. Habit sketch of the conidiophore

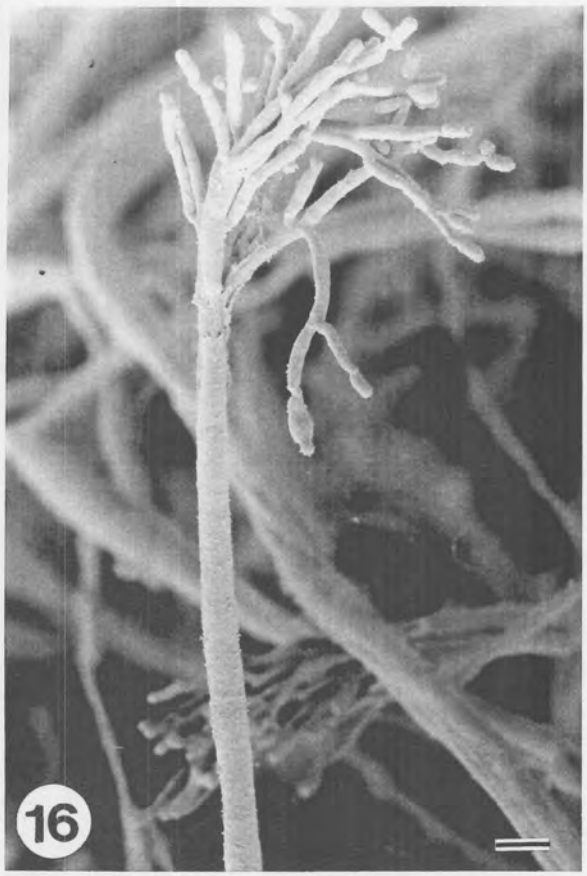
(Bar = 20 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).

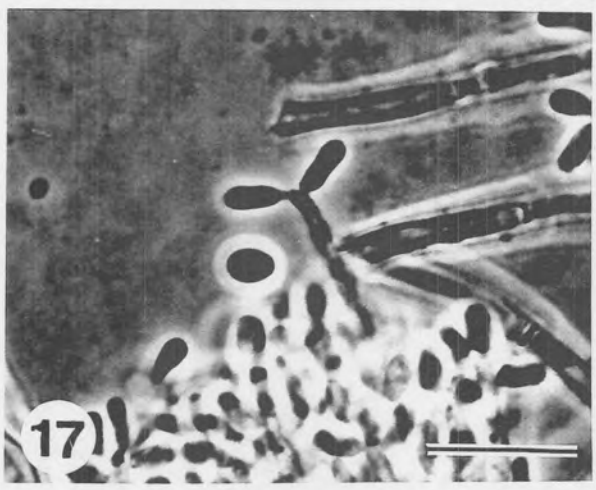


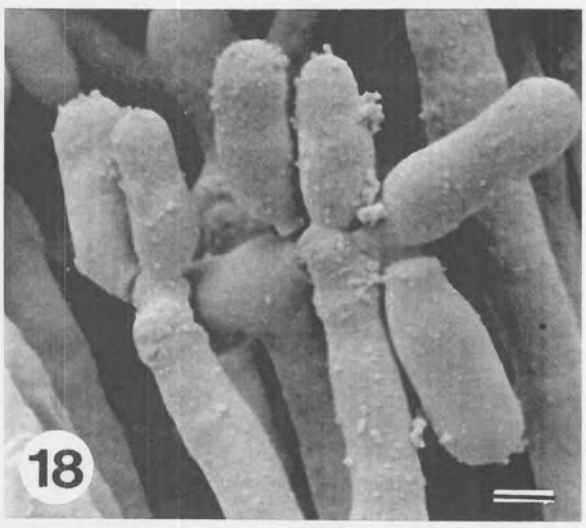
4

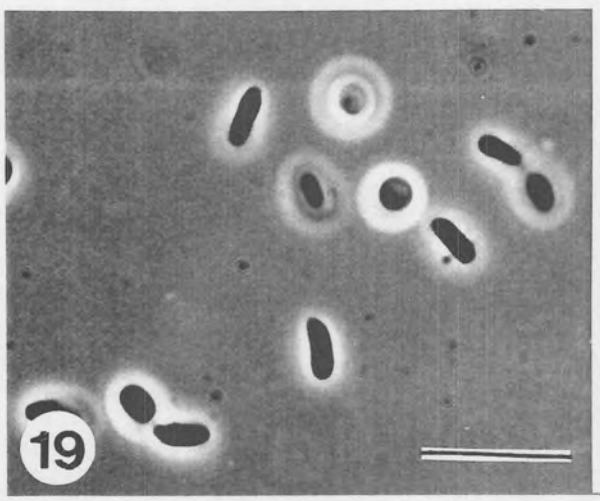
Fig. 15 - 20. *Leptographium peucophilum* (CMW 2876). **Fig. 15.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 µm). **Fig. 16.** Complex conidiogenous apparatus (Bar = 10 µm). **Fig. 17.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10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











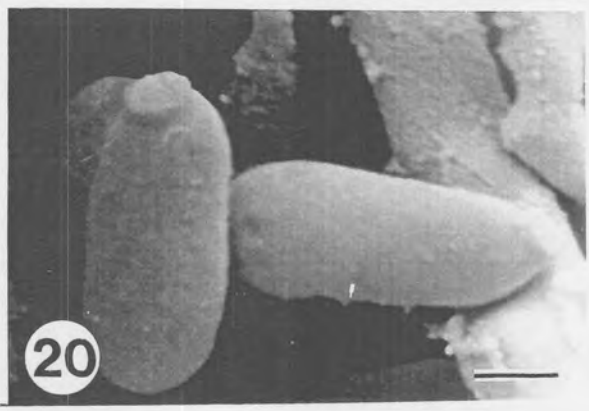
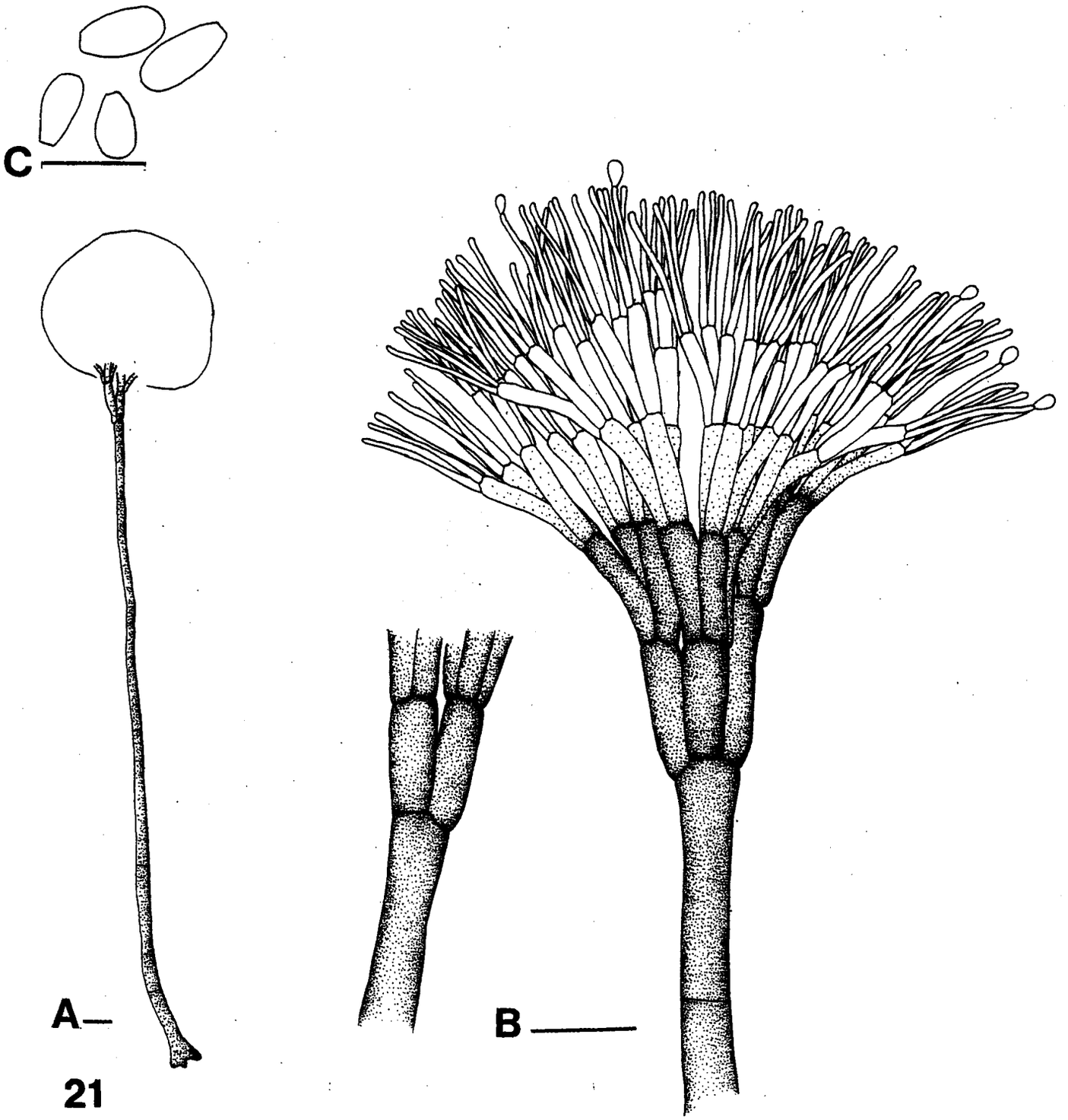


Fig. 21. *Leptographium peucophilum* (CMW 2876). A. Habit sketch of the conidiophore (Bar = 10 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 5 µm).



21

Chapter 8

Jacobs, K., Wingfield, M.J., Pashenova, N.V. & Vetrova, V.P. (1999). A new *Leptographium* species from Russia. Mycological Research (submitted).

A new *Leptographium* species from Russia

Leptographium spp. are well known inhabitants of conifers in the Northern hemisphere, where they cause blue-stain. These fungi are also known to be associated with insects, especially bark beetles (Coleoptera: Scolytidae). Surveys of dying stands of Siberian fir (*Abies sibirica)* have resulted in the consistent isolation of an unknown *Leptographium* species from the galleries of the fir sawyer beetle, *Monochamus urussovi* (Coleoptera: Cerambycidae). Comparison with known *Leptographium* spp. has led us to conclude that the species from Siberian fir has not been previously described and we, therefore, provide the name *Leptographium sibiricum* sp. nov. for it here.

Keywords: *Abies sibirica, Monochamus urussovi,* blue-stain.

Introduction

Species of *Leptographium* can generally be recognized by their mononematous conidiophores with pigmented stipes and complex conidiogenous apparatuses. Single celled, hyaline conidia are produced through percurrent annelidic proliferation of the conidiogenous cells (Kendrick, 1962). The conidiogenous cells are furthermore, characterized by delayed secession of the conidia, giving the conidiogenous cells a sympodial appearance (Van Wyk, Wingfield & Marasas, 1988). The conidia accumulate in slimy masses at the apices of the conidiophores, making these fungi ideal for dispersal by insects.

Most species in *Leptographium* are associated with insects, especially bark beetles (Solheim, 1992a,b, 1995). The relationship between fungi and their insect vectors remains uncertain (Paine, Raffa & Harrington, 1990; Hobson, Parmeter & Wood, 1991; Lévieux *et al.,* 1994; Raffa, 1995; Wingfield, Harrington & Solheim, 1995). In some cases, it appears as if these insects serve only as vectors of the fungi, which are essentially saprophytes (Harrington, 1988, 1993). Some evidence suggests that they play a role in tree death (Wingfield, 1986) and in some cases they provide nutrition for the insects (Six & Paine, 1996). In association with insects, *Leptographium* spp. are known for their ability to cause blue-stain of lumber (Solheim & Långström, 1991; Solheim, 1992a,b, 1995). Furthermore, the three varieties of *L. wageneri* cause the serious, black stain root disease of conifers in the North Western United States (Cobb, 1988; Harrington & Cobb, 1984, Harrington, 1993).

Leptographium spp. are generally known to inhabit conifers (Lagerberg *et al.* 1927; Kendrick 1962; Harrington 1988), although some exceptions occur (Davidson 1942; 1958; 1971; 1976; Jooste 1978; Weber *et al.* 1996). In the Northern hemisphere, several new species have recently been described from conifers (Van der Westhuizen *et al.,* 1995; Jacobs, Wingfield & Bergdahl, 1997; Jacobs *et al.,* 1998). In all cases, the species were found to be restricted to their

relatively specific niches. Surveys between 1988 and 1998 of dying *Abies sibirica* Ledeb. in Siberia have led to the consistent isolation of a *Leptographium* sp. (Vetrova *et al.*, 1992; Pashenova *et al.*, 1994). This fungus was found to occur in the galleries of the fir sawyer beetle (*Monochamus urussovi* Fisch) (Coleoptera: Cerambycidae). The aim of this study was to compare isolates of this *Leptographium* sp. from Siberia with known species of *Leptographium* and to establish its identity.

Materials and Methods

A survey of dying *Abies sibirica* trees in Krasnoyarsk Territory (Central Siberia, Russia, between 53 and 60 of north latitude and 90 and 94 of east longitude) resulted in the consistent isolation of an unknown *Leptographium* sp. from the galleries of *M. urussovi.* Frequency of the *Leptographium* sp. in *M. urussovi* galleries varied from 70-100% (Pashenova et al., 1995; 1998). Conidiophores of the fungus were found in all parts of *M. urussovi* galleries in trunks of Siberian fir. The ability of the *Leptographium* sp. to develop in phloem and sapwood was confirmed by laboratory and field experiments (Pashenova *et al.*, 1994).

Spore masses were transferred from the apices of conidiophores to 2 % malt extract (MEA) (20g Biolab malt extract, 20g Biolab agar and 1000 ml distilled water) plates amended with 0.5 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 were determined with the aid of colour charts (Rayner 1970).

The optimal temperature for growth of representative isolates (CMW 4484, CMW 4481) was determined by inoculating eight MEA plates for each temperature with

6.0 mm diameter agar disks taken from the actively growing margins of a fresh isolates. The plates were incubated at temperatures ranging from 5 to 35 °C at 5 °C intervals. Colony diameters were measured on the fourth and the eight day after commencing the experiment, and the colony diameters computed as an average of eight readings.

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 Jeol JSM 840 Scanning Electron microscope.

Cycloheximide tolerance of isolates (CMW 4484, CMW 4481) was determined by growing them on 2 % MEA amended with 0.5g/l cycloheximide. Dishes were incubated in the dark at 25 °C for eight days and two colony diameters were measured. Five replicate plates were used and the growth rate (mm/day) was determined based on the average of ten diameter readings.

Results

The *Leptographium* sp. from *A. sibirica* is characterized by short, light olivaceous conidiophores with up to three series of branches. It has an optimum growth temperature of 25 °C and can tolerate high concentrations of cycloheximide in culture. It is furthermore characterized by small oblong to obovoid conidia. Comparison with known species of *Leptographium* revealed that this species is new and it is, therefore, described as follows:

PLEASE NOTE THAT THE FOLLOWING SPECIES DESCRIPTION SHOULD NOT BE CITED AND ARE PRINTED HERE IN PRELIMINARY FORM. THESE WILL BE FORMALLY PRINTED ELSEWHERE.

***Leptographium sibiricum* Jacobs & Wingfield sp. nov**

Latin

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 31 mm in diameter in 7 days. No growth below 10 °C or above 35 °C. Able to withstand high concentrations of cycloheximide with a no reduction in growth on 0.5 g/l cycloheximide after days 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 (-7.0) µm.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (109-) 165 (-238) µm in length, rhizoid-like structures absent (Fig. 1, 7a). Stipe light olivaceous, smooth, cylindrical, simple, 2-7 septate, (68-) 128 (-200) µm long (from first basal septum to below primary branches), 4.5 – 5.5 µm wide below primary branches, apical cell not swollen, (3.0-) 5.5 (-8.0) µm wide at base, basal cell not swollen. Conidiogenous apparatus (26-) 40 (-56) long, excluding the conidial mass, with 2 to 3 series of cylindrical branches; 2-3 primary branches, light olivaceous, smooth, cylindrical, aseptate, (8.0-) 14 (-25) µm long and (2.0-) 4.0 (5.0) µm wide, secondary branches hyaline, light olivaceous aseptate, (8.0-) 11 (-17) µm long, (2.0-) 2.5 (-3.0) µm wide, tertiary branches hyaline, aseptate, (5.0-) 9.0 (-12) µm long, (1.0–) 2.0 (-3.0) µm wide (Fig. 2, 7b). Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (6.0-) 13 (-20) µm long and (1.0-) 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, Kirk and Sutton, 1982; 1983; Van Wyk *et al.,* 1988) (Fig. 3, 4). Conidia oblong, (2.0-) 4.0 (-6.0) x (1.0–) 2.0 (-3.0) µm (Fig. 5, 6, 7c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Holotype: CMW 4484 (=DEG 27/94), isolated from a larval gallery of *M. urussovi* in phloem of *Abies sibirica*, Yartzevo, Krasnoyarsk Territory, Russia (about 60 of north latitude and 90 of east longitude); collected: V.P. Vetrova, Aug.,1993

Additional specimens: CMW 4479 (=DEG 10/96) and CMW 4481 (=DEG 30/96), isolated from egg chambers of *M. urussovi* in the phloem of *A. sibirica* damaged by the Siberian moth, *Dendrolimus superans sibiricus* Tschetv., Taseevo, Krasnoyarsk Territory, Russia (about 57 of north latitude and 94 of east longitude); collected: V.P. Vetrova, July.,1996. CMW4487 (=DEG 06/96), isolated from pupal chambers of *M. urussovi* in sapwood damaged by the Siberian moth, *D. s. sibiricus*, Taseevo, Krasnoyarsk Territory, Russia (about 57 of north latitude and 94 of east longitude); collected: V.P. Vetrova, July,1996.

Discussion

Leptographium sibiricum has short delicate conidiophores similar to those found in isolates of *L. brachiatum, L. elegans, L. antibioticum* and the *Leptographium* anamorphs of *Ophiostoma grandifoliae* and *O. leptographioides* (Davidson, 1942; Kendrick, 1962; Davidson, 1976; Wingfield, Crous & Tzean, 1994). It can, however, be distinguished from these species based on various morphological characters. *Leptographium sibiricum* and *L. antibioticum* are both characterized by short conidiophores, although those of *L. antibioticum* can be slightly longer (Table 1). Furthermore, both species have oblong to obovoid conidia of equal length. These species can be distinguished from each other based on the

number of primary branches of the conidiophores. *Leptographium sibiricum* has two or three branches, whereas *L. antibioticum* can have up to five primary branches. These species can also be distinguished ecologically. *Leptographium antibioticum* has been isolated from pine and spruce in North America and is not known to be associated with any insects (Kendrick, 1962; Mielke, 1979; Harrington, 1988). In contrast, *L. sibiricum* appears to be consistently and specifically associated with the siberian fir sawyer beetle on fir in Siberia.

Leptographium sibiricum is morphologically similar to *L. brachiatum.* These two species have conidiophores of similar length. They also have conidia of similar shape and size (Table 1). These species can be distinguished based on the presence of rhizoids in *L. brachiatum* and the absence of these structures in *L. sibiricum.* The lateral branches on the conidiophores, which is one of the most obvious characters of *L. brachiatum,* are absent in *L. sibiricum.* As in the case of *L. antibioticum, L. brachiatum* originates from spruce in North America and is not associated with insects (Kendrick, 1962), while *L. sibiricum* originates from fir and is associated with insects.

Leptographium sibiricum and *L. elegans* are morphologically similar and cannot be distinguished based on conidiphore length, conidium shape and size or the number of primary conidiophore branches. Both species are characterized by the absence of rhizoids. However, these species can be distinguished based on the presence of a *Sporothrix* synanamorph in *L. elegans* and the absence of this state in *L. sibiricum.* Furthermore, these species also differ in host specificity and insect association. *Leptographium elegans* occurs on *Chamaecyparis formosensis* wood and has not been associated with insect activity (Wingfield *et al.,* 1994).

Leptographium sibiricum, *Ophiostoma grandifoliae* and *O. leptographioides* can not be distinguished based on conidiophore length or conidial shape (Davidson, 1942; 1976). However, the conidia of *O. leptographioides* are almost twice as

long [(4.0-) 6.0 (-12) µm] as those of *O. grandifoliae* [(2.5-) 3.5 (-4.0) µm] and *L. sibiricum* [(2.0-) 4.0 (-6.0) µm]. *Ophiostoma leptographioides* and *O. grandifoliae* are characterized by rhizoids at the bases of the conidiophores, in contrast to *L. sibiricum* where these structures are absent. Both *O. grandifoliae* and *O. leptographioides* have been isolated from non-coniferous hosts (Davidson, 1942, 1976), while *L. sibiricum* that is known to occur on a conifer. Also, *Leptographium sibiricum* is consistently associated with an insect, while *O. grandifoliae* and *O. leptographioides* have no known insect associates (Davidson, 1942; 1976).

The fir sawyer beetle (*M. urussovi*) appears to be the main vector of *L. sibiricum* in Central Siberia. This beetle is one of the most destructive xylophages in Europe and Asia. Its distribution extends from Finland and Poland at the west to the Russian shore of the Pacific Ocean, excluding Chukotka and Kamchatka, at the east. The southern boundary of the area corresponds with a zone of conifer forests in the European part of Russia and runs to the northern regions of Kazakhstan, Mongolia, China and Korea in Asia (Isaev et al., 1988). Krasnoyarsk Territory, where our collections were made, is at the center of the *M. urussovi* distribution. The fir sawyer beetle inhabits mainly dark coniferous forests (taiga) and although it can infest many conifers belonging to the Pinaceae, Siberian fir (*A. sibiricum*) is the main host plant of the beetle in Siberia (Isaev et al., 1988). The role of *L. sibiricum* in the life cycle of the beetle is not known, although it does contribute to blue stain.

The fir sawyer beetle breeds in the trunks of fir trees. Female beetles lay eggs in the phloem of trunk, and the larvae bore galleries in the phloem, sapwood and heartwood. Pupal chambers are in the sapwood near to surface of trunk. Upon leaving the pupal chamber, juvenile (imago stage) beetles undergo maturation feeding in the crowns of trees. While feeding, the beetles cause injury to the branches. Therefore, additional feeding on the crowns results in desiccation of branches and weakened trees. The weakened trees then become susceptible to

stem colonization by the beetles. It has been suggested that fungi, carried by *M. urussovi,* have a role in the desiccation of branches (Isaev et al., 1988).

Despite of the consistent association between *L. sibiricum* and the fir sawyer beetle, the fungus was not found in fir branches injured by juvenile beetles when this material was collected in the forests. It appears that *L. sibiricum* is inoculated into the phloem of Siberian fir during oviposition. This results in the development of lesions between 40-60 mm (2-3 times greater than the control) after wound inoculations (Vetrova et al., 1992, 1999; Pashenova et al., 1994). Very little is known about the biology of *L. sibiricum.* The fungus appears to be inoculated into stressed trees during oviposition. Phoretic mites or some other secondary vectors might transmit *L. sibiricum* to trees. Such an association has been suggested for *Ophiostoma* spp. found in the galleries of *Monochamus* spp. in North America (Wingfield & Blanchette, 1983). Additional studies on the pathogenicity and insect associates of *L. sibiricum* are planned for the future.

Literature cited

Cobb, F.W. (jr.) (1988). *Leptographium wageneri,* cause of black-stain root disease: a review of its discovery , occurrence and biology with emphasis on pinyon and ponderosa pine. In: *Leptographium* root diseases on conifers. American Phytopathological Society, St Paul, Minnesota, pp. 41-62.

Davidson, R.W. (1942). Some additional species of *Ceratostomella* in the United States. *Mycologia* **34**, 650-662.

Davidson, R.W. (1958). Additional species of Ophiostomataceae from Colorado. *Mycologia* **50**, 661-670.

Davidson, R.W. (1971). New species of *Ceratocystis*. *Mycologia* **63**, 5-15.

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

Harrington, T. C. (1988). *Leptographium* species, their distributions, hosts and insect vectors. In: *Leptographium* root diseases on conifers. American Phytopathological Society, St. Paul, Minnesota, pp. 1-39.

Harrington, T.C. (1993), Diseases of conifers caused by species of *Ophiostoma* and *Leptographium* In: *Ceratocystis* and *Ophiostoma:* Taxonomy, Ecology and Pathogenicity (eds. M.J. Wingfield, K.A. Seifert and J.F. Webber), American Phytopathological Society, St. Paul, Minnesota, pp. 161-172.

Harrington, T.C. and Cobb, F.W. (jr.) (1984). Host specialization of three morphological variants of *Verticicladiella wageneri. Phytopathology* **74**, 286-290.

Hobson, K.R., Parmeter, J.R. and Wood, D.L. (1991). Bluestain fungi and the xylem occlusion enigma. Proceedings: North American Forest Insect Work Conference. (ed: D.C. Allen & L.P. Abrahamson), pp156-157.

Isaev A.S., Rozhkov A.S., Kiselev V.V.(1988) Fir sawyer beetle Monochamus urussovi (Fisch.)-Novosibirsk:"Nauka". -270p. (in Russian)

Jacobs, K; Wingfield, M.J. and Bergdahl, D. (1997). A new species of *Ophiostoma* from North America, similar to *Ophiostoma penicillatum. Canadian Journal of Botany* **75,** 1315-1322.

Jacobs, K., Wingfield, M.J, Wingfield, B.D. and Yamaoka, Y. (1998). Comparison of *Ophiostoma huntii* and *O. europhioides* and description of *O. aenigmaticum* sp. nov. *Mycological Research* **102,** 289-294.

Jooste, W.J. (1978). *Leptographium reconditum* sp. nov. and observations on conidiogenesis in *Verticladiella. Transactions of the British Mycological Society* **70**, 152-155.

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

Lagerberg, T., Lundberg, G and Melin, E. (1927). Biological and practical researches into blueing in pine and spruce. *Svenska Skogsvårdsföreningens Tidskrift* **25**, 145-272, 561-691.

Lévieux, J., Piou, D., Cassier, P., André, M. and Guillaumin, D. (1994). Association of phytopathogenic fungi for the scots pine (*Pinus sylvestris* L.) with the European pine weevil *Hylobius abietis* (L.) (Col. Curculionidae). *The Canadian Entomologist* **126,** 929-936.

Mielke, M.E. (1979). *Verticicladiella* species and associated root stain and decay fungi isolated from symptomatic Northern Rocky Mountain conifers. M.Sc. thesis, University of Idaho, Moscow.

Minter, D.W., Kirk, P.M. and Sutton, B.C. (1982). Holoblastic phialides. *Transactions British Mycological Society* **79**, 75-93.

Minter, D.W., Kirk, P.M. and Sutton, B.C. (1983). Thallic phialides. *Transactions British Mycological Society* **80**, 39-66.

Paine, T.D., Raffa, K.F. & Harrington, T.C. (1990). Interactions among Scolytid bark beetles, their associated fungi, and live host conifers. *Annual Review of Entomology* **42,** 179-206.

Pashenova N.V., Vidryakova G.A., Vetrova V.P. (1994). Phytopathogenic micromycetes associated with black fir sawyer.Lesovedenie.N 3. P. 39-47 (in Russian)

Pashenova N.V., Vetrova V.P., Matrenina R.M., Aphanasova E.N. (1995). The blue-stain fungi associated with aggressive xylophagous insects on conifers in Middle Siberia (Russia) / "Bark Beetles, Blue-stain Fungi, and Conifer Defence

System". Proceedings IUFRO S7.01 Symp., 31 July - 2 Aug., 1995, As (Norway) Aktuelt fra scogforsk. N 6. P.40.

Pashenova N.V., Vishnyakova Z.V., Vetrova V.P. (1998). Structural changes of bark and wood mycobiota in conifers after damage by Siberian moth and xylophagous pests. Lesovedenie. N 4. P. 11-19 (in Russian)

Raffa, K.F. (1995). Bark beetles, fungi, trees and humans: Four perspectives, four agendas. Proceedings from a symposium held at the Norwegian Forest Research Institute, Ås, Norway (ed. E. Christiansen). pp. 7-9.

Rayner, R.W. (1970). A Mycological color chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey and British Mycological Society.

Six, D.L. and Paine, T.D. (1996). *Leptographium pyrinum* is a mycangial fungus of *Dendroctonus adjunctus*. *Mycologia* **88**, 739-744.

Solheim, H. (1992a). Fungal succession in sapwood of Norway spruce infested by the bark beetle *Ips typographus*. *European Journal of Forest Pathology* **22**, 136-148.

Solheim, H. (1992b). The early stages of fungal invasion in Norway spruce infested by the bark beetle *Ips typographus*. *Canadian Journal of Botany* **70**, 1-5.

Solheim, H. (1995). Blue-stain fungi associated with the spruce beetles *Dendroctonus rufipennis*. Proceedings from a symposium held at the Norwegian Forest Research Institute, Ås, Norway (ed. E. Christiansen). pp. 43.

Solheim, H. and Långström, B. (1991). Blue-stain fungi associated with *Tomicus piniperda* in Sweden and preliminary observations on their pathogenicity. *Ann. Sci. For.* **48**, 149-156.

Van der Westhuizen, K., Wingfield, M.J., Yamaoka, Y., Kemp, G.H.J. and Crous, P.W. (1995). A new species of *Ophiostoma* with a *Leptographium* anamorph from Larch in Japan. *Mycological Research* **99**, 1334-1338.

Van Wyk, P., Wingfield, M.J. and 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.

Vetrova V.P., Pashenova N.V., Grodnitsky D.L. (1992). Response of Siberian fir to inoculation with fungi-simbionts of black fir sawyer. Lesovedenie. N 3.P. 24-32 (in Russian)

Vetrova V.P., Stasova V.V., and Pashenova N.V. (1999). Effect of defoliation on resistance response of *Abies sibirica* Ledeb. to inoculation with blue stain-fungi. /"Physiology and Genetics of Tree - Phytophage Interactions". Proceedings IUFRO S7.01 Symp., 31 Aug. - 5 Sept., 1997. Gujan(France) - Ed. INRA,Paris.- P.287-297

Weber, G., Spaaij, F. and Wingfield, M.J. (1996). *Leptographium costaricense* sp. nov., a new species from roots of *Talauma sambuensis*. *Mycological Research* **100**, 732-736.

Wingfield, M.J. (1986). Pathogenicity of *Leptographium procerum* and *L. terebrantis* on *Pinus strobus* seedlings and established trees. *European Journal of Forest Pathology* **16,** 299-308.

Wingfield, M.J. and Blanchette, R.A. (1983). The pine-wood nematode, *Bursaphelenchus xylophilus,* in Minnesota and Wisconsin: insect associates and transmission studies. *Canadian Journal of Forest Research* **13,** 1068-1976.

Wingfield, M. J., Crous, P.W. and Tzean, S.S. (1994). *Leptographium elegans*: a new species from Japan. *Mycological Research* **98**, 781-785.

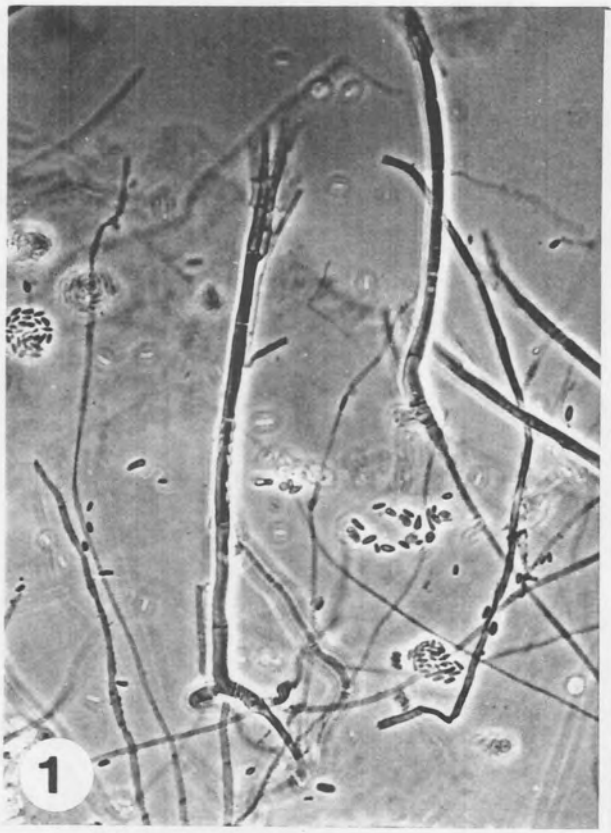
Wingfield, M.J., Harrington, T.C. and Solheim, H. (1995). Do conifer bark beetles require fungi to kill trees ? In: Bark Beetles, Blue-stain Fungi, and Conifer Defence System". Proceedings IUFRO S7.01 Symp., 31 July - 2 Aug., 1995, As (Norway) Aktuelt fra scogforsk. (ed. E. Christiansen). pp. 6

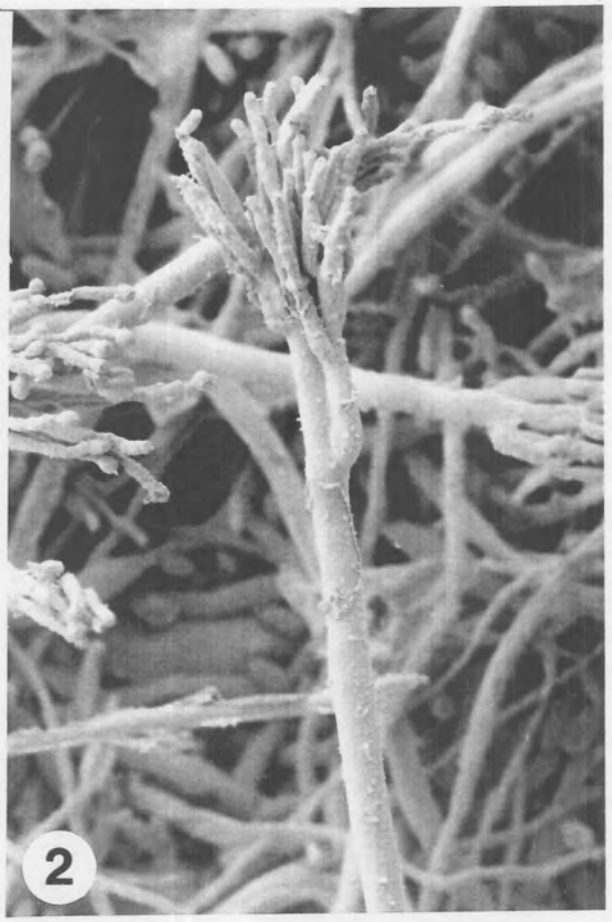
Table 1. Characteristics of *L. sibiricum* compared with those of morphologically similar species.

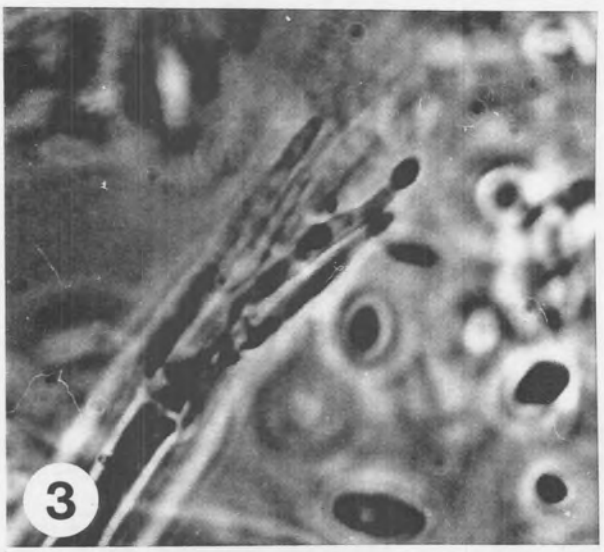
	L. sibiricum	***L. antibioticum***	***L. brachiatum***	***L. elegans***	***O. grandifoliae***	***O. leptographioides***
Substrate	*Abies sibirica*	*Pinus contorta, P. monticola, Abies lasiocarpa, Thuja plicata*[1]	*Psuedostuga menziesii, Picea mariana*[2]	*Chamaecyparis formosensis*[3]	*Fagus grandifolae*[4]	*Quercus* sp. [5]
Insect association	*Monochamus urrussovi*	not known	not known	not known	not known	not known
Conidiophore length	(109-) 165 (-238) µm	(110-) 223 (-407) µm	(73-) 116 (-186) µm	(102-) 234 (-432) µm	(80-) 179 (-397) µm	(77-) 140 (-237) µm
Conidium shape	oblong to obovoid	oblong to obovoid	oblong to obovoid	oblong	obovoid	oblong to obovoid
Conidium size	(2.0-) 4.0 (-6.0) µm	(2.5-) 4.0 (-5.0) µm	(3.0-) 4.0 (-5.5) µm	(3.0-) 4.0 (-5.0) µm	(2.5-) 3.5 (-4.0) µm	(4.0-) 6.0 (-12.0) µm
Teleomorph	absent	absent	absent	absent	*Ophiostoma*	*Ophiostoma*
Rhizoids	absent	present	present	absent	present	present
Primary branches	2-3	2-5	2	2-3	2-3	2-3
Lateral branches	absent	absent	present	absent	absent	absent

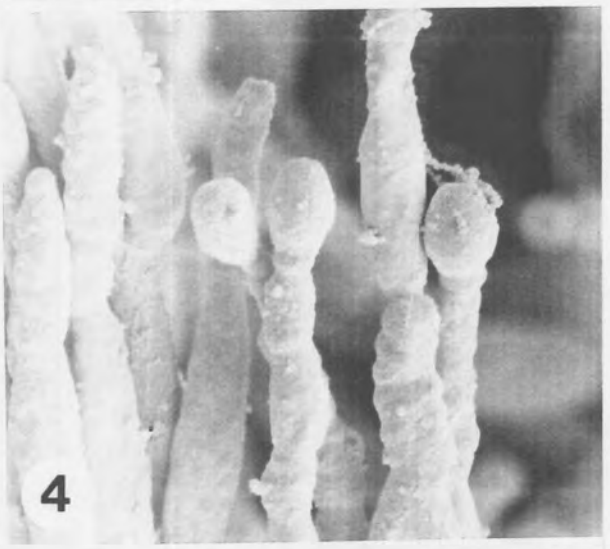
1. Kendrick, 1962; Mielke, 1979; Harrington, 1988. 2. Kendrick, 1962. 3. Wingfield, Crous & Tzean, 1994. 4. Davidson, 1976. 5. Davidson, 1942.

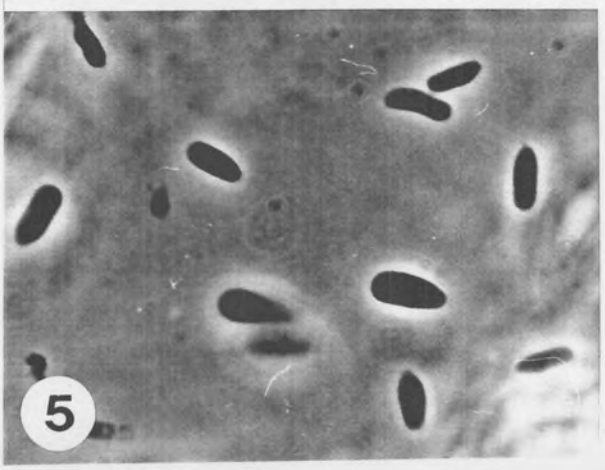
Fig. 1 - 6. *Leptographium sibiricum* (CMW 4484). **Fig. 1.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 10 µm). **Fig. 2.** Complex conidiogenous apparatus (Bar = 10 µm). **Fig. 3.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 µm). **Fig. 4.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 5 µm). **Fig. 5.** Conidia (Bar = 10 µm). **Fig. 6.** Conidia (Bar = 1 µm).











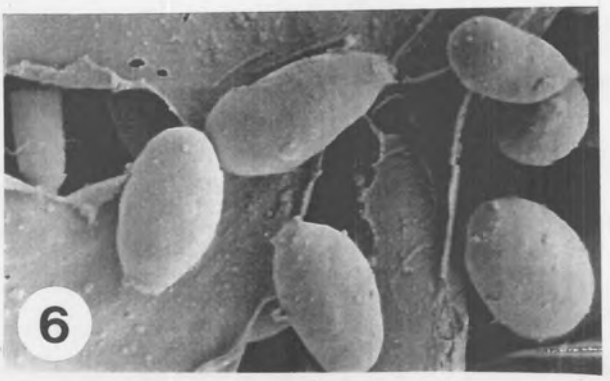
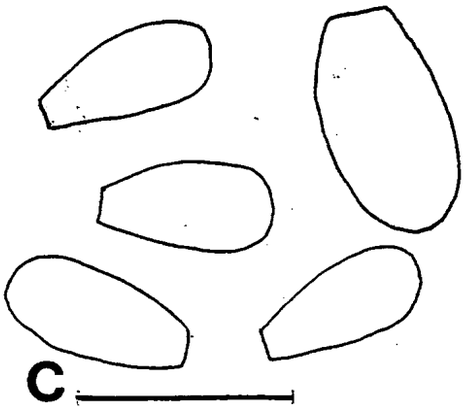
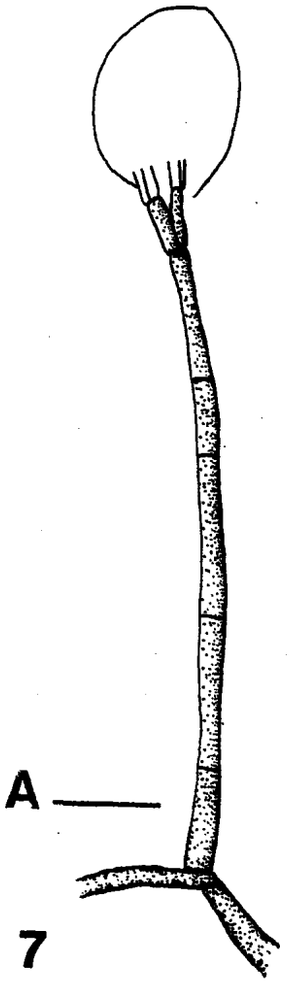
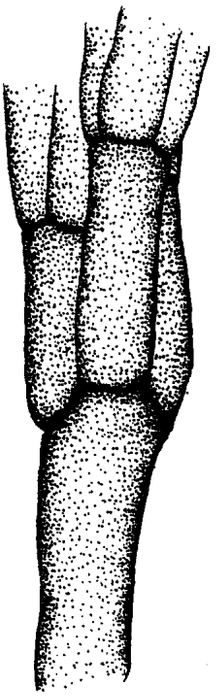
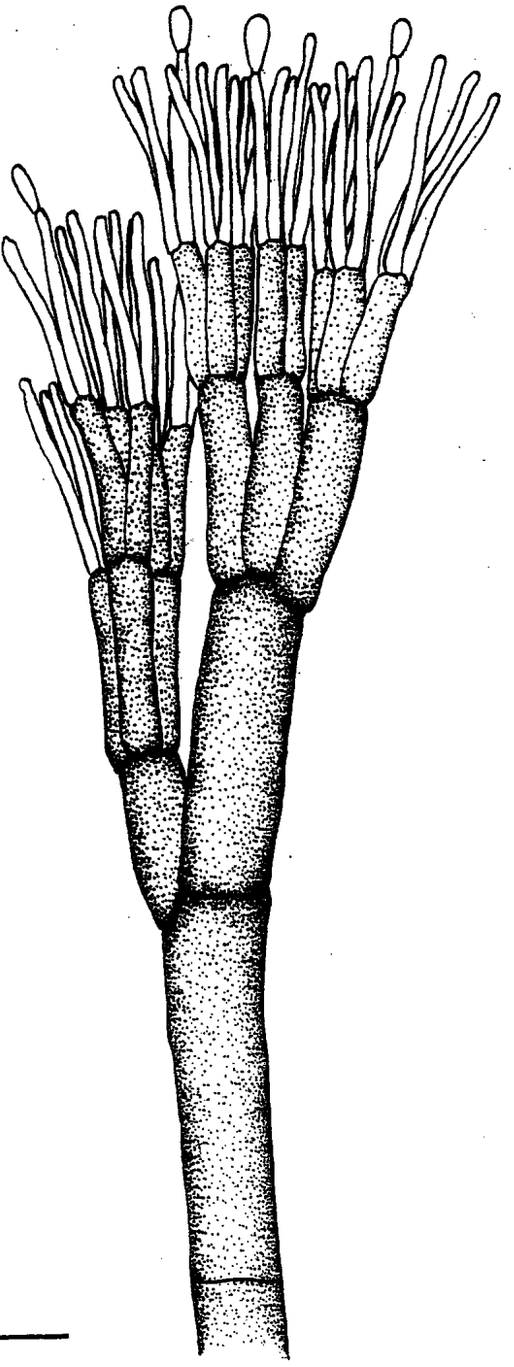


Fig. 7. *Leptographium sibiricum* (CMW 4484). A. Habit sketch of the conidiophore (Bar = 50 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).









7