THE GENUS *LEPTOGRAPHIUM:* A CRITICAL TAXONOMIC ANALYSIS

by

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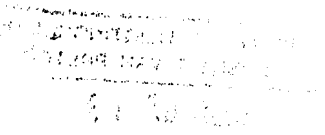
Dedicated to my husband and best friend

Rudi



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PREFACE

The genus *Leptographium* Lagerb. & Melin dates back to early in the twentieth Century, when it was established for species of fungi that cause blue-stain in the sapwood of pine and spruce. The taxonomic history of *Leptographium* has been characterized by considerable confusion, with several species having been transferred between various genera. *Leptographium* includes many species that are morphologically similar. This fact makes the accurate identification of species extremely difficult, even for experienced mycologists. This is an especially relevant impediment where correct identification of potential pathogens is important. As a result of the morphological similarity of species of *Leptographium*, misidentification of important taxa can have serious economic implications. The lack of a comprehensive key to species of *Leptographium* has exacerbated this problem. Correct identification of species in this genus could, in the past, be achieved only through comparison with herbarium type specimens and original descriptions. This was not always possible due to a lack of well-preserved herbarium specimens or cultures. These problems have emphasized the need for a key to both the asexual forms, as well as the sexual states of *Leptographium* spp.

Some species of *Leptographium* are economically important and are well known as agents of blue stain of timber. Other species are regarded as saprophytes. The staining of sapwood, although it does not affect the integrity of the wood, can have major economic implications for commercial wood production. The stain fungi rarely kill trees, but they reduce the value of the timber, which makes this an undesirable trait.

Apart from the species of *Leptographium* that cause blue stain, there are a few species that are pathogens. The best known of these are the three varieties of *Leptographium wageneri,* that are responsible for a serious root disease of conifers in the Western United States. Another suspected pathogen is *L. procerum,* which has

been associated with pine root disease and decline chiefly in Eastern North America. The role of this fungus in the disease complex, is however, still a matter of debate.

This thesis is divided into two parts. The first part is a monograph that deals with all species described in *Leptographium*. The aim of this part of the thesis is to aid in the identification of *Leptographium* spp. The second part of the thesis represents a variety of studies that, over the course of four years, has made it possible to develop the monograph.

Part I

The monograph aims to provide a comprehensive review of all the known species of *Leptographium,* including complete descriptions and illustrations of each species. *Leptographium* anamorphs, known only by their teleomorph states, are provided with names. We believe that this is necessary, since most species have no known teleomorph. Where teleomorph structures are known, they are in most cases represented by poor herbarium material (Shaw & Hubert, 1952; Samuels & Seifert, 1995). This makes comparisons difficult, or even impossible. Most species of *Leptographium* are typically found only in the anamorph state and this is also the state which is typically used in identification.

Part II

The second part of the thesis consists of eight chapters. Chapter one attempts to determine the phylogenetic relationships amongst species of *Leptographium*, as well as to verify placement of the genus at an ordinal level. With the exception of three species, all *Leptographium* spp. are compared based on ribosomal DNA sequence analysis. Considerable difficulty has been experienced in amplifying DNA of this region, and thus this study is restricted to the ITS 2 (internal transcribed spacer region) and part of the large subunit of the rDNA. A sub-set of *Leptographium* spp.

are also used in a comparison with other Ascomycete genera to confirm their ordinal placement. Morphological data generated in the monograph were coded and analyzed in the same manner as the molecular data. Taxonomically important characters were preferentially weighted and analyzed. The resulting dendrograms were then compared with those generated from the molecular analysis, in an attempt to assess the phylogenetic value of morphological characteristics.

The second chapter deals with *L. abietinum* that is found in association with various bark beetles on conifers in North America and is characterized by distinctly curved conidia. A second species, *L. engelmannii,* is also characterized by curved conidia and is indistinguishable from *L. abietinum.* These species are compared morphologically and *L. engelmannii* is formally synonomised with *L. abietinum.* Kendrick (1962) examined several isolates identified as *L. abietinum.* Two of these isolates were, however, isolated from unusual hosts, unlike other isolates of this species. These isolates originated from Borneo and a similar isolate from Vietnam was made available to us. These isolates are carefully compared with authenticated isolates of *L. abietinum.* Consequently, a new species, *L. hughesii* sp. nov., is described in this chapter.

Ophiostoma europhioides is a well-known associate of the bark beetle, *Ips typographus,* and is known to cause blue-stain of conifers. *Ophiostoma piceaperdum* is also an associate of bark beetles in North America and is known to cause blue-stain. These species are indistinguishable based on morphology and previous studies have suggested that they may be the same. In chapter three, these species are compared morphologically. *Ophiostoma europhioides* is synonomised with *O. piceaperdum.* The taxonomic placement of *Ceratocystis pseudoeurophioides* is also considered in this chapter.

Chapter four provides a critical re-evaluation of *Phialocephala phycomyces.* This species is characterized by reddish-brown conidiophores, which is unlike other species of *Phialocephala.* In addition, the phialides are not as deep-seated as those of the type of *Phialocephala,* i.e. *P. dimorphospora.* The taxonomic placement of *P.*

phycomyces is re-evaluated based on light microscopy, scanning and transmission electron microscopy and molecular comparisons. As a result, a new genus, *Kendrickiella* gen. nov. is proposed for *P. phycomyces*.

In chapter five, a new species of *Leptographium, L. eucalyptophilum* sp. nov., is described. This species is unique in that it has been isolated from the Congo in Central Africa. In addition, this species is found on *Eucalyptus,* which is an unusual habitat for members of this genus. This is the first report of a *Leptographium* sp. from *Eucalyptus.* Some comments are also made regarding the pathogenicity and ecology of this species.

Leptographium procerum has been associated with a root decline of white pine (*Pinus strobus*) particularly in the Eastern USA. However, controversy still surrounds the pathogenicity of this fungus, with several studies indicating that it is, at best, a weak pathogen. During the past two decades, a large collection of isolates, tentatively identified as *L. procerum,* has been assembled. Examination of these isolates has revealed four different morphological groups, including *L. procerum s.str.* In chapter six, three new species of *Leptographium,* similar to *L. procerum* are described.

Leptographium spp. have mostly been described from conifers in the Northern hemisphere. In recent years, several new species have been described from other hosts and geographical areas. Chapter seven includes the description of three new *Leptographium* spp. The first of these originates from Indonesia. The other two species are described from red spruce (*Picea rubra*) and balsam fir (*Abies balsamea*) growing on high elevation sites in Eastern North America.

Despite the fact that large parts of Russia and especially Siberia are occupied by coniferous forests, little is known regarding the occurrence of blue-stain fungi in these areas. A survey of conifer diseases in Russia has led to the consistent isolation of an unknown *Leptographium* sp. Chapter eight provides a description of this species.

The overall aim of this thesis is to aid in the identification of known *Leptographium* spp., as well as previously undescribed species. It is also my hope that this dissertation will renew scientific interest in *Leptographium* and that it will lead to the description of many more species, especially from regions where this group of fungi has not been previously considered.

Summary

Leptographium have been known since the early part of the 20th Century and include of many species causing blue stain of timber. Among these species are several species known or believed to be involved in causing diseases of trees. *Leptographium* spp. occur mainly on conifers and many species are recognized as anamorphs of *Ophiostoma.* Similar to *Ophiostoma, Leptographium* spp. are closely associated with insects. Their morphology thus reflects this association, and they thus have upright conidiophores with slimy masses that are produced in beetle galleries.

Leptographium spp. are morphologically very similar to each other and this makes their accurate identification difficult. The first part of this thesis, presents dichotomous, as well as synoptic keys for the identification of these species. These keys are supported by comprehensive descriptions accompanied by both photographs and line drawings.

The second part of this thesis deals with several key taxonomic questions pertaining to *Leptographium*. Chapter one represents a phylogenetic study of the majority of species in *Leptographium.* Morphological characters were coded and analyzed. The results of the molecular and the morphological analyses are compared to determine whether any morphological characters might be used to infer phylogeny. The results indicate that morphology does not infer phylogenetic relatedness.

Chapter two represents a comparison between *Leptographium abietinum* and *L. engelmannii.* These species are morphologically similar, and various authors have suggested that they are synonyms. Based on morphology, *L. engelmanni* was synonomised with *L. abietinum.* Furthermore, examination of various atypical isolates led to the description of the new species, *L. hughesii.*

In chapter three, *Ophiostoma europhioides, O. piceaperdum* and *Ceratocystis pseudoeurophioides* are compared. These species have *Leptographium* anamorphs and are morphologically identical. Both *O. europhioides* and *C. pseudoeurophioides* are synonymised with *O. piceaperdum,* and a name is provided for the anamorph of *O. piceaperdum.*

Chapter four represents a re-evaluation of *Phialocephala phycomyces.* The inconspicuous collarettes, characteristic of this fungus, are unlike the deep-seated collarettes of the type species of *Phialocephala* (*P. dimorphospora*). Scanning and transmission electron microscopy revealed that conidiogenesis in *P. phycomyces* is phialidic, placing this species among other *Phialocephala* spp. However, *P. phycomyces* is able to tolerate high concentrations of cycloheximide, characteristic of *Leptographium* spp. DNA analysis indicates that this species does not belong in either *Phialocephala* or *Leptographium.* A new genus *Kendrickiella* is described to accommodate this species.

In chapter five, a new species of *Leptographium, L. eucalyptophilum,* is described. This species is unique in that it occurs on *Eucalyptus,* which is an unusual host for this species. In addition, this species is one of several described from tropical regions and it is apparently adapted to this habitat.

Chapter six represents a critical re-evaluation of isolates identified as *L. procerum.* Morphological comparison of these isolates revealed that *L. procerum sensu lato*, represents more than one taxon. From this study, three new species of *Leptographium* were described. These are *L. alethinum, L. pityophilum* and *L. euphyes.* These species can easily be distinguished from *L. procerum s. str.* and their incorrect identification is probably as a result of their shared habitat.

In chapter seven, I describe an additional three species of *Leptographium.* Like most other *Leptographium* spp., these were isolated from conifers. The first of these, *L. pineti,* originates from Indonesia. The other two species is found in

high elevation sites in Eastern North America. These are *L. abicolens* and *L. peucophilum.* These species are unique in that they are associated with the conifer swift moth, which is an unusual insect associate of *Leptographium.*

Chapter eighth presents a description of a new species of *Leptographium* from Russia. This species, *L. sibiricum,* is associated with staining and mortality in siberian fir (*Abies sibirica*). The role of the fungus in the disease complex is still unknown, and awaits further study.

This thesis represents a comprehensive review of all known, as well as newly described species. It should greatly facilitate plant pathologists and mycologists in the identification of *Leptographium* spp. This should lead to extensive pathogenicity tests, to determine the economic impact of species in this genus as blue-stain fungi and pathogens. It is my sincere wish that it will renew interest in this group of fungi, and will lead to the description of many more species in this genus.

Opsomming

Leptographium is bekend sedert vroeg in the 20ste eeu en bevat verskeie spesie wat verkleuring van hout veroorsaak. Onder hierdie is ook verskeie spesies wat siektes veroorsaak of moontlik veroorsaak. *Leptographium* spp. kom meestal voor of konifere en verskeie spesies is bekend as anamorf stadia van *Ophiostoma.* Soortgelyk aan *Ophiostoma,* is *Leptographium* spp. nou geassosieer met insekte. Dit word gereflekteer in die morfologie van hierdie fungi, met hul regop konidiofore met spoordruppels wat in the baskewer tonnels gevorm word.

Leptographium spp. is morfologies soortgelyk, wat die identifikasie van spesies moeilik maak. Die eerste deel van die tesis verskaf digotome, sowel as sinoptiese sleutes vir die identifikasie van spesies. Die sleutels word verder ondersteun deur foto's en lynsketse.

Die tweede deel van die tesis behandel verskeie sleutel vrae oor die taksonomie van *Leptographium.* Hoofstuk een verteenwoordig 'n filogenetiese studie van die meerderheid spesies in *Leptographium.* Morfologiese karakters is gekodeer en geanaliseer. Die resultate van die molekulêre en die morfologiese analises is vergelyk om te bepaal of sekere morfologiese karakters filogenie bepaal. Die resultate van hierdie studie bevestig dat geen enkele morfologiese karakter filogenie bepaal nie.

Hoofstuk twee verteenwoordig 'n vergelyking tussen *Leptographium abietinum* en *L. engelmannii.* Hierdie spesies is morfologies soortgelyk en verskeie werkers het voorgestel dat hulle sinonieme is. *Leptographium engelmannii* is sinoniem gemaak met *L. abietinum,* gebaseer op morfologie. Verdere bestudering van atipiese isolate het gelei tot die beskrywing van 'n nuwe spesie, *L. hughesii.*

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THE GENUS LEPTOGRAPHIUM : A CRITICAL TAXONOMIC ANALYSIS

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Part 1

Leptographium species: tree pathogens, insect associates and agents of blue-stain

INTRODUCTION

The genus *Leptographium* is characterized by dark mononematous conidiophores that give rise to a series of branches, terminating in conidiogenous cells in brush-like heads (Kendrick, 1962). The conidiogenous cells produce single-celled, hyaline or faintly pigmented conidia through enteroblastic ontogeny and holoblastic proliferation. Conidia accumulate in slimy masses at the apices of conidiophores, making them ideal for dispersal by insects (Molnar, 1965; Wingfield, 1993a). In association with the insects, some well known species of *Leptographium* have the ability to cause diseases of trees (Grosmann, 1932; Kendrick, 1962; Barras & Perry, 1971a; Harrington & Cobb, 1988). Numerous other species are typically saprophytic or weakly pathogenic and their ecological role remains to be determined (Harrington, 1988).

Leptographium spp. are known to have teleomorphs in *Ophiostoma*. As in the case of *Ophiostoma, Leptographium* spp. are tolerant to high concentrations of the antibiotic cycloheximide and are characterized by the presence of cellulose, rhamnose and chitin in their cell walls (Rosinski & Campana, 1964; Spencer & Gorin, 1971; Weijman & de Hoog, 1975; Marais & Wingfield, 1999a,b). However, in most cases where the teleomorph is known, the anamorph has not been named and only brief reference has been made to its presence. This often leads to taxonomic confusion, as the teleomorph structures are rarely produced in culture, making identification extremely difficult.

Several authors have reviewed the taxonomy of *Leptographium* and its related teleomorph genera, *Ophiostoma* Sydow & P. Sydow, *Ceratocystis* Halst. *sensu lato* and *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. In addition, several keys to selected species of these genera have been published (Hunt, 1956; Kendrick, 1964a; Upadhyay, 1981; Hutchison & Reid, 1988). However, no comprehensive key, to all species of *Leptographium* or *Ophiostoma* with *Leptographium* states exists, which makes this a most difficult group of fungi to treat.

Most descriptions of *Leptographium* spp. are based on living cultures and herbarium material, which may have deteriorated over time. In some taxa holotype material is altogether lacking (Harrington, 1988). The need for a comprehensive monograph reviewing all the known species of *Leptographium,* and a key to species in this genus is long overdue (Harrington & Cobb, 1988; Harrington, 1988; Wingfield, Capretti & Mackenzie, 1988; Wingfield, 1993a). During the past 20 years, M.J. Wingfield has actively collected and preserved *Leptographium* spp. from a wide variety of sources. These collections form the basis of this study. My aim has been to provide a comprehensive key to all known *Leptographium* spp., or *Ophiostoma* spp. with *Leptographium* states. I have also attempted to support this with detailed descriptions, as well as with photographs and line drawings for all species.

TAXONOMY

Anamorph genera similar to or synonymous with *Leptographium*

Scopularia Preuss

The first anamorph genus associated with the taxonomic history of *Leptographium* is *Scopularia,* based on the single species, *S. venusta* Preuss. The vague description of this genus provided by Preuss (1851), was amended and redescribed by Saccardo (1886) and later again by Lindau (1907). The original illustration by Preuss was, however, the source of considerable confusion and the accuracy of his description was placed in doubt by Saccardo (1886). In addition, the type specimen of *S. venusta* was lost, making comparative studies and verification of characters reported for this genus, impossible (Kendrick, 1964b). In a study of fungi causing blue-stain of timber, Lagerberg, Lundberg and Melin (1927) found that some of their isolates resembled the characters reported for *Scopularia.* However, these could not be verified as a result of the lost type specimen. This led to the establishment of *Leptographium* in 1927, based on the single species, *L. lundbergii* (Lagerberg *et al.*, 1927).

Goidanich (1936) argued against the use of *Leptographium* in place of *Scopularia,*

although the former name was used by most authors at that time. He consequently transferred several species described in *Leptographium* to *Scopularia* (Goidanich, 1936). Shaw and Hubert (1952) reviewed the nomenclature of these related genera and found that *Scopularia* Preuss was a later homonym of *Scopularia* Lindley and was, therefore, invalid. *Leptographium* was thus accepted as the valid name for this genus. Rediscovery of the type material of *Scopularia* led Kendrick (1964b) to conclude that *Scopularia* could have been a synonym of *Leptographium.* The state of the material was, however, poor and it was impossible to make any definite conclusions in this regard (Kendrick, 1964b).

Hantzschia Auersw.

Grosmann (1932) regarded the genus *Scopularia* unsuitable for a new species found on spruce in Europe and concluded that the undescribed species would best reside in *Hantzschia* (Kendrick, 1964b). The genus *Hantzschia* was established in 1862 for a single species, *H. phycomyces* Auersw. (Kendrick, 1964b). However, Grosmann (1932) reduced *Hantzschia* to synonymy with *Leptographium* and retained the latter name because the description for *Hantzschia,* as in the case of *Scopularia,* was unclear and insufficient for taxonomic purposes. *Hantzschia phycomyces* subsequently became *L. phycomyces* (Auersw.) Grosmann. Shaw and Hubert (1952) also declared *Hantzschia* invalid based on the existence of an earlier described algal genus, *Hantzschia* Grunow. Hughes (1953) distinguished *Hantzschia* and *Leptographium* based on their different modes of conidium development, phialidic in the case of *Hantzschia* and annelidic in the case of *Leptographium. Leptographium phycomyces,* was later transferred to a new genus, *Phialocephala* W.B. Kendr. based on the phialidic production of conidia (Kendrick, 1964a).

Phialocephala Kendrick

Phialocephala was established for species producing conidia in phialides with periclinal thickening and prominent collarettes (Kendrick, 1961; 1963a). The type

species was described as *P. dimorphospora* W.B. Kendr., based on its well differentiated conidiophores and unmistakable phialides. The generic description was subsequently amended by Crane (1971) to include species that are once or twice branched at the stipe, while Onofri and Zucconi (1984) included species with conidiogenous cells originating directly from the stipe. Several additional species have been added to the genus (Kendrick, 1961; 1963a,b, 1964a; Crane, 1971; Jong & Davis, 1972; Sivasithamparam, 1975; Onofri & Zucconi, 1984, Siegfried, Seifert & Bilmer, 1992), which now deserves revision.

In contrast to *Leptographium* spp., which occur mainly on coniferous hosts, the habitat of *Phialocephala* is usually decaying wood and bark or processed timber and living trees (Kendrick, 1961). No definite relationship with bark beetles has been established and no connection to any teleomorph genus has been found (Harrington, 1988). This is also in contrast to *Leptographium* spp. that have a definite and unique relationship with insects (Solheim, 1986; Harrington, 1988; Perry, 1991; Malloch and Blackwell, 1993; Harrington, 1993; Krokene & Solheim, 1996) and have teleomorphs in *Ophiostoma* (Grosmann, 1932; Harrington, 1987; Wingfield, 1993a; Van der Westhuizen *et al.*, 1995; Jacobs *et al.*, 1997).

Wingfield, Van Wyk and Wingfield (1987) questioned the placement of anamorphs of *Ophiostoma* in *Phialocephala*. After a study of various species of *Phialocephala*, they concluded that the anamorphs of *Ophiostoma* with *Leptographium*-like conidiophores would best be accommodated in *Leptographium* and not *Phialocephala*. Harrington (1988) supported the exclusion of *Phialocephala* from the anamorphs of *Ophiostoma*. These findings were further supported by Mouton, Wingfield and Van Wyk (1992) who found that closely packed annelations at the apices of conidiogenous cells cannot be seen with the light microscope. These annelations lead to the impression that conidia are produced at the same level, without percurrent proliferation, giving the false interpretation of phialides, when viewed with the light microscope. Based on these findings, they suggested that the only proposed *Phialocephala* anamorph in *Ophiostoma*, i.e. *O. francke-grosmanniae*, should reside in *Leptographium*.

Wingfield *et al.* (1987) found that the genus *Phialocephala* could be divided into two groups based on the mode of conidium development. Species displaying

replacement wall building (Minter, Kirk & Sutton, 1983) remained in *Phialocephala*, whereas those with ring wall building (Minter *et al.*, 1983) were accommodated in *Sporendocladiella* G. Arnaud, Nag Raj & W.B. Kendr. Although *Phialocephala* is now more clearly defined than it was in the past, it remains in need of closer investigation (Wingfield, 1993a).

Verticicladiella S. Hughes

The genus *Verticicladiella* was separated from *Leptographium* based on different modes of conidium development. *Verticicladiella*, together with its type *V. abietina* (Peck) S. Hughes, was established by Hughes (1953) to accommodate species that produce conidia sympodially. Kendrick (1962) provided a re-description for this genus and its type, and transferred several species from *Leptographium* to *Verticicladiella.* Several new species were also described in the genus (Kendrick, 1962).

The separation of *Verticicladiella* and *Leptographium* was not universally accepted. Jooste (1978) commented on the conidiogenesis of *V. abietina* in a study undertaken to compare conidiogenesis of certain species in *Verticicladiella* and *Leptographium*. He noted the delayed secession of conidia observed in species of *Verticicladiella,* as well as annelations characteristic of *Leptographium,* and suggested that further studies would be needed to clarify these discrepancies. Wingfield (1985), after a thorough electron microscope study of many species residing in the two genera, reduced *Verticicladiella* to synonymy with *Leptographium.* This synonymy was based on the fact that species in the two genera were indistinguishable under the light microscope. Scanning electron microscopy revealed that species in both *Leptographium* and *Verticicladiella* displayed annelidic as well as sympodial conidiogenesis. Their findings were confirmed by Van Wyk and Wingfield (1987) and Van Wyk, Wingfield and Marasas (1987) who showed that delayed secession of the conidia, developing percurrently, can lead to a false impression of sympodial development when viewed under the light microscope (Fig. 1). This synonymy was also supported by Harrington (1988), in his review of species in *Leptographium.*

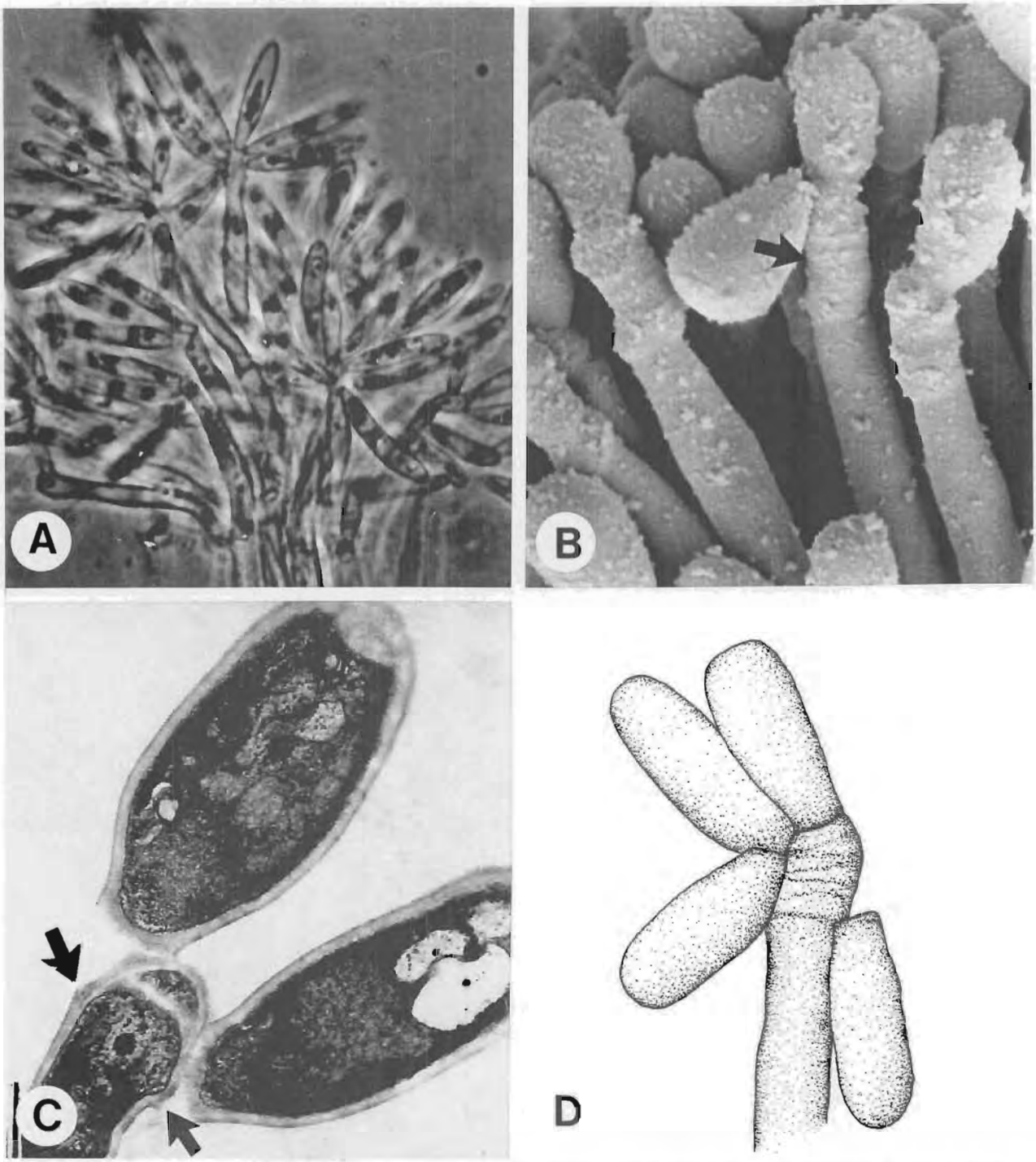

Fig. 1. Conidiogenesis in *Leptographium*. **A.** Light micrograph showing conidiogenous cells and conidia that appear to develop sympodially. **B.** Scanning electron micrograph showing percurrent proliferation of conidiogenous cells. Note the distinct annelations (arrows) and the fact that delayed secession gives a false impression of sympodial conidium development. **C.** Transmission electron micrograph showing annelations (arrows) at the apex of conidiogenous cells. **D.** Schematic representation of conidium development in *Leptographium* spp.

Teleomorphic genera associated with *Leptographium*

Some *Leptographium* spp. have described teleomorphs in *Ophiostoma.* Grosmann (1931, 1932) described the first species of *Leptographium* associated with *Ceratostomella* Saccardo, which was later reduced to synonymy with *Ophiostoma* (Von Arx, 1952). The history of *Ophiostoma* is characterized by several name changes that can be traced back to the early part of the 20th Century. A few years after Grosmann's description of *L. penicillatum,* Goidanich (1936) described the teleomorph genus *Grosmannia* Goid. for all the *Leptographium* species that had been associated with teleomorphs. *Endoconidiophora* had been established for species with *Chalara* - like anamorphs (Samuels, 1993). Von Arx (1952), however, reduced *Grosmannia, Endoconidiophora* Münch and *Ceratostomella* to synonymy with *Ophiostoma,* and transferred all species to that genus. Parker (1957a) described *Europhium* A.K. Parker for one species of *Leptographium, L. trinacriforme,* with a cleistothecial-like teleomorph that lacked the typical long necks of *Ophiostoma* (Parker, 1957a). Robinson-Jeffrey and Davidson (1968) described a further three species in this genus. All of these species were later transferred to *Ophiostoma* (Harrington, 1987).

Ceratocystis is another important genus that has been associated with species of *Leptographium.* There are many similarities between *Ophiostoma* and *Ceratocystis.* Most notable are the long necks of the ascomata and a close association with insects. These similarities have led to considerable debate as to the validity of the genera. This debate has now been resolved and the two genera are widely accepted as being phylogenetically unrelated (Hausner, Reid & Klassen, 1993a, Spatafora & Blackwell, 1994). Thus, *Ceratocystis* can be distinguished from *Ophiostoma* based on its *Chalara* (Corda) Rabenh. anamorphs (Ellis & Halsted, 1890; De Hoog & Scheffer, 1984), intolerance to the antibiotic cycloheximide (Fergus 1956; Harrington, 1981; Marais & Wingfield, 1999b), absence of cellulose, chitin and rhamnose in its cell walls (Smith, Patik & Rosinski, 1967; Spencer & Gorin, 1971; Jewell, 1974; Weijman & de Hoog, 1975; Marais & Wingfield, 1999a) and differences in ascospore development and morphology (Van Wyk & Wingfield, 1990; 1991; Van Wyk, Wingfield & Van Wyk, 1991). In contrast, species of *Ophiostoma* are characterized by anamorphs other than *Chalara* (De Hoog &

Scheffer, 1984). These include *Leptographium, Graphium, Sporothrix* and *Hyalorhinocladiella* (Harrington, 1988; Wingfield, 1993a, Seifert & Okada, 1993; De Hoog, 1993; Mouton, Wingfield & Van Wyk, 1994). *Ophiostoma* spp. are also characterized by a marked resistance to high concentrations of cycloheximide (Fergus, 1956; Hicks, 1973; Harrington, 1988; Marais & Wingfield, 1999b) and the presence of cellulose, chitin and rhamnose in their cell walls (Rosinski & Campana, 1964; Smith, Patik & Rosinski, 1967; Spencer & Gorin, 1971; Jewell, 1974; Weijman & de Hoog, 1975).

The separation of *Ceratocystis* and *Ophiostoma* was debated for many decades. Hunt (1956) considered *Ophiostoma* and *Ceratocystis* to be synonyms and supported the synonymy of *Grosmannia* with *Ophiostoma.* He, however, divided *Ceratocystis* into two groups based on the mode of conidium development of their anamorphs, namely exoconidia (*Leptographium* - like) and endoconidia (*Chalara* - like). This synonymy was supported by Olchowecki and Reid (1974) who placed all species of *Ophiostoma* including those with *Leptographium* anamorphs in *Ceratocystis.* They further divided *Ceratocystis* into four groups based on ascospore shape. Other than being a convenient arrangement of taxa, this situation did not provide an indication of the natural division of species in the genus (Harrington, 1988).

De Hoog (1974) divided *Ceratocystis sensu lato* into *Ophiostoma* and *Ceratocystis sensu stricto.* This separation was based on two distinct anamorph groups (those with exoconidia and those with endoconidia), previously noted by Hunt (1956). Weijman and de Hoog (1975), as well as Samuels and Müller (1978) distinguished between *Ceratocystis* and *Ophiostoma* based on cell wall composition as well as conidium development. In his monograph, Upadhyay (1981) disregarded the separation of *Ceratocystis* and *Ophiostoma* proposed by De Hoog (1974), Weijman and De Hoog, (1975) and Samuels and Müller (1978), and treated all species in these genera as either *Ceratocystis* or *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. Thus, *Leptographium* species were again treated as anamorphs of *Ceratocystis.*

Ceratocystis s.l. was once again split by De Hoog and Scheffer (1984) based on the two different anamorph groups. Species with anamorphs other than *Chalara* were

moved to *Ophiostoma*. Following the same trend a further 11 species with *Leptographium* anamorphs were later transferred to *Ophiostoma* by Harrington (1987). He also suggested that *Leptographium* spp. with a tolerance to cycloheximide implies a strong affinity to *Ophiostoma* (Harrington, 1988).

Ceratocystiopsis was described by Upadhyay and Kendrick (1975) for species with falcate ascospores. Although De Hoog and Scheffer (1984) considered *Ceratocystiopsis* to be a well-defined genus, Wingfield (1988; 1993b) proposed a reconsideration of *Ceratocystiopsis* because this genus is separated from *Ophiostoma* and *Ceratocystis,* based solely on the shape of the ascospores. *Ceratocystiopsis crassivaginata* (H.D. Griffin) H.P. Upadh., was the only species in this genus with a *Leptographium* anamorph and it was consequently transferred to *Ophiostoma* as *O. crassivaginatum* (H.D. Griffin) T.C. Harr. (Harrington, 1987).

Hausner, Reid and Klassen (1993b) compared *Ophiostoma, Ceratocystis* and *Ceratocystiopsis* at the molecular level and concluded that *Ceratocystiopsis* and *Ophiostoma* should be synonomised. Most species previously treated in *Ceratocystiopsis,* were moved to *Ophiostoma*. Currently, and as a result of the above-mentioned studies, all *Leptographium* spp. with known teleomorphs are found in *Ophiostoma*. Studies at the molecular level have provided strong support for the fact that *Ophiostoma* and *Ceratocystis* are distinct and phylogenetically unrelated (Hausner *et al.,* 1993a; Samuels, 1993; Spatafora & Blackwell, 1994; Wingfield *et al.,* 1994; Samuels & Seifert, 1995; Wingfield *et al.,* 1996; Wingfield, Viljoen & Wingfield, 1999).

Ceratocystiopsis is generally treated as a synonym of *Ophiostoma* (Wingfield, 1988; Wingfield, 1993b; Hausner *et al.,* 1993a, b). Two species of *Ceratocystiopsis, C. falcata* and *C. proteae,* were not transferred to *Ophiostoma* by Hausner (1993b). Subsequent studies have treated these species and *C. falcata* now resides in the monotypic genus *Cornuvesica* Viljoen, Wingfield & Jacobs, as *Cornuvesica falcata* Viljoen, Wingfield & Jacobs (Viljoen *et al.,* 1999). *Ceratocystiopsis proteae* resides in *Gondwannamyces* Marais & M.J. Wingf. as *G. proteae* (M.J. Wingf., P.S. van Wyk & Marasas) Marais & M.J. Wingf., together with *G. capensis* (M.J. Wingf. & P.S. van Wyk) Marais & M.J. Wingf. (Marais *et al.,* 1998). It has been suggested that *Ophiostoma* could represent a number of well defined genera, possibly

separated by different ascospore forms, although this has yet to be clearly shown (Wingfield, Viljoen & Wingfield, 1999).

The teleomorph structures of *Ophiostoma* spp. with *Leptographium* states are characterized by small, hyaline ascospores and evanescent asci (Fig. 2). In all cases the ascospores are surrounded by a gelatinous sheath. This is in contrast to certain other *Ophiostoma* spp. that are characterized by ascospores without sheaths (Van Wyk, Wingfield & Van Wyk, 1993). The ascocarps are darkly pigmented with, in most cases, well-developed necks and ostioles. Sticky ascospores accumulate at the apices of the necks, and are well adapted for insect dispersal (Harrington, 1988; Malloch & Blackwell, 1993). Although this similarity in morphology can lead to the impression that *Ophiostoma* and *Ceratocystis* are closely related, this might not be the case at all. These similarities are most probably the result of adaptation to their habitat, which in most cases constitutes the tunnels of insects formed in the inner bark of trees (Lagerberg *et al.*, 1927; Craighead, 1928), and convergent evolution (Wingfield, 1993a).

In a review of *Leptographium* spp., Harrington (1988) listed 20 species of *Ophiostoma* with *Leptographium* anamorphs. Since then several additional species have been described. Many species of *Leptographium* are not associated with a teleomorph or alternatively, the teleomorph has been seen seldom or only once, as in the case of *Ophiostoma wageneri* (Goheen & Cobb) Harrington (Goheen & Cobb, 1978). In such cases, the anamorph might be considered as the holomorph (Wingfield, 1993a). Harrington (1988) suggested that in species of *Ophiostoma* with *Leptographium* anamorphs, a name for the anamorph is unnecessary and that the teleomorph name should preferably be used. This can, however, lead to confusion, as in most cases, the teleomorph is not readily formed in culture. This confusion is compounded where mycologists rely on published names and descriptions for identification. For the purpose of this study, we have chosen to provide names for *Leptographium* states of the small number of *Ophiostoma* spp. where such names have not been provided previously. Although we fully recognized the arguments for not doing so, we believe that this group is exceptional, in that a very small number of species have not been treated in this way. We also believe that this will simplify the task of pathologists who are unlikely to ever see a teleomorph in most of these species.

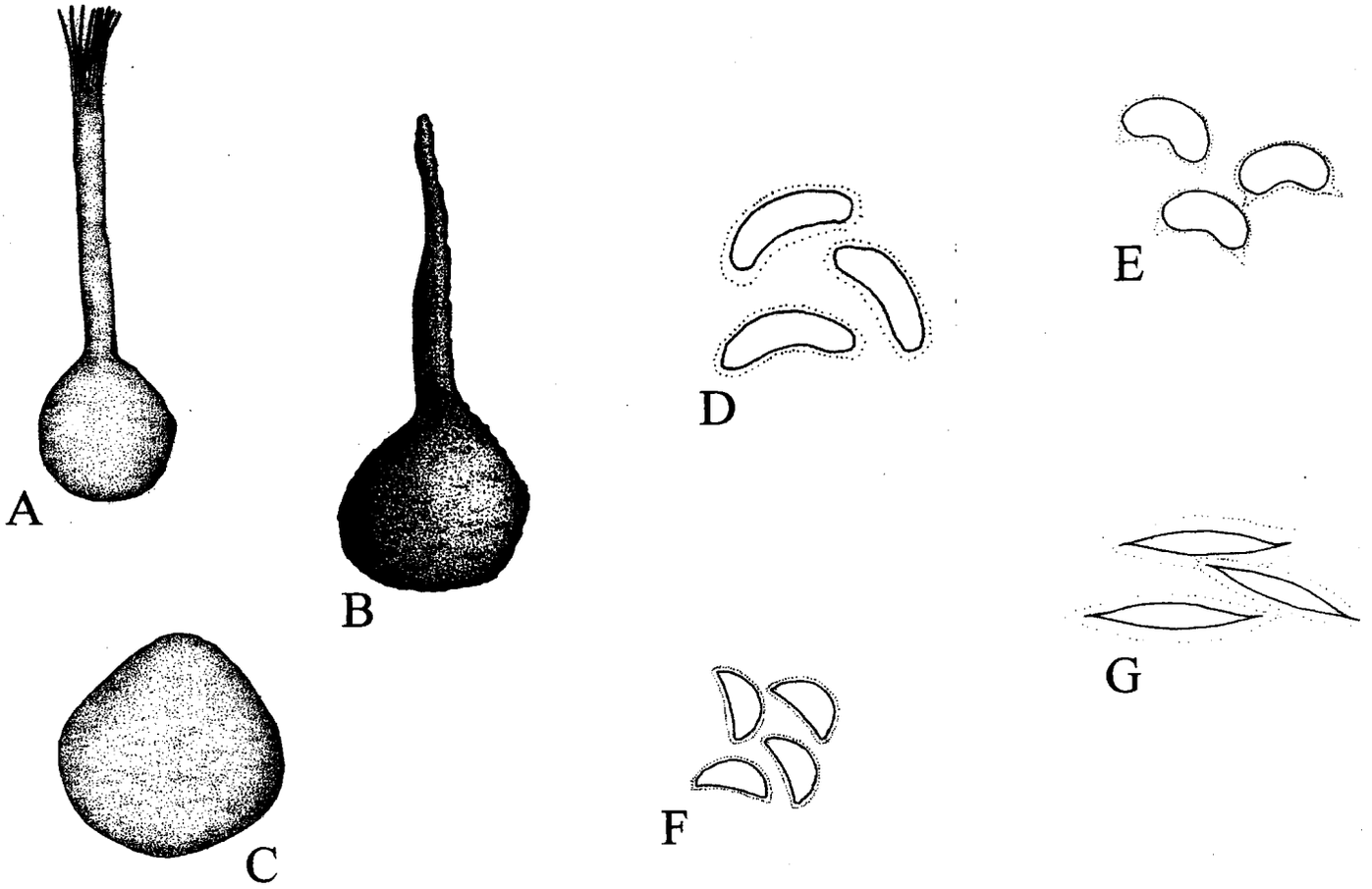

Fig. 2. Teleomorph structures associated with *Leptographium* spp. Perithecia can be with (A,B) or without (C) necks. Ostiolar hyphae can be present (A) or absent (B). Ascospores can be allantoid (D), cucullate (E), orange-section shaped (F) or elongate (G).

DISEASES ASSOCIATED WITH SPECIES OF *LEPTOGRAPHIUM*

Some species in *Leptographium* are associated with serious diseases of trees that cause devastation in forests, resulting in major economic losses (Harrington & Cobb, 1988; Solheim, 1992a,b; Wingfield, Seifert & Webber, 1993). The best known of these are certainly the three varieties of *Leptographium wageneri* that are responsible for black stain root disease (BSRD) of conifers in the North Western United States (Wagener & Mielke, 1961; Cobb, Lawson & Popenuck, 1987; Cobb, 1988; Harrington, 1993). Other species considered to play an important role in disease are *L. procerum*, associated with a root disease of pines, *L. serpens,* associated with pine disease in Italy and South Africa (Wingfield & Marasas, 1980; 1981), *L. terebrantis,* that is known to cause extensive lesions on pines (Wingfield,

1986) and *L. calophylli,* associated with the wilt of the takamaka tree (*Calophyllum inophyllum*) in Mauritius and the Seychelles (Wiehe, 1949; Webber *et al.,* 1999). Most *Leptographium* spp. are, however, best known for their association with blue-stain of sapwood in conifers.

While species of *Leptographium* might have been isolated from diseased trees, their role in causing disease is often unknown (Kulhavy, Chako & Partridge, 1978). The disease complexes in which these fungi are involved, usually include the fungus, the host, which in most cases would be a coniferous tree, and in certain cases insects. Most species of *Leptographium* are, however, non-pathogenic and are probably saprotrophic (Harrington, 1988; Wingfield *et al.,* 1988). Results obtained from wound inoculation studies should also be interpreted with care, as these fungi have extremely complex relationships with insects and the development of lesions need not necessarily imply a primary role in disease (Harrington, 1988; Wingfield *et al.,* 1988). At this stage, only *L. wageneri* and *L. calophylli* are considered to be true primary pathogens. The role of *L. procerum* and *L. serpens* as pathogens is still debated (Wingfield *et al.*, 1988).

Black-stain root disease

Leptographium wageneri is responsible for a disease known as black stain root disease (BSRD). This disease was first recorded in 1939 on *Pinus* spp. in California (Wagener & Mielke, 1961), but was later also described from other conifers (Harrington & Cobb, 1987). Wagener and Mielke (1961) first described the symptoms and factors associated with the disease. Kendrick (1962) provided the name *Verticicladiella wageneri* Kendrick for the causal agent of BSRD. Although several species of *Leptographium* have been isolated from trees showing symptoms of BSRD, Harrington and Cobb (1983) showed conclusively that the disease is caused by the single species, *Leptographium wageneri.* The role of the fungus had probably been overlooked for a considerable time because of the presence of bark beetles in diseased trees and the fact that people attributed tree death to insect infestation (Cobb, 1988). Other *Leptographium* spp. were frequently isolated from trees with BSRD, but these are probably only secondary invaders (Partridge & Bertagnole, 1980).

BSRD is restricted to the western United States (Walters & Walters, 1977; Harrington, 1982; Cobb, 1988). It was found to spread rapidly, and is capable of causing extensive losses in forests (Byler, Cobb & Rowney, 1979; Cobb *et al.*, 1982; Cobb, 1988). Economic impacts are not restricted only to direct losses such as reduced growth and death. Indirect losses also occur through the build-up of populations of secondary fungal pathogens and insects (Smith, 1974). BSRD is also of particular importance since it is capable of killing European conifers, and could be a serious threat to forests of Britain and Europe, if it were to be introduced into that part of the world (Webber & Hansen, 1990).

BSRD occurs on trees of all ages and predisposes the host to further attacks by bark beetles (Helms, Cobb & Whitney, 1971; Morrison & Hunt, 1988). Although BSRD has been grouped with the major root pathogens, it also displays symptoms characteristic of vascular wilt pathogens on hardwoods (Leaphart, 1960; Smith, 1967; Harrington, 1982). These include the fact that it is restricted to the xylem, and the fact that it spreads specifically in the vascular system of trees (Smith, 1967; Goheen & Cobb, 1978; Harrington, 1982; Hessburg & Hansen, 1982; Cobb *et al.*, 1984; Bertagnole, Partridge & LeTourneau, 1987).

The host specificity of strains of *L. wageneri* has been noted by various researchers (Wagener & Mielke, 1961; Smith, 1967; Harrington, 1982; Cobb *et al.*, 1984; Harrington & Cobb, 1984; Cobb, Lawson & Popenuck, 1987). Three varieties of this fungus are currently known and these are referred to as *L. wageneri* var. *wageneri* occurring on pinyon pines (*Pinus monophylla; P. edulis*) (Kendrick, 1962; Harrington, 1993), *L. wageneri* var. *pseudotsuga* occurring on douglas-fir (*Pseudotsuga menziesii*) (Cobb & Platt, 1967; Harrington & Cobb, 1987; Harrington, 1993) and *L. wageneri* var. *ponderosum* occurring on hard pines (*P. ponderosa, P. contorta, P. jeffreyi*) (Harrington & Cobb, 1987; Harrington, 1993). These varieties can be distinguished based on various characters such as morphology (Harrington, 1982), differences in virulence (Otrosina, Cobb & Popenuck, 1987), isozymes (Otrosina, 1986; Otrosina & Cobb, 1987; Zambino & Harrington, 1987; Zambino, Harrington & O'Malley, 1987; Zambino & Harrington, 1989), Random Amplified Polymorphic DNA markers (RAPD's) (Witthuhn *et al.*, 1997) and ribosomal DNA sequences (Jacobs *et al.*, unpublished).

All three varieties of *L. wageneri* are able to infect tree species other than those from which they were isolated, but this characteristic is rare in nature (Cobb, & Platt, 1967; Harrington & Cobb, 1984; Diamandis, Epstein & Cobb, 1987). This can be attributed to several factors, including symptoms that might not be expressed on certain hosts, feeding activities of insects that carry the fungi, and the fact that seedlings used in the pathogenicity tests might not have displayed the resistance expressed in older trees (Harrington & Cobb, 1984). Zambino & Harrington (1989) suggested that the host specialization and designation of three varieties of *L. wageneri* is possibly the result of limited recombination, or the lack thereof, in nature. This conclusion is based on the fact that there is no or very limited sexual recombination in the natural populations of *L. wageneri* (Goheen, 1976; Goheen & Cobb, 1978; Zambino & Harrington, 1989).

Goheen and Cobb (1978) described *Ceratocystis wageneri* as the teleomorph of *L. wageneri.* This state has never been seen again and it is possible that teleomorph structures were not appropriately linked to *L. wageneri.* Zambino and Harrington (1989, 1990) found a low level in gene diversity, suggesting a low level of recombination amongst isolates of the three varieties. Population studies of this species indicate that the three varieties of *L. wageneri* represent homogenous populations with essentially asexual reproduction (Zambino & Harrington, 1990). The presence of a teleomorph in nature, thus, seems unlikely.

Symptoms associated with BSRD include reduced leader and branch growth, chlorosis, reduced needle size, needle retention and resinous lesions on the lower stems (Leaphart, 1960; Hunt & Morrison, 1980; Witcosky, 1981) (Fig. 3). Other symptoms are severe needle chlorosis, needle cast and a pronounced reduction in height growth (Cobb & Platt, 1967; Lawson & Cobb, 1987a). Infected trees appear to form more heartwood than uninfected trees, which reduces water conduction (Lawson & Cobb, 1987b). The pathogen causes severe reduction in photosynthesis and transpiration as a result of water stress and stomatal closure (Helms *et al.*, 1971), which is most probably the result of phytotoxins (Cobb, 1988).

The stain resulting from infection by *L. wageneri* is streaky and occurs in the tracheids (Wagener & Mielke, 1961), extending from the roots upwards in the tree (Cobb, 1988) (Fig. 4). This is the most characteristic symptom of the disease

(Cobb, 1988). The difference between this staining pattern and that of blue-stain, is the result of the hyphae that are located only in the tracheids (Goheen & Cobb, 1978; Harrington, 1982; Hessburg & Hansen, 1982; Cobb *et al.*, 1984; Bertagnole *et al.*, 1987), and not in the parenchyma as is the case of typical blue-stain fungi (Cobb, 1988). This results in the black streaked patterns associated with this disease, in contrast to the wedge-shaped staining patterns associated with blue-staining organisms (Wagener & Mielke, 1961; Cobb, 1988) (Fig. 4).

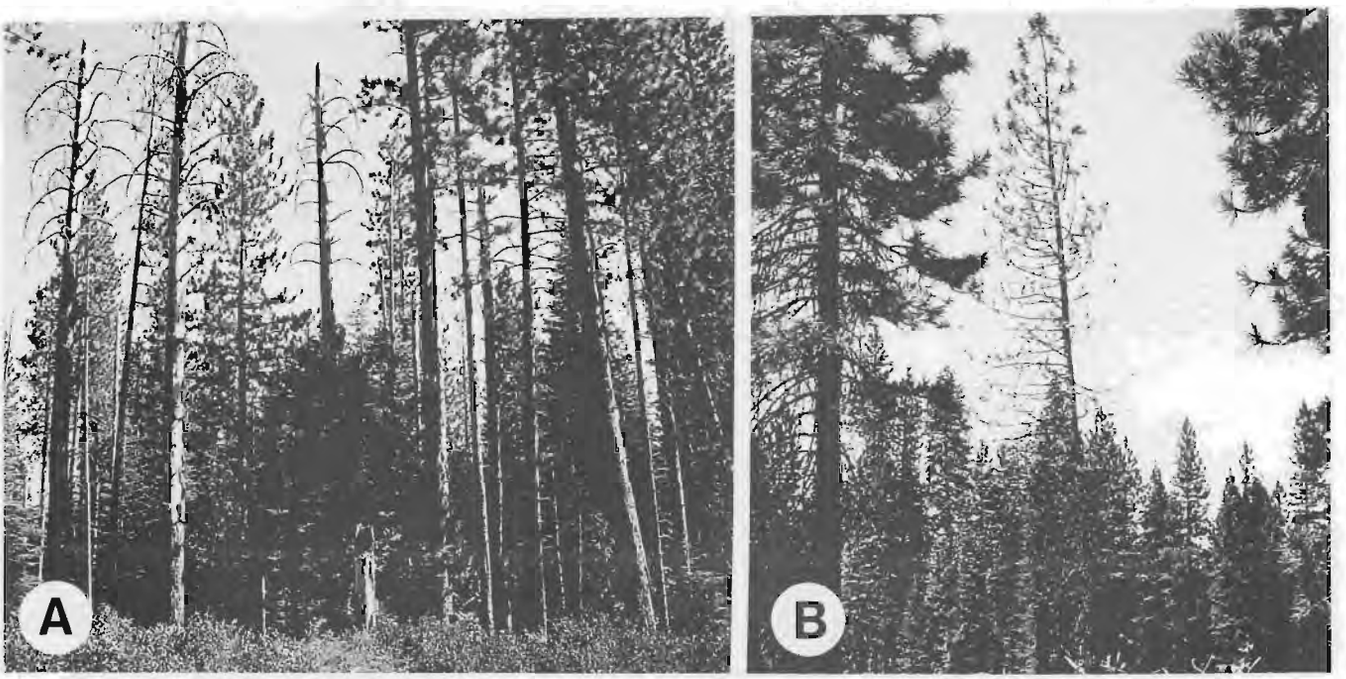

Fig. 3. Symptoms associated with BSRD in *Pinus ponderosa*. **A.** Dying tree in an infection centre. **B.** Dying tree showing distinct crown thinning (Photos taken by Fields W. Cobb).

The invasion of the tracheids by *L. wageneri* leads to a decrease in sap flow, which ultimately results in tree death (Hessburg & Hansen, 1987). Resinosis appears on the outer surface of pine roots, but this is more apparent in douglas-fir than in other conifers. Foliar symptoms can be seen in some cases, but bark beetles usually kill the trees before these symptoms appear (Cobb, 1988). In douglas-fir, the symptoms are generally similar to those found in pine. Growth of trees is reduced for 2-3 years before death, the crown thins and the foliage becomes chlorotic (Hansen *et al*, 1988) (Fig. 3).

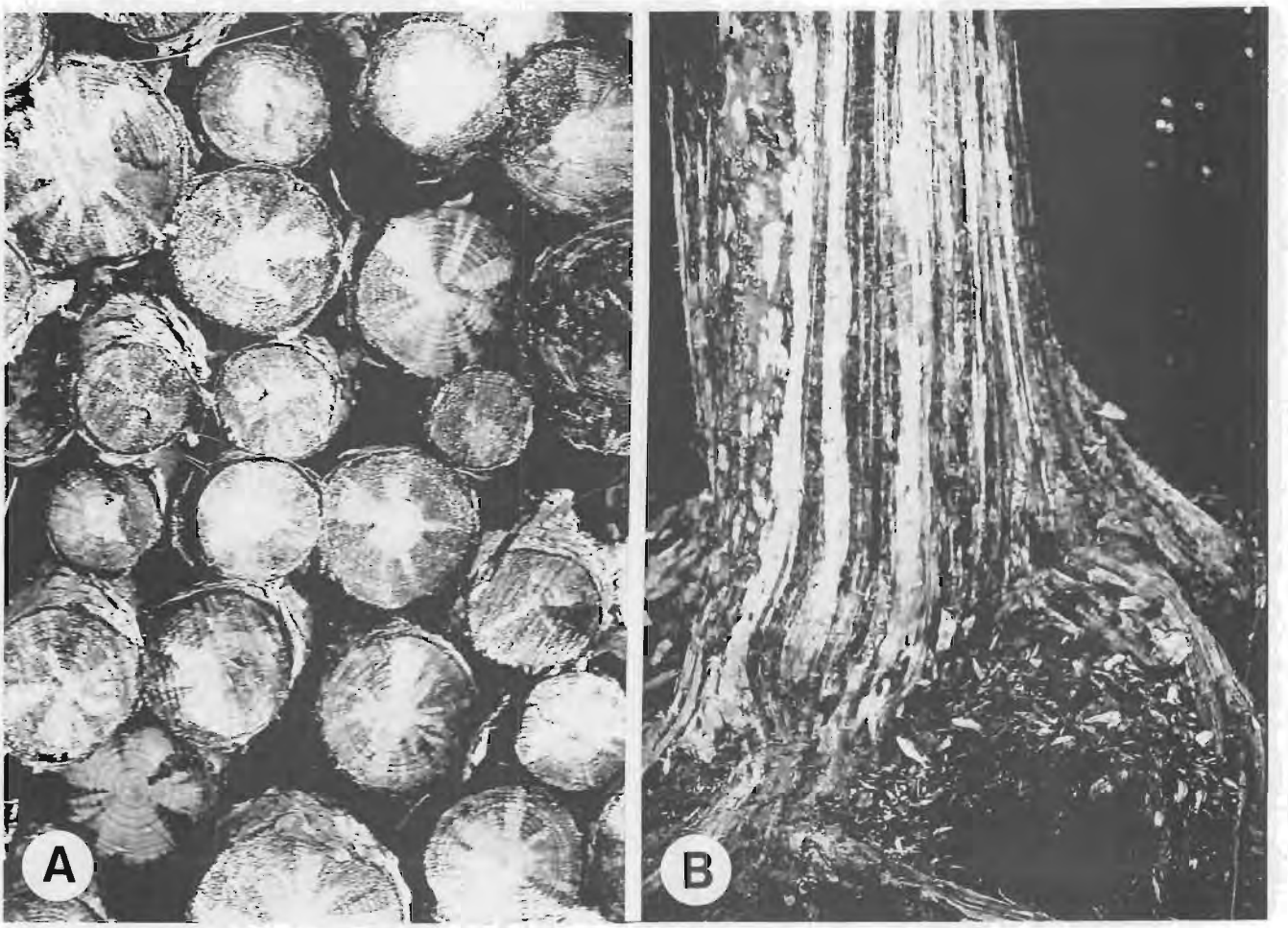

Fig. 4. Patterns of wood colonization associated with *Leptographium* spp. **A.** Pie-shaped lesions associated with most *Leptographium* spp. and other blue stain fungi that colonize both tracheids and ray parenchyma. **B.** Typical staining pattern associated with infection in *L. wageneri*, where the fungus is restricted to tracheids and does not colonize parenchyma (photographs supplied by Fields W. Cobb).

Reports of the mode of infection of *L. wageneri* are conflicting, possibly as a result of different hosts and environmental conditions that are associated with this disease. On the one hand, *L. wageneri* has been found to be able to infect healthy trees in the absence of traumatic wounds (Cobb, 1988). In contrast, the fungus was found to be able to colonize only non-living tracheids and was never found to infect living tissue (Hansen *et al.*, 1988).

Infection by *L. wageneri* occurs through the roots (Cobb & Platt, 1967; Smith,

1967), and because *L. wageneri* is unable to break down or utilize cellulose, spreads through the trees via the pit membranes (Smith, 1969). *Leptographium wageneri* occasionally spreads short distances from tree to tree across root grafts and major contacts. The most common origin of infections is through small rootlets (Wagener & Mielke; 1961; Goheen, 1976; Hansen, 1978; Hessburg & Hansen, 1986a). Although it might increase infection, contact between roots of different trees is not necessary for spread of the disease (Hessburg & Hansen, 1986a). The mechanism of spread between roots is unknown (Hansen *et al.*, 1988), but long distance spread requires insect vectors (Hansen, 1978). *Leptographium wageneri* has also been isolated from soils around diseased roots and might be able to survive saprophytically in this environment (Hicks, 1973).

BSRD can predispose trees to infestation by bark beetles. Thus, diseased trees have been found to be more likely to become bark-beetle infested than healthy trees (Goheen & Cobb, 1980; Goheen *et al.*, 1985; Hansen *et al.*, 1988). Weakened trees then serve as a food base for beetle populations to increase. When these populations become high in number, mass attacks can occur and healthy, as well as diseased trees are affected (Cobb *et al.*, 1974; Cobb, 1988). Diseased trees usually occur in groups or centres (Cobb, 1988). A disease centre appears as a group of dead trees mixed with uninfected trees (Goheen & Hansen, 1978). Disease centres can be established by insect vectors attracted to stressed trees. The disease then spreads further by points of contact between diseased and healthy trees (Morrison & Hunt, 1988). The rate of infection and the expansion of disease centres appears to slow with the aging of the tree (Hansen & Goheen, 1988).

Leptographium wageneri can be found in trees infested by species of *Dendroctonus* (Cobb *et al.*, 1974), *Pissodes fasciatus, Steremnius carinatus* and the root bark beetle, *Hylastes nigrinus* (Cobb *et al.*, 1984; Hansen *et al.*, 1988; Witcosky & Hansen, 1985; Witcosky, Schowalter & Hansen, 1986). Although there was initially no firm evidence for insect transmission (Cobb *et al.*, 1974), insects are now known to serve as vectors for this fungus (Hansen *et al.*, 1988; Harrington, Cobb & Lownsberry, 1985; Witcosky & Hansen, 1985; Witcosky *et al.*, 1986). *Hylastes nigrinus* appears to be the primary vector of the douglas-fir variant of *L. wageneri* (Cobb, 1988).

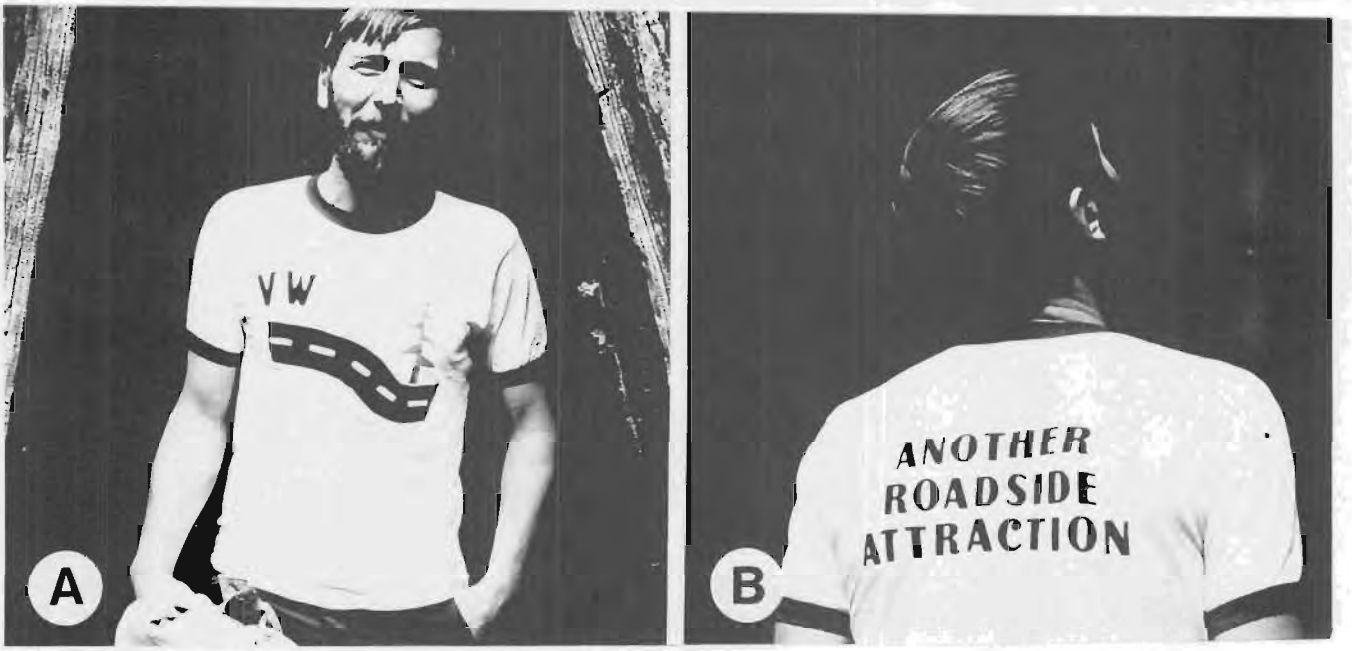

Fig. 5. Black stain root disease centres tend to occur at roadsides. These two pictures are of Dr. Everett Hansen wearing a T-shirt to illustrate this point. **A.** A tree growing at a roadside with VW referring to *Verticicladiella wageneri* (now *Leptographium wageneri*). **B.** The second picture illustrates the roadside nature of the disease.

Cobb (1988) proposed that *L. wageneri* renders sufficient trees susceptible to bark beetle infestation to maintain a high beetle population that is able to attack healthy trees. The adult beetles create wounds through their maturation feeding habits, and introduce the pathogen through these wounds (Harrington *et al.*, 1985; Hansen *et al.*, 1988). How the beetles detect a diseased or stressed tree is still unknown, although the incidence of root disease is directly correlated with the incidence of beetle infestation (Cobb, 1988).

Factors influencing BSRD can, in most cases, be associated with disturbances in the environment (Harrington *et al.*, 1983). BSRD appears to be more severe in places that have been disturbed by human activity, such as near roads or railroad tracks, where logging has occurred or where the thinning of trees is practiced (Fig. 5) (Hansen, 1978; Harrington, 1982; Harrington *et al.*, 1983; Cobb, 1988; Hansen *et al.*, 1988). This feature of the disease is believed to be associated with insect activity.

Leptographium wageneri is a temperature sensitive fungus that grows best at temperatures below 20°C (Wagener & Mielke, 1961; Smith, 1967; Hicks, 1973; Harrington, 1982; Hessburg & Hansen, 1983). Thus, BSRD occurs mostly in soils with bedrock near the surface and on well-drained coarse textured soils that have been disturbed (Morrison & Hunt, 1988). Soil moisture also influences the occurrence of this disease. BSRD is favored by high soil moisture and cooler temperatures (Goheen, 1976; Landis & Helburg, 1976; Goheen, Cobb & McKibbin, 1978; Cobb *et al.,* 1984; Wilks, Gesper & Cobb, 1985; Hessburg & Hansen, 1986a; Cobb, 1988). Fenn, Dunn and Wilborn (1990) found that increased levels of ozone tend to lead to an increase in disease incidence in ponderosa pine. Stressed trees are also especially susceptible to the disease (Hansen, 1978). Virulence of *L. wageneri* appears to increase with the increase of manganese concentrations and soil moisture (Goheen, 1976; Wilks, Gersper & Cobb, 1983).

Disease management strategies may include replacement of old trees with more vigorous trees, less prone to attack by bark beetles and spacing of trees to prevent spread through root contact (Goheen *et al.*, 1978). Some other strategies include planting mixed stands instead of trees in monoculture (Goheen *et al.*, 1978), minimizing stand and site disturbance, and selection of disease-resistant trees (Cobb, 1988; Hansen *et al.,* 1988). Sanitation through the removal of diseased trees or chemical treatment has also been suggested (Witcosky, 1989). In the case of douglas-fir, thinning after insect flight will reduce activity of vectors. If species other than douglas-fir are planted and site disturbances and tree injury are minimized, this will also reduce the incidence of BSRD. *Leptographium wageneri* has a short survival span after infected trees have been felled, indicating that the site where the disease occurs can be regenerated in a short period of time (Hunt & Morrison, 1986). An integrated pest management plan, making use of sanitation, resistant species and desirable cultural practices provides an ideal strategy for reducing the impact of BSRD (Witcosky, 1989).

White pine root decline

White pine root decline (WPRD) was first reported in the Eastern United States and was later found that *Leptographium procerum* is consistently associated with this

disease symptom (Kendrick, 1962; Dochinger, 1967). The role of the fungus in causing this disease has, however, been a matter of considerable debate (Lackner & Alexander, 1982; Harrington & Cobb, 1983; Wingfield, 1983; 1986). White pine root decline refers to a symptom. The consistent association of *L. procerum* with diseased trees need not imply that the fungus causes the disease. The association of the fungus with opportunistic insects that feed in the roots and root collars of stressed trees implies that *L. procerum* is commonly found in these parts of trees displaying symptoms of WPRD (Wingfield, 1983; Wingfield *et al.*, 1988). WPRD results in major economic losses in the Christmas tree industry in the USA (Lackner & Alexander, 1982).

Leptographium procerum is able to infect various species of pine other than *Pinus strobus*, but the symptoms and disease development in these species have been found to differ from those in *P. strobus* (Horner & Alexander, 1983a,b). The fungus has also been isolated from dying red pine (*P. resinosa*) and Scots pine (*P. sylvestris*) (Sinclair & Hudler, 1980). A disease similar to WPRD has been reported from Croatia and New Zealand, and the causal agent was speculated to be *L. procerum* (Orlic *et al.*, 1973; Halambek, 1976; Shaw & Dick, 1980; Halambek, 1981). The presence of WPRD in New Zealand was later confirmed by Mackenzie and Dick (1984). White pine root decline is now known to occur in various parts of the world in various ecosystems, and is not only restricted to forest trees (Livingston & Wingfield, 1982; Morelet, 1986; Alexander, Horner & Lewis, 1988; Morrison & Hunt, 1988; Smith, 1991). The extent of damage associated with WPRD has also not been fully assessed (Towers, 1977; Meyer, Hindal & Quinn, 1983).

Symptoms associated with WPRD include extended periods of bud break, retardation of shoot elongation, crooking of growing shoots, retention of needles, needle wilt, browning of needles and resin soaked black-streaked wood at the bases of stems, as well as basal cankers (Pest Alert, 1977; Towers, 1977; Anderson & Alexander, 1979; Mackenzie & Dick, 1984; Alexander *et al.*, 1988) (Fig. 6). The disease begins with a dark brown discoloration of the cambium at the base of trees. In the case of severe infection, marked resin exudation is observed (Alexander *et al.*, 1988). Colonized roots are resin-soaked and cross-sections of the stems reveal prominent wedges of blue-stained wood. Discoloration of the sapwood is consistent with the patterns and physiology of blue-stain fungi

(Alexander *et al.*, 1988). Electron microscopic examination has shown that *L. procerum* erodes the cell walls, and spreads from cell to cell via pits (Kilbertus, Mangenot & Radtke, 1980). Reduced water potential in symptomatic trees supports the notion that this root disease is associated with xylem dysfunction (Horner, Alexander & Lewis, 1987). Tree death occurs when the xylem is blocked by resin, resulting in desiccation (Alexander *et al.*, 1988).

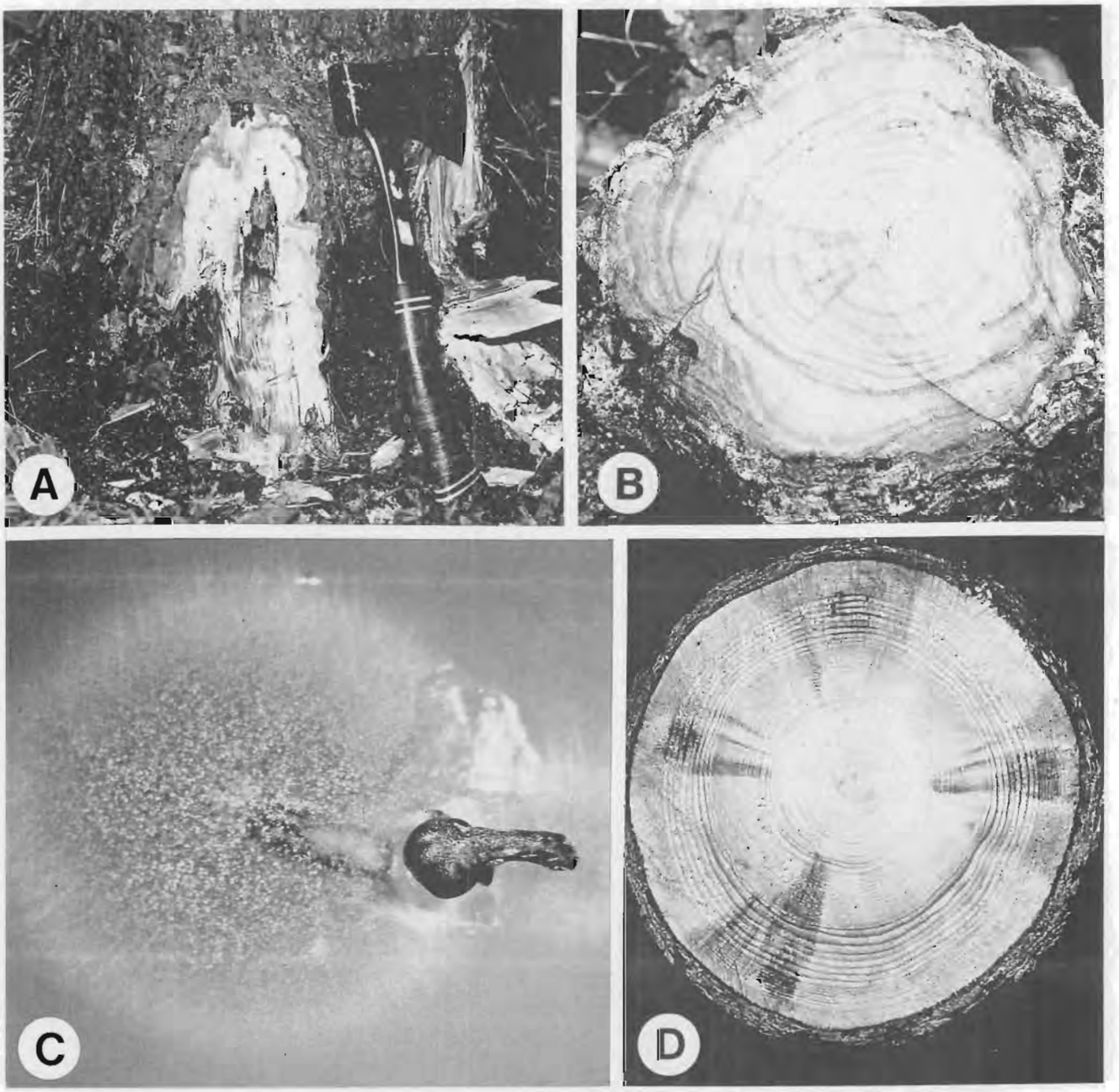

Fig. 6. Symptoms and insects associated with WPRD. **A.** Resinous lesion at the base of a mature *P. strobus* tree. **B.** Base of a *P. sylvestris* tree infested by the pine root collar weevil and colonized by *L. procerum*. **C.** *Leptographium procerum* growing out of body parts of a pine root collar weevil on agar. **D.** Typical staining pattern in pine wood inoculated with *L. procerum*.

Insect activity is evident at the bases of trees infected with *L. procerum* (Alexander *et al.*, 1988). Various reports exist where trees infected with *L. procerum,* were also infested with insects that may act as vectors for the fungus (Alexander *et al.*, 1988). It appears that weevils (Coleoptera: Curculionidae) are the main vectors, with bark beetles less commonly associated with the fungus (Wingfield, 1983; Lewis, 1985; Lewis & Alexander, 1986; Horner *et al.*, 1987; Alexander *et al.*, 1988) (Fig. 6). Volatiles such as ethanol and turpenes are often released from trees infected with *L. procerum.* The release of these volatiles is thought to play an important role in the association of the vectors with the trees (Nevill & Alexander, 1992a). The severity of WPRD is also affected by the breeding and feeding activities of the bark beetles that are secondary invaders (Alexander *et al.*, 1988).

Leptographium procerum is transmitted by insects, and it has also been speculated to spread through the soil. Air-borne dispersal has been ruled out as a means of spread (Alexander *et al.*, 1988). Propagules of *L. procerum* are able to survive in the soil around infected hosts for short periods of time (Lackner & Alexander, 1984; Alexander *et al.*, 1988). It appears that colonized roots are the main source of these propagules in the soil (Alexander *et al.*, 1988). The propagules occurring in the soil were later found to be relatively unimportant in the spread of the pathogen (Lewis, 1985; Lewis & Alexander, 1985; Alexander *et al.*, 1988). *Leptographium procerum* is also not uniformly distributed through the soil and is, therefore, unlikely to be a relevant source of infection. It has, thus, been proposed that insects are the main source of inoculum (Lewis, Alexander & Horner, 1987).

The pathogenicity of *L. procerum* has been a matter of substantial debate, and some studies have indicated that *L. procerum* is only a weak pathogen (Towers, 1977; Livingston & Wingfield, 1982; Wingfield, 1982; Wingfield, 1986; Wingfield *et al.,* 1988; Harrington, 1993). This can be illustrated by the fact that in some cases, only the symptoms of the disease have been reported, without any trace of a vector or *Leptographium* sp. The cause of these symptoms has, therefore, been attributed to other factors such as soil moisture (Prey, 1975) and not the fungus.

Leptographium procerum has been isolated from severely diseased trees (Leaphart, 1960; Dochinger, 1967). However, Houston (1969) found with inoculation studies that *L. procerum* does not kill as many trees as other pathogens.

Sinclair and Hudler (1980) indicated that it is frequently associated with mortality of red pine on poorly draining soils. However, there is no evidence to suggest that *L. procerum* is directly responsible for the mortality. Harrington and Cobb (1983) indicated that *L. procerum* is not virulent and is unable to kill wounded or unwounded douglas-fir. This was confirmed by Wingfield (1983, 1986) who considered *L. procerum* to be a weak pathogen. This is contrast to studies of Lackner (1981) and Lackner and Alexander (1982), who viewed the fungus as the cause of severe losses in Christmas tree plantations. In contrast to the results of Harrington and Cobb (1983) and Wingfield (1983, 1986), pathogenicity tests done on seedlings with isolates of this fungus confirmed its ability to kill seedlings. (Halambek, 1981; Alexander *et al.*, 1988). Nevill and Alexander (1992a) postulated that the lack of foliar symptoms as observed by Wingfield (1986), might be as a result of a long latent period of this fungus. In a separate study, however, *Leptographium procerum* did not produce lesions that were significantly longer than those of the controls in *P. taeda* (Nevill *et al.,* 1995).

Control and management of WPRD includes the planting of trees on sites suitable for the species, the control of weevils and bark beetles, removal of slash in and around the plantation and the control of weeds (Alexander *et al.*, 1988). It is also advisable to allow sites to lie fallow for one year or to consider planting non-susceptible trees (Lewis, 1985). WPRD affects trees more seriously when they are planted on wet sites (Anderson & Alexander, 1979). Poor site drainage has also been reported to promote disease development (Smith, 1991).

Dochinger (1967) speculated that soil moisture and temperature play an important role in the ecology of the fungus that causes WPRD. Excessive soil moisture can increase the severity of WPRD (Alexander *et al.*, 1988). *L. procerum* has also been found to be associated with root damage along roads (Alexander *et al.*, 1988), which is probably due to insect activity as in the case of *L wageneri* (Cobb *et al.,* 1984; Hansen *et al.*, 1988; Witcosky & Hansen, 1985; Witcosky *et al.,* 1986). Lackner (1981) and Lackner and Alexander (1983) found that *P. strobus* trees subjected to air pollution were more susceptible to root disease, presumably caused by *L. procerum* and insect infestation.

The debate surrounding the role of *L. procerum* as a conifer pathogen has perhaps

not fully been resolved. The fungus is substantially less virulent than *L. wageneri* and a general consensus seems that it cannot kill trees independently. It is commonly associated with root and root collar insects (Wingfield, 1983). Symptoms associated with insects such as pine root collar weevil (*Hylobius radicis*) on young trees are similar to those reported for WPRD and this has perhaps led to confusion relating to the role of *L. procerum* as pathogen (Wingfield, 1986). White pine root decline is a distinct disease syndrome on *Pinus strobus,* particularly in Christmas tree plantations and it is probably pertinent to view this disease alone and not confuse it with the occurrence of *L. procerum* on other pine species. The role of *L. procerum* in the development of WPRD and in the ecology of root and root collar insects remains to be fully understood.

Other diseases associated with species of *Leptographium*

Pole blight

The disease known as pole blight occurred exclusively on western white pine (*Pinus monticola* Douglas) in the 1950's (Gill & Andrews, 1949; Gill, Leaphart & Andrews, 1951; Hubert, 1953), where it caused serious damage (French, 1949; Foster, 1957; Leaphart, Copeland & Graham, 1957). Leaphart (1956) isolated a species of *Leptographium* from trees with pole blight symptoms. From the description of the corkscrew-like or wavy appearance of the mycelium, this fungus was thought to be *L. serpens* (Leaphart, 1956). However, inoculation studies on trees with this fungus did not conclusively result in symptoms (Leaphart, 1958).

Hubert (1953) suggested that the *Leptographium* sp. associated with pole blight is not the primary cause of the disease. These findings were supported by Leaphart and Gill (1959) in their study of the effect of several species of *Leptographium* on western white pine. They found that species of *Leptographium* were pathogenic to pine, but that they were not the causal agents of pole blight.

Ophiostoma trinacriforme has also been implicated as a possible cause of pole blight. However, a study by Parker (1957b) showed that this fungus is unable to produce the typical lesions associated with the disease. It is more likely a

secondary invader of lesions created by the causal agent of pole blight.

Leptographium terebrantis - associated disease

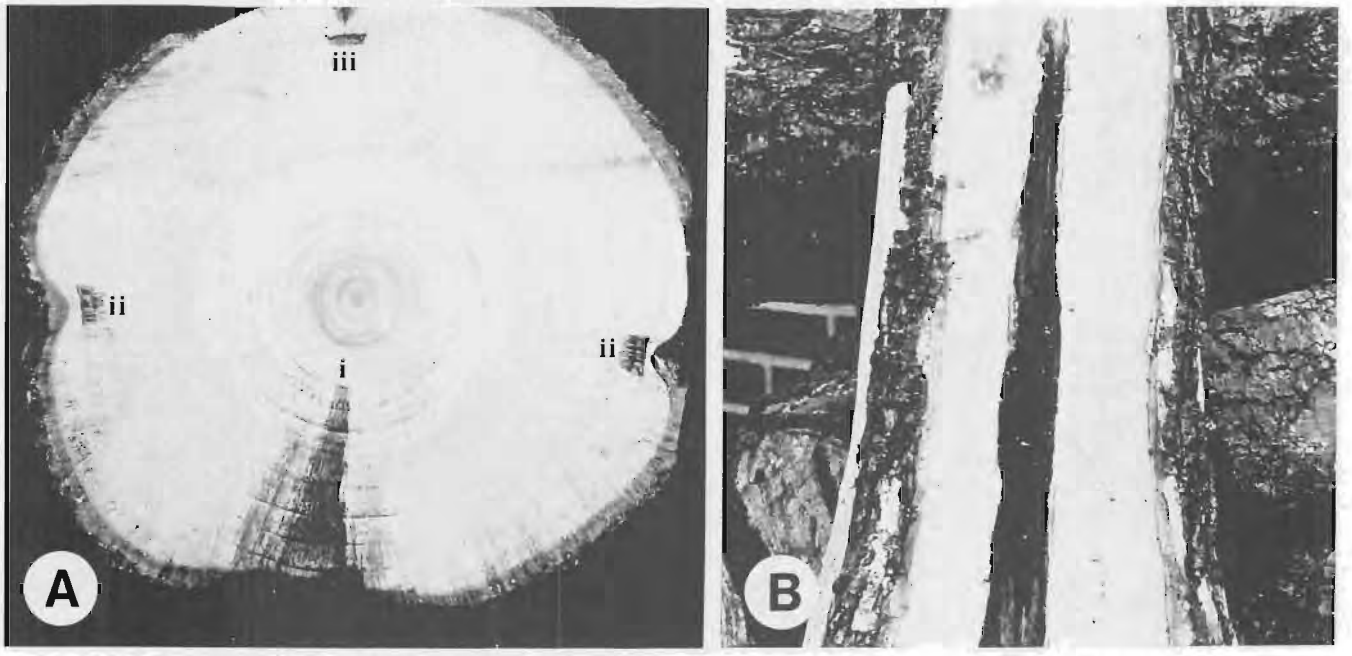

Fig. 7. Lesions in *Pinus strobus* five months after inoculations with *L. terebrantis* and *L. procerum*. **A.** Section through a stem inoculated with *L. terebrantis* (i), *L. procerum* (ii) and control (iii). **B.** Face view of an extensive lesion caused by *L. terebrantis* five months after inoculation.

Leptographium terebrantis is a common blue-stain fungus that is associated with a wide range of bark beetles, particularly *Dendroctonus terebrans* (Bennet & Tattar, 1988). Although the fungus has never been considered as a primary cause of tree disease, it has a high level of pathogenicity. Thus, Harrington & Cobb (1983) were able to kill pine seedlings with this fungus while, in the same study, *L. procerum* was not able to kill the plants. Similarly, Wingfield (1986) showed that *L. terebrantis* could kill inoculated seedlings and cause extensive lesion development in established trees (Fig. 7). This was unlike *L. procerum* that did not kill seedlings and gave rise to very limited lesion development, which was hardly different to the controls. The pathogenicity of this fungus to Japanese and Scots pine was confirmed by Bennet & Tattar (1988), Ross, Fenn & Stephan, (1992) and Nevill *et al.*, (1995). They found that this fungus caused severe resinosis and lesion development. Otrosina *et al.*, (1997) isolated *L. terebrantis* from lesions in trees

attacked by the Southern pine beetle. However, no conclusions were made regarding its pathogenicity to pine.

Leptographium terebrantis has also been found in the roots of *Pinus resinosa* with symptoms of red pine decline (RPD). In association with two other fungi, *O. ips* and *O. nigrocarpum,* it was thought to play a role in red pine death in the Lake States (Smalley *et al.,* 1993). Inoculation studies with other species of *Ophiostoma* and *Leptographium* suggest that *L. terebrantis* is the primary cause of root disease in red pine. This species is also known to be associated with the red turpentine beetle that infests *P. resinosa* (Smalley *et al.,* 1993).

Leptographium serpens - associated diseases

Leptographium serpens has been associated with a root disease of *Pinus pinea* in Italy (Lorenzini & Gambogi, 1976). A similar disease was later found in on *Pinus radiata* and *P. pinaster* in South Africa (Wingfield & Knox-Davies, 1980a). The causal agent of the root disease in South Africa was described as *Leptographium alacris* M.J. Wingf. & Marasas (Wingfield & Marasas, 1980), but this species was later synonomised with *L. serpens* (Wingfield & Marasas, 1981). There have been some reports of this fungus from the USA, although these are of doubtful authenticity (Harrington, 1988).

Wingfield *et al.* (1988) concluded that the pathogenicity of *L. serpens* has not been conclusively established and that the combined feeding activity of the insects and the subsequent colonization by the fungus may result in tree death. *Leptographium serpens* colonizes both the ray parenchyma as well as the tracheids resulting in a wedge shape discoloration of infected wood (Wingfield *et al.*, 1988). Two root feeding insects, *Hylurgus ligniperda* and *Hylastes angustatus,* are associated with this fungus and can act as possible vectors. The disease, thought to be associated with *L. serpens* is also characterized by distinct infection centers in plantations (Wingfield *et al.,* 1988).

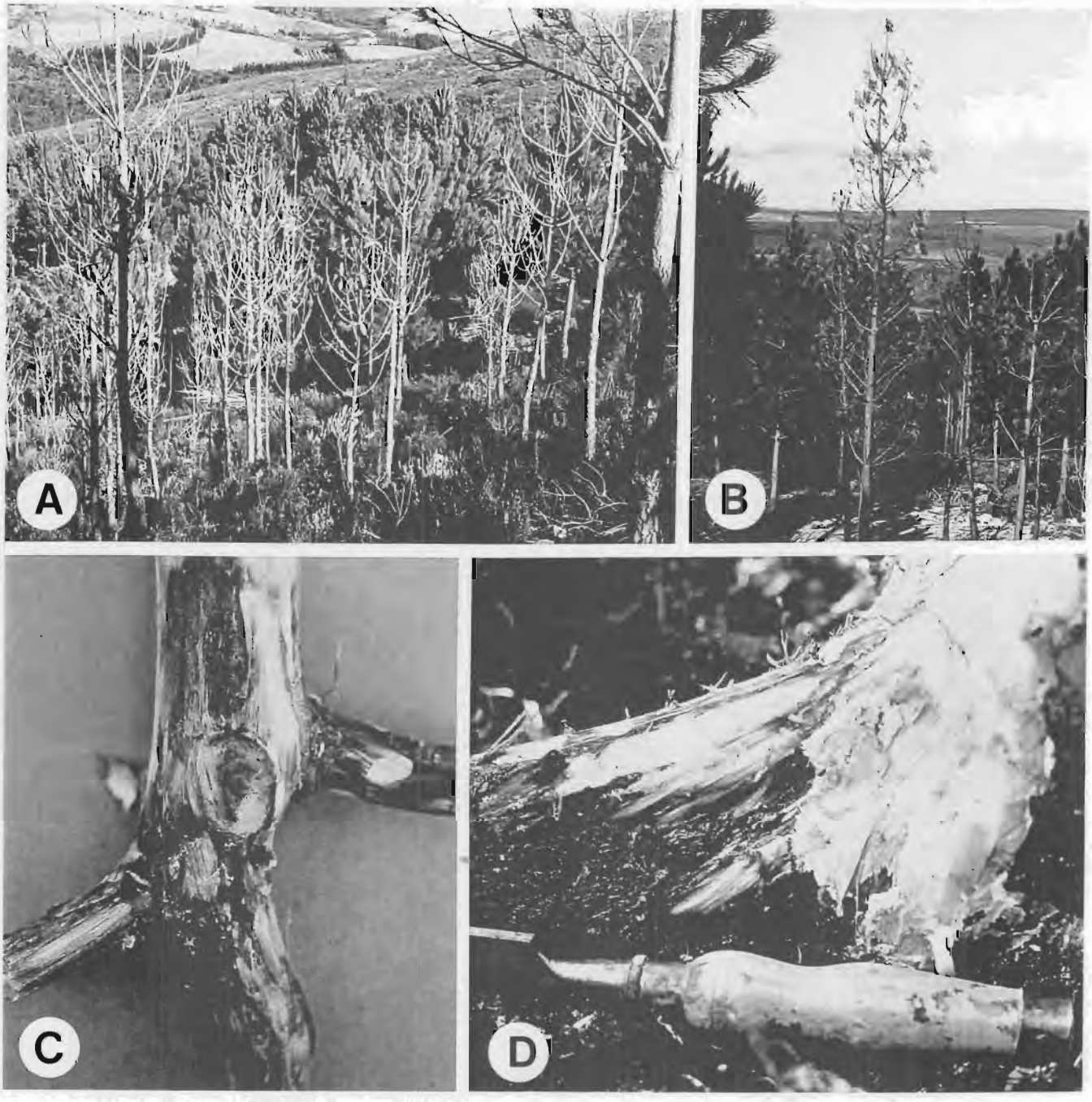

Fig. 8. Symptoms associated with *P. pinaster* trees infected with *L. serpens* in South Africa. **A.** Dead trees in a discrete patch-like infection centre. **B.** Dying tree at the edge of an infection centre. **C.** Diseased root system of young tree. **D.** Stained root on trees.

Symptoms of the disease associated with *L. serpens* includes scant, yellowish foliage in the upper crown of trees, reduced needle length, sudden marked

decrease in height growth and dark stained areas on roots (Wingfield & Marasas, 1983 Wingfield *et al.*, 1988). The trees retain their dead needles after death, in contrast to other diseases where trees will loose needles before they die (Wingfield & Knox-Davies, 1980a, b) (Fig. 8).

Takamaka disease

Takamaka disease occurs on Takamaka (*Calophyllum inophyllum*) trees, which are indigenous to the Seychelles and Mauritius (Wiehe, 1949; Wainhouse *et al.*, 1998; Webber *et al.*, 1999). The fungus associated with this severe wilting disease was initially identified as a species of *Haplographium* (Wiehe, 1949), and Gams (1971) transferred it to *Verticillium*. In recent study of this fungus, it was shown that it is unlike other *Verticillium* spp. and was subsequently transferred to *Leptographium* as *L. calophylli* (Webber *et al.*, 1999).

Fig. 9. Symptoms Takamaka disease on *Calophyllum inophyllum* in the Seychelles. **A.** Dying trees on beach front. **B.** Thinning crown of a dying tree. (Photographs supplied by Dr. D. Wainhouse).

Takamaka disease is characterized by wilting of the crowns of trees. The leaves loose their shine, curl inwards and dry out (Fig. 9). The leaves dry suddenly and remain attached to the trees for up to two weeks (Wiehe, 1949). No apparent lesions on the trunks, branches or roots are associated with this disease. However, brownish streaks are visible in the tracheids of trees (Wiehe, 1949).

Fungal infection occurs through wounds on the branches and twigs. These wounds can be as a result of mechanical wounding by strong winds or bark beetle activity. Bark beetle tunnels are frequently associated with this disease and their feeding and breeding habit can cause wounds (Wiehe, 1949). The bark beetle, *Cryphalus trypanus,* has been identified as the principal vector of *L. calophylli* (Wainhouse *et al.*, 1998)

Blue-stain

Blue-stain of conifer wood refers to the discoloration of sapwood that results from the presence of fungal hyphae (Münch, 1907; Lagerberg, 1927; Seifert, 1993) and can be recognized by its wedge-shaped appearance in the logs (Gibbs, 1993). The discoloration can range from bluish to grey (Seifert, 1993). However, the color of the mycelium does not necessarily influence the color of the stain (Lagerberg *et al.,* 1927). Two different categories of blue-stain are recognized, namely log-blueing and surface-blueing, and different fungi are associated with these symptoms (Lagerberg *et al.,* 1927). Blue-stain fungi generally do not kill trees, although Nelson (1934) found with experiments using dye that the stained areas in the wood interfered with transpiration.

Many species of *Leptographium* are associated with blue stain in conifer lumber (Lagerberg *et al.,* 1927; Solheim, 1992a,b, 1995a,b,c; Solheim, Långström & Hellqvist, 1993). This was first recognized, when Lagerberg *et al.* (1927) studied the causal agents of blue-stain in pine and spruce. This study led to the description of *Leptographium* (Lagerberg *et al.,* 1927) and its type species, *Leptographium lundbergii*, as discussed earlier. Various examples of *Leptographium* spp. causing blue-stain are known, for example *L. penicillatum* and *L. piceaperdum* associated with *Ips typographus* L. on Norway spruce (Solheim, 1992b; Wingfield *et al.,* 1993).

Leptographium wingfieldii and *L. terebrantis* have, apart from their blue-stain properties, also been shown to be pathogenic to their hosts (Wingfield, 1986; Solheim & Långström, 1991; Gibbs & Inman, 1991; Solheim *et al.,* 1993).

Insect activity is also associated with blue-stain and the frequency of the blue-stain is determined by the frequency of the beetle attack (Highley & Tattar, 1985). Insects lower the resistance of trees and allow fungi to colonize trees (Francke-Grosmann, 1965; Livingston *et al.,* 1983; Kulhavy, Partridge & Stark, 1984; Wingfield *et al.,* 1988; Lieutier, Cheniclet & Garcia, 1989; Solheim, 1993a; Krokene & Solheim, 1996). Hobson, Parmeter and Wood (1991) found that blue-stain fungi were generally absent from the xylem of dying pine trees. These fungi were found to colonize trees later when the xylem had been debilitated.

INSECT ASSOCIATIONS

Insects are commonly associated with *Leptographium* spp. (Münch, 1907; Lagerberg *et al.,* 1927; Kendrick, 1962; Harrington, 1988; Wingfield & Gibbs, 1991; Wingfield, Harrington & Crous, 1994) (Table 1) (Fig. 10). Currently, there are two hypotheses to explain the relationship between *Leptographium* spp. and insects. One is that these fungi are mostly transported, with little primary benefit to the insects (Leach, Orr & Christensen, 1934; Bramble & Holst, 1935, 1940; Mathre, 1964; Hinds, 1972; Goheen & Cobb, 1978; Witcosky & Hansen, 1985; Lewis & Alexander, 1986). The fungus on the other hand might serve as a source of food for the insects or play some role in the development of the brood (Nelson, 1934; Leach *et al.,* 1934). A second hypothesis is that the association of the insects and the fungi might be co-incidental. The fungi would then be considered as "weeds" in the habitat of the beetles (Harrington, 1993).

The conidia of *Leptographium* spp. are sticky and adhere easily to the body surfaces of insects (Harrington, 1993; Malloch & Blackwell, 1993) (Fig. 10). However, several species of *Ophiostoma* and *Leptographium* are carried in the mycangia of their associated insects (Francke-Grosmann, 1965; Whitney & Farris, 1970; Barras & Perry, 1971b; Ross & Solheim, 1995; Six & Paine 1996; Solheim, 1995a). Mycangial fungi have been shown to be important to the beetles and the

removal of these structures can lead to a reduction of the progeny and development of the pine beetle brood (Barras, 1973). Some evidence is also available to suggests that the fungi provide nutrition for the beetles (Batra, 1963; Francke-Grosmann, 1967; Hinds, 1972; Brand *et al.* 1976; Six & Paine, 1996).

Phoretic mites associated with bark beetles might serve as a vectors of blue stain fungi. It has for example been found that the mites associated with *Ips typographus,* carry one or more spores of different fungi (Moser, 1985; Moser, Perry & Solheim, 1989) and these represent an example of secondary phoresy (Blackwell *et al.,* 1986). However, the role of the fungi in the life cycle of the insects is still uncertain and much debated (Robinson, 1962; Lieutier *et al.,* 1988; Redfern, 1989; Paine, Raffa & Harrington, 1990; Hobson, Parmeter & Wood, 1991; Lévieux *et al.,* 1994; Raffa, 1995; Six & Paine, 1995; Wingfield, Harrington & Solheim, 1995; Otrosina *et al.,* 1997).

Insects associated with species in *Leptographium* mostly occur on conifers, especially bark beetles (Coleoptera: Scolytidae) (Grosmann, 1931; Harrington, 1988; Paine *et al.*, 1990). These insects can be primary bark beetles that attack and kill healthy trees, or secondary bark beetles that rarely kill their hosts (Berryman, 1972; Paine *et al.,* 1990). Most insects associated with *Leptographium* spp. are quite specific to the fungi they carry. Although one species of insect may carry two or more *Leptographium* spp., these relationships give a very clear insight into the taxonomy of the fungi (Grosmann, 1931; Leach *et al.,* 1934; Mathiesen, 1951; Griffin, 1968; Olchowecki & Reid, 1974; Horntvedt *et al.,* 1983; Harrington, 1988; Wingfield *et al.,* 1988; Furniss, Solheim & Christiansen, 1990; Gibbs & Inman, 1991). In other cases, the insects associated with the fungi can be diverse and the relationship appears to be casual (Olchowecki & Reid, 1974; Harrington, 1988) (Table 1). It is, however, important to distinguish between the pathogenic cycle where the insect introduces a pathogenic fungus into a tree, and a saprophytic cycle, where the dying trees provide food and brood material for the insects and sites for sporulation of the fungi (Brand *et al.,* 1976; Wingfield *et al.,* 1988).

Several studies indicate that root disease and blue stain fungi predispose the trees to further attack by bark beetles (Francke-Grosmann, 1965; Livingston *et al.,* 1983;

Kulhavy *et al.*, 1984; Lieutier, *et al.*, 1989; Solheim, 1993a; Krokene & Solheim, 1996). Fungi infecting the roots, such as *L. terebrantis* and *L. procerum,* might also predispose trees to further beetle-attack by diminishing the tree defenses as a result of the lesions caused by these fungi (Otrosina *et al.*, 1997). Cobb *et al.* (1974) showed a high degree of association between root disease and species of *Dendroctonus* that infest trees. Krokene (1996) and Krokene & Solheim, (1996) indicted that aggressive beetles vector pathogenic fungi, whereas non-aggressive beetles tend to carry less pathogenic fungi.

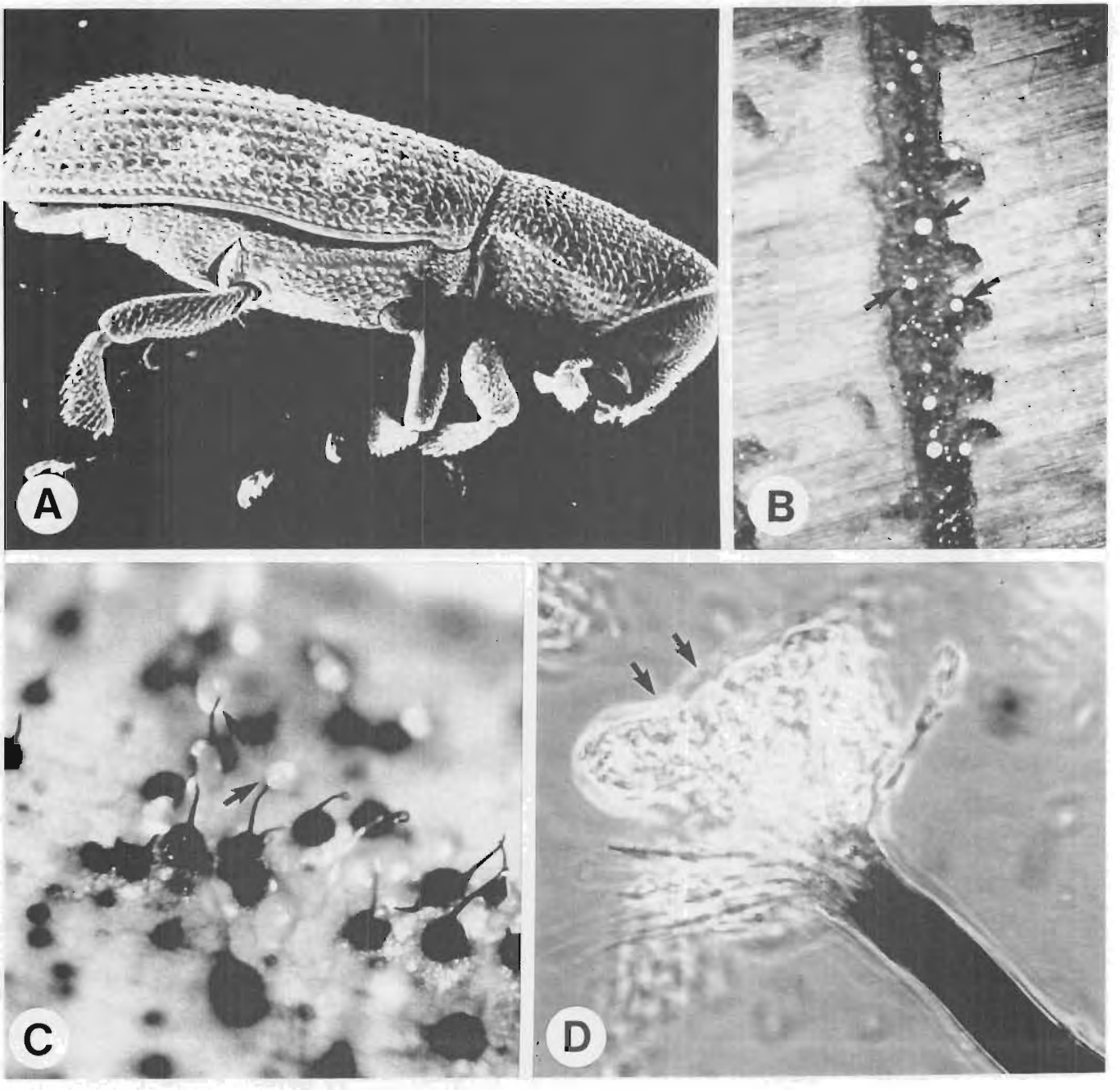

Fig. 10. Most *Leptographium* spp. are vectored by bark beetles such as the root-feeding beetle *Hylastes angustatus* (A). Fungal structures are adapted to insect dispersal with conidiophores (B) and perithecia (C, D) produced in galleries with spores in slimy masses (arrows) at the apices.

Table 1. Insects associated with *Leptographium* spp. and *Ophiostoma* spp. with *Leptographium* anamorphs.

Fungus	Insect	Reference
Leptographium abietinum	*Dendroctonus rufipennis*	Davidson, 1955; Kendrick, 1962; Harrington, 1988; Perry, 1991; Reynolds, 1992; Solheim, 1995a,b; Werner, 1995
	Dendroctonus pseudotsugae	Harrington, 1988; Perry, 1991; Lewinsohn *et al.*, 1994; Ross & Solheim, 1995; Solheim & Krokene, 1998
	Hylastes longicollis	Harrington, 1982, 1988
	Hylurgops porosus	Wagner, 1977
	Hylurgops planirostris	Harrington, 1988
Leptographium abicolens	*Korscheltellus gracilus*	Jacobs, Wingfield & Bergdahl, 1999
Ophiostoma abiocarpum	*Ips* spp.	Davidson, 1966
	Polygraphus rufipennis	Harrington, 1988
	Dryocoetus confusus	"
Ophiostoma aenigmaticum	*Ips typographus* f. *japonicus*	Jacobs *et al.*, 1998
Leptographium albopini	*Hylastes* spp.	Wingfield *et al.*, 1994
Leptographium alethinum	*Hylobius abietis*	Jacobs *et al.*, (1999)
Ophiostoma americanum	*Dendroctonus simplex*	Jacobs, Wingfield & Bergdahl, 1997
Ophiostoma aureum	*Dendroctonus sp.*	Robinson-Jeffrey & Davidson, 1968; Perry, 1991
	Hylurgops porosus	Harrington, 1988
Ophiostoma brevicolle	*Trypodendron retusus*	Davidson, 1958; Harrington, 1988
Leptographium calophylli	*Cryphalus trypanus*	Webber *et al.*, 1999
Ophiostoma crassivaginatum	*Trypodendron retusus*	Harrington, 1988
	Epuraea spp.	Hinds, 1972
	Colopterus truncatus	"
	Glischrochilus moratus	"

Table 1. cont.

Ophiostoma crassivaginatum (cont.)	*Glischrochilus vittatus*	"
	Rhizophagus brunneus	"
	Nudobius coricalis	"
Leptographium douglasii	*Hylastes nigrinus*	Wingfield *et al.*, 1994
Ophiostoma dryocoetidis	*Dryocoetus confusus*	Kendrick & Molnar, 1965; Molnar, 1965; Harrington, 1988
Leptographium euphyes	*Tomicus piniperda*	Jacobs *et al.*, 1999
Ophiostoma francke-grosmanniae	*Hylecoetus dermestoides*	Davidson, 1971
Leptographium guttulatum	*Dryocoetus autographus*	Jacobs *et al.*, 1999
	Hylastes ater	Wingfield & Gibbs, 1991
	Hylastes opacus	"
	Hylurgops palliatus	Mathiesen, 1950; Harrington, 1988; Wingfield & Gibbs, 1991; Jacobs *et al.*, 1999
	Hylurgops glabratus	Jacobs *et al.*, 1999
	Ips typographus	Mathiesen, 1950
	Tetropium sp.	"
	Tomicus piniperda	Jacobs *et al.*, 1999
Ophiostoma huntii	*Dendroctonus ponderosae*	Robinson-Jeffrey & Grinchenko, 1964; Harrington, 1988; Perry, 1991; Solheim, 1995c
	Hylastes ater	Jacobs *et al.*, 1998
	Hylastes macer	Harrington, 1988
	Ips pini	Davidson & Robinson-Jeffrey, 1965; Harrington, 1988
	Tomicus piniperda	Gibbs & Inman, 1991
Ophiostoma laricis	*Ips cembrae*	Van der Westhuizen *et al.*, 1995; Yamaoka *et al.*, 1998
Leptographium lundbergii	*Bursaphelenchus xylophilus*	Kaneko & Harrington, 1990
	Blastophagus minor	Mathiesen-Käärik, 1953
	Blastophagus piniperda	"

Table 1. cont.

Leptographium lundbergii (cont).	*Dendroctonus ponderosae*	Rumbold, 1931
	Hylastes angustatus	Wingfield & Marasas, 1983; Harrington, 1988; Wingfield *et al.*, 1988
	Hylastes ater	Harrington, 1988
	Hylastes opacus	Wingfield & Gibbs, 1991
	Hylurgus ligniperda	Harrington, 1988
	Hylurgops palliatus	Wingfield & Gibbs, 1991
	Ips acuminatus	Mathiesen-Kääńk, 1953; Harrington, 1988
	Myelophilus minor	Harrington, 1988
	Myelophilus piniperda	"
	Orthotomicus proximus	Mathiesen-Käärik, 1953; Harrington, 1988
	Pissodes pini	Mathiesen-Käärik, 1953
	Pityogenes quadridens	"
	Tomicus piniperda	Gibbs & Inman, 1991
	Trypodendron lineatum	Harrington, 1988; Bakshi, 1950
Ophiostoma penicillatum	*Dendroctonus rufipennis*	Perry, 1991
	Hylastes ater	Mathiesen, 1950; Mathiesen-Käärik, 1953; Harrington, 1988
	Hylastes cunicularis	Mathiesen-Käärik, 1953; Harrington, 1988
	Hylurgus ligniperda	"
	Hylurgops porosus	Wagner, 1977
	Hylurgops palliatus	Mathiesen, 1950; Mathiesen-Käärik, 1953; Harrington, 1988
	Dryocoetus confusus	Davidson, 1958
	Ips typographus f. *japonicus*	Yamaoka *et al.*, 1997
	Ips typographus	Goidanich, 1936; Kendrick, 1962, Mathiesen, 1950; Grosmann, 1931; Rennerfelt, 1950; Mathiesen-Kääńk, 1953; Solheim, 1986, 1992a; Harrington, 1988; Furniss *et al.*, 1990; Solheim, 1993b; Krokene, 1996; Krokene & Solheim, 1996; Viiri, 1997
	Ips duplicatus	Valkama, 1995; Krokene, 1996; Krokene & Solheim, 1996
	Pityogenes chalcographus	Goidanich, 1936; Mathiesen, 1950; Grosmann, 1931; Mathiesen-Käärik, 1953
	Pityogenes quadridens	Harrington, 1988
	Polygraphus poligraphus	Krokene, 1996; Krokene & Solheim, 1996

Table 1. cont.

Ophiostoma penicillatum (cont).	*Tetropium* sp	Mathiesen, 1950; Mathiesen-Kääńk, 1953
	Trypodendron lineatum	Harrington, 1988
Leptographium peucophilum	*Korscheltellus gracilus*	Jacobs *et al.*, 1999
Ophiostoma piceaperdum	*Dendroctonus ponderosae*	Perry, 1991
	Dendroctonus pseudotsugae	Solheim & Krokene, 1998
	Dendroctonus rufipennis	Harrington, 1988; Perry, 1991
	Dendroctonus valens	Perry, 1991
	Dryocoetus sp.	Davidson & Robinson-Jeffrey, 1965; Harrington, 1988
	Hylurgops palliatus	Harrington, 1988; Krokene & Solheim, 1996
	Ips typographus f. *japonicus*	Yamaoka *et al.*, 1997
	Ips typographus	Harrington, 1988; Solheim, 1986, 1992a, 1993b; Harding, 1995; Viiri, 1997
	Ips duplicatus	Krokene, 1996; Krokene & Solheim, 1996
	Pityogenes chalcographus	Harrington, 1988
	Polygraphus poligraphus	Krokene, 1996; Krokene & Solheim, 1996
Leptographium pineti	*Ips* spp.	Jacobs *et al.*, 1999
Leptographium procerum	*Dendroctonus frontalis*	Otrosina *et al.*, 1997
	Dendroctonus valens	Wingfield, 1983; Harrington, 1988
	Dendroctonus terebrans	Harrington, 1988; Perry, 1991
	Hylastes sp.	Lewis & Alexander, 1986; Alexander *et al.*, 1988
	Hylastes ater	Mackenzie & Dick, 1984
	Hylastes opacus	Wingfield & Gibbs, 1991
	Hylobius abietis	Lévieux *et al.*, 1994
	Hylobius pales	Lackner & Alexander, 1982; Wingfield, 1983; Lewis & Alexander, 1986; Alexander *et al.*, 1988; Nevill & Alexander, 1992a, b
	Hylobius radicis	Wingfield, 1982; Wingfield, 1983; Alexander *et al.*, 1988
	Hylobius rhizophagus	"
	Hylurgus ligniperda	Mackenzie & Dick, 1984
	Hylurgops palliatus	Wingfield & Gibbs, 1991
	Hylurgops porosus	Wagner, 1977

Table 1. cont.

Leptographium procerum (cont.)	*Ips typographus*	Harrington, 1988
	Orthotomicus spp.	Lewis & Alexander, 1986; Alexander *et al.*, 1988
	Pachylobius picivorus	Wingfield, 1983; Alexander *et al.*, 1988
	Pissodes spp.	Lewis & Alexander, 1986
	Pissodes approximatus	Lackner & Alexander, 1982; Alexander *et al.*, 1988
	Pissodes nemorensis	Nevill & Alexander, 1992a, b
	Pissodes pini	Kendrick, 1962; Livingston & Wingfield, 1982
	Pityokteines sp.	Lackner & Alexander, 1984; Alexander *et al.*, 1988
	Pityogenes sp.	Lackner & Alexander, 1984; Lewis & Alexander, 1986; Harrington, 1988; Alexander *et al.*, 1988
	Pityophthorus sp.	Lackner & Alexander, 1984; Alexander *et al.*, 1988
	Tomicus piniperda	Gibbs & Inman, 1991
	Xyleborus sp.	Lewis & Alexander, 1986; Alexander *et al.*, 1988
Leptographium pyrinum	*Dendroctonus adjunctus*	Davidson, 1978; Harrington, 1988; Perry, 1991; Six & Paine, 1996
Ophiostoma robustum	*Dendroctonus sp.*	Robinson-Jeffrey & Davidson, 1968; Perry, 1991
Ophiostoma serpens	*Hylastes angustatus*	Harrington, 1988; Wingfield *et al.*, 1988
	Hylastes ater	Wingfield & Gibbs, 1991
	Hylastes linearis	Harrington, 1988
	Hylobius pales	Nevill & Alexander, 1992
	Hylurgus ligniperda	Harrington, 1988; Wingfield *et al.*, 1988; Wingfield & Knox-Davies, 1980a
	Myelophilus piniperda	Siemaszko, 1939; Harrington, 1988
	Orthotomicus erosus	Wingfield & Knox-Davies, 1980a
	Pissodes nemorensis	Nevill & Alexander, 1992
Leptographium sibiricum	*Monochamus urrusovi*	Jacobs *et al.*, 1999
Leptographium terebrantis	*Dendroctonus frontalis*	Otrosina *et al.*, 1997
	Dendroctonus pseudotsugae	Lewinsohn *et al.*, 1994

Table 1. cont.

Leptographium terebrantis (cont.)	*Dendroctonus terebrans*	Barras & Perry, 1971a; Wingfield, 1983; Highley & Tattar, 1985; Highley & Tattar, 1987; Bennet & Tattar, 1988; Harrington, 1988; Perry, 1991
	Dendroctonus valens	Harrington, 1982; Harrington & Cobb, 1983; Harrington, 1988; Perry, 1991
	Hylurgops porosus	Harrington, 1982; Harrington & Cobb, 1983; Harrington, 1988
	Hylobius radicis	Wingfield, 1983
	Hylobius rhizophagus	"
	Ips pini	Bennet & Tattar, 1988
Leptographium wageneri	*Dendroctonus brevicomis*	Wagener & Mielke, 1961; Goheen, 1976; Goheen & Cobb, 1980
	Dendroctonus ponderosae	Goheen, 1976; Goheen & Cobb, 1980; Hunt & Morrison, 1986; Morrison & Hunt, 1988
	Dendroctonus valens	Goheen, 1976; Harrington & Cobb, 1983; Harrington, 1988; Perry, 1991
	Hylastes macer	Goheen, 1976; Goheen & Cobb, 1978; Harrington, 1982; Harrington & Cobb, 1983; Harrington, 1988
	Hylastes nigrinus	Witcosky, 1981, 1989; Harrington, 1982; Harrington & Cobb, 1983; Witcosky *et al.*, 1986; Harrington, 1988; Jacobi, 1992
	Hylurgops porosus	Wagner, 1977; Harrington, 1982
	Ips latidens	Morrison & Hunt, 1988
	Ips mexicanus	"
	Pissodes fasciatus	Witcosky, 1981, 1989; Witcosky *et al.*, 1986; Jacobi, 1992
	Steremnius carinatus	"
Leptographium wingfieldii	*Hyalstes opacus*	Wingfield & Gibbs, 1991
	Hylurgops palliatus	"
	Tomicus piniperda	Morelet, 1988; Lieutier *et al.*, 1989a,b; Wingfield & Gibbs, 1991; Gibbs & Inman, 1991; Solheim & Långström, 1991; Masuya *et al.*, 1998
Leptographium yunnanensis	*Tomicus piniperda*	Zhou *et al.*, 1999

HOSTS AND GEOGRAPHIC DISTRIBUTION OF *LEPTOGRAPHIUM* SPP.

Species of *Leptographium* are known from various parts of the world and occur on a wide variety of hosts. In the northern hemisphere, *Leptographium* spp. have been recorded from the U.S.A (Davidson, 1942; Davidson, 1958; Robinson-Jeffrey & Davidson, 1968; Wingfield *et al*, 1994), Canada (Hunt, 1956; Parker, 1957a; Wright & Cain, 1961; Kendrick, 1962; Robinson-Jeffrey & Grinchenko, 1964; Olchowecki & Reid, 1974), Europe [Croatia (Halambek, 1981), Germany (Grosmann, 1932), Italy (Goidanich, 1936) and Norway (Solheim, 1986, 1992a)] and Asian countries such as Japan (Van der Westhuizen *et al.*, 1995; Yamaoka *et al.*, 1997), Vietnam (Jacobs *et al.,* 1999), Indonesia (Jacobs *et al.,* 1999) and Taiwan (Wingfield, Crous & Tzean, 1994). In the southern hemisphere, *Leptographium* spp. have been reported from New Zealand (Shaw & Dick, 1980; Wingfield & Marasas, 1983; Mackenzie & Dick, 1984; Hutchison & Reid, 1988; Farrell *et al.,* 1997), South Africa (Wingfield & Knox-Davies, 1980a, b; Wingfield & Marasas, 1980; 1983), Central Africa (Jacobs, Wingfield & Roux, 1999), and Australia (Jacobs *et al.*, 1998) (Fig. 11).

In most cases, *Leptographium* spp. occur on conifers (Kendrick, 1962; Harrington, 1988; Wingfield *et al.,* 1994) (Table 2). Only a small number of species occur on deciduous trees, or other substrates (Davidson, 1958, 1971, 1976; Jooste, 1978; Kendrick, 1962, Jacobs *et al.,* 1998). Some *Leptographium* spp. are highly specific, and are often closely linked to insects that infest trees. Host, insect associations and area of occurrence, can thus be helpful in species identification. In some cases, the host can be used to distinguish between different species, for example the *L. wageneri* varieties (Kendrick, 1962; Harrington & Cobb, 1986, 1987). Thus, Hunt (1956) used this host specificity as a character in his key to species in several genera, including *Ophiostoma* and *Ceratocystis.*

Most *Leptographium* spp. are known from the Northern Hemisphere where conifers are native (Kendrick, 1962; Harrington, 1988). Virtually all species that have been recorded from the Southern Hemisphere have been introduced into that region with pine infesting bark beetles. Thus, a number of species of *Leptographium* (*L. lundbergii, L. procerum* and *L. huntii*) have been introduced into New Zealand and Australia from Europe with *Hylastes ater* and *Hylurgus ligniperda.* In South Africa,

L. serpens, *L. procerum* and *L. lundbergii* have been introduced into exotic pine plantations together with *H. angustatus* and *Hylurgus ligniperda* (Table 1, 2).

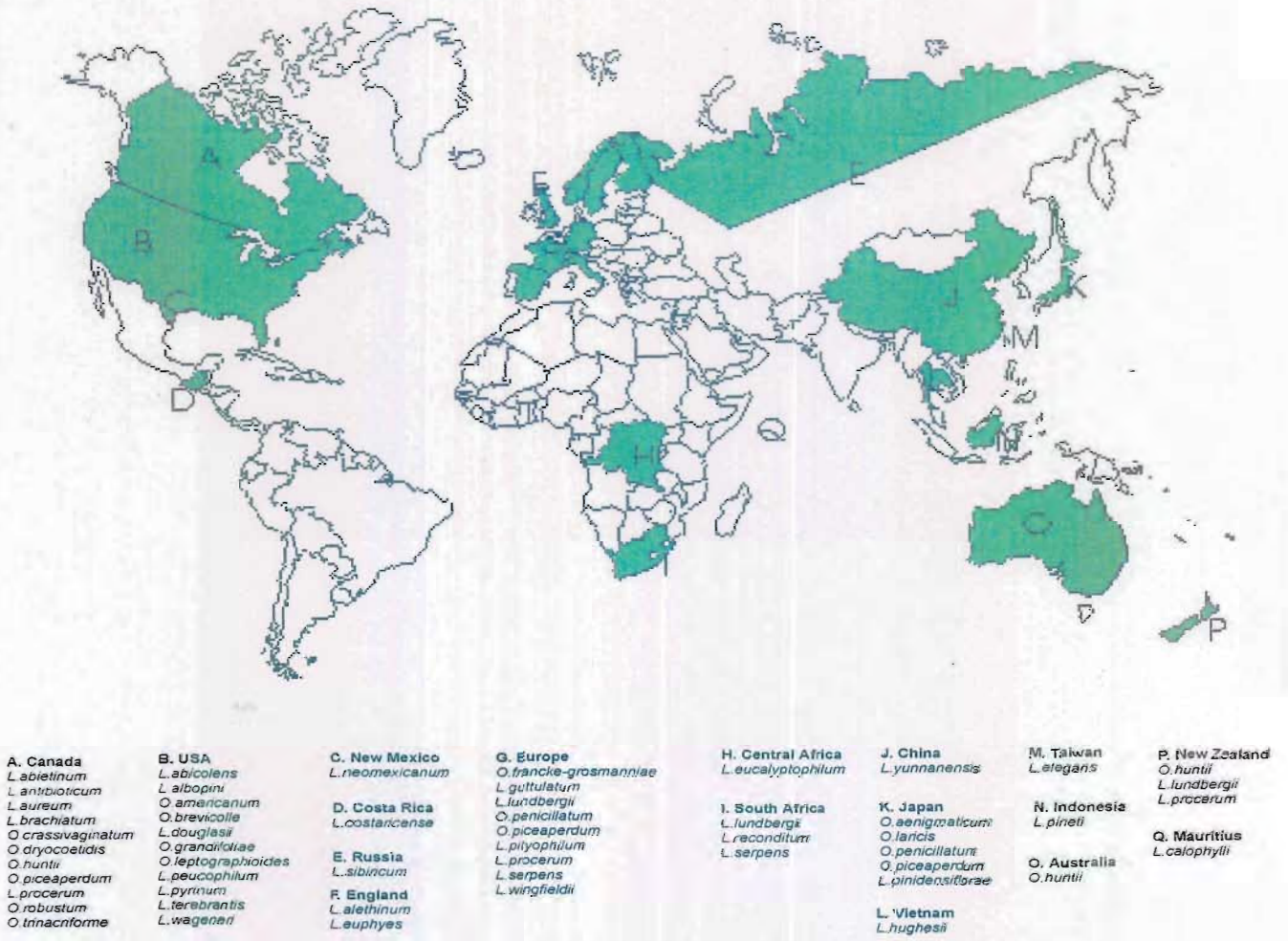

Fig. 11. World map showing the distribution of known *Leptographium* spp.

Table 2. Hosts associated with *Leptographium* spp. and *Ophiostoma* with *Leptographium* anamorphs.

Fungus	Host	Reference
Leptographium abietinum	*Melia* sp.	Kendrick, 1962
	Picea mariana	"
	Picea engelmannii	Davidson, 1955; Solheim 1995b
	Picea glauca	Solheim, 1995b
	Pseudotsuga menziesii	Mielke, 1979; Lewinsohn *et al.*, 1994; Solheim & Krokene, 1998
	Pinus contorta	Mielke, 1979
	Pinus monticola	Kulhavy *et al.*, 1978; Mielke, 1979
	Pinus sylvestris	Mielke, 1979
	Pinus ponderosa	"
	Pinus aristata	"
	Pinus mugo	"
Leptographium abicolens	*Abies balsamea*	Jacobs *et al.*, 1999
Ophiostoma aenigmaticum	*Picea jezoensis*	Jacobs *et al.*, 1998
Leptographium albopini	*Pinus edulis*	Wingfield *et al.*, 1994
	Pinus strobus	"
Leptographium alethinum	*Picea* spp.	Jacobs *et al.*, 1999
Ophiostoma americanum	*Larix decidua*	Jacobs *et al.*, 1998
Leptographium antibioticum	*Pinus contorta*	Mielke, 1979
	Pinus monticola	Kulhavy *et al.*, 1978; Mielke, 1979
	Abies lasiocarpa	Mielke, 1979
	Abies balsamea	Harrington, 1988
	Pinus albicaulis	Mielke, 1979
Ophiostoma aureum	*Pinus contorta var. latifolia*	Robinson-Jeffrey & Davidson, 1968
	Pinus ponderosa	Harrington, 1988

Table 2. cont.

Leptographium brachiatum	*Pinus edulis*	"
	Pseudotsuga menziesii	Kendrick, 1962
	Picea mariana	"
	Pinus pinaster	Morelet, 1986
	P. strobus	"
	P. sylvestris	"
Ophiostoma brevicolle	*Populus tremuloides*	Davidson, 1958
Leptographium calophylli	*Calophyllum inophyllum* var. *tacamaha*	Webber *et al.*, 1999
Leptographium costaricense	Rhizosphere of *Talauma sambuensis*	Weber, Spaaij & Wingfield, 1996
Ophiostoma crassivaginatum	*Picea mariana*	Griffin, 1968; Olchowecki & Reid, 1974
	Picea glauca	Olchowecki & Reid, 1974
	Pinus resinosa	"
	Pinus strobus	"
	Pinus sylvestris	"
	Populus grandidentata	Griffin, 1968
	Populus tremuloides	Griffin, 1968; Hinds, 1972
	Fraxinus nigra	Olchowecki & Reid, 1974
Leptographium douglasii	*Pseudotsuga menziesii*	Wingfield *et al.*, 1994
Ophiostoma dryocoetidis	*Abies lasiocarpa*	Kendrick & Molnar, 1965; Molnar, 1965
Leptographium elegans	*Chamaecyparis formosensis*	Wingfield *et al* ., 1994
Leptographium eucalyptophilum	*Eucalyptus urophylla* X *E. pellita* hybrid	Jacobs *et al.*, 1999
Leptographium euphyes	*Pinus* spp.	Jacobs *et al.*, 1999
Ophiostoma francke-grosmanniae	*Quercus* spp.	Davidson, 1971

Table 2. cont.

Ophiostoma grandifoliae	*Fagus grandifolia*	Davidson, 1976
Leptographium guttulatum	*Pinus sylvestris*	Wingfield & Gibbs, 1991; Jacobs *et al.*, 1999
	Picea abies	Jacobs *et al.*, 1999
Leptographium hughesii	*Parashorea plicata*	Kendrick, 1962; Jacobs *et al.*, 1999
Ophiostoma huntii	*Pinus contorta* var. *latifolia*	Robinson-Jeffrey & Grinchenko, 1964; Solheim, 1995c
	Pinus strobus	Davidson & Robinson-Jeffrey, 1965
	Pinus ponderosa	"
	Pinus monticola	"
	Pinus banksiana	Olchowecki & Reid, 1974
	Picea mariana	"
Ophiostoma laricis	*Larix* sp.	Van der Westhuizen *et al.*, 1995
	Larix kaempferi	Yamaoka *et al.*, 1998
Ophiostoma leptographioides	*Quercus* spp.	Davidson, 1942
Leptographium lundbergii	*Pinus* spp.	Lagerberg *et al.*, 1927
	Pinus densiflora	Kaneko & Harrington, 1990
	Pinus ponderosae	Rumbold, 1931
	Pinus taeda	Wingfield & Marasas, 1983; Wingfield *et al.*, 1988
	Pinus pinaster	Morelet, 1986
	Pinus radiata	Wingfield & Marasas, 1983
	Pinus strobus	Wingfield & Marasas, 1983; Morelet, 1986
	Pinus sylvestris	Morelet, 1986; Wingfield & Gibbs, 1991
	Pinus thunbergii	Kaneko & Harrington, 1990
	Larix leptolepis	Bakshi, 1950
	Picea spp.	Lagerberg *et al.*, 1927
	Picea abies	Bakshi, 1950; Hallaksela, 1977
Leptographium neomexicanum	*Pinus ponderosa*	Wingfield *et al.*, 1994

Table 2. cont.

Ophiostoma penicillatum	*Abies lasiocarpa*	Davidson, 1958
	Picea sp.	Mathiesen, 1951; Mathiesen-Käärik, 1960; Aoshima, 1965
	Picea abies	Grosmann, 1931; Goidanich, 1936; Siemaszko, 1939; Kendrick, 1962; Solheim, 1986; 1992a, 1993
	Picea jezoensis	Yamaoka *et al.*, 1997
	Pinus sp.	Mathiesen, 1951; Mathiesen-Käärik, 1960; Aoshima, 1965
	Pinus contorta	Mielke, 1979
	Pinus monticola	Kulhavy *et al.*, 1978; Mielke, 1979
	Pinus strobus	Morelet, 1986
	Pinus sylvestris	Mielke, 1979; Morelet, 1986
	Pinus pinaster	Morelet, 1986
	Pinus ponderosa	Mielke, 1979
Leptographium peucophilum	*Picea rubra*	Jacobs *et al.*, 1999
Ophiostoma piceaperdum	*Picea abies*	Solheim, 1986, 1992a; 1993
	Picea glauca	Rumbold, 1936
	Picea mariana	Wright & Cain, 1961
	Picea jezoensis	Yamaoka *et al.*, 1997
	Pinus glauca	Wright & Cain, 1961
	Pinus nigra	Hutchison & Reid, 1988
	Pinus radiata	"
	Pinus resinosa	Wright & Cain, 1961; Griffin, 1968
	Pinus strobus	"
	Pinus sylvestris	"
	Pinus taeda	Hutchison & Reid, 1988
	Pinus banksiana	Olchowecki & Reid, 1974
	Pseudotsuga menziesii	Davidson & Robinson-Jeffrey, 1965
Leptographium pineti	*Pinus* spp	Jacobs *et al.*, 1999
Leptographium pityophilum	*Pinus nigra*	Jacobs *et al.*, 1999

Table 2. cont.

Leptographium procerum	*Abies grandis*	Lane & Goheen, 1979
	Picea abies	Hallaksela, 1977; Alexander *et al.*, 1988
	Picea fraseri	Alexander *et al.*, 1988
	Pinus banksiana	Kendrick, 1962; Wingfield, 1982, 1983; Alexander *et al.*, 1988
	Pinus contorta	Mielke, 1979; Alexander *et al.*, 1988
	Pinus clausa	Barnard *et al.*, 1985; Alexander *et al.*, 1988
	Pinus echinata	Horner & Alexander, 1983a; Alexander *et al.*, 1988
	Pinus elliotii	"
	Pinus monticola	Alexander *et al.*, 1988
	Pinus nigra	Lackner & Alexander, 1982; Wingfield, 1982; Alexander *et al.*, 1988
	Pinus pinaster	Morelet, 1986
	Pinus ponderosa	Mielke, 1979; Wingfield, 1982; Alexander *et al.*, 1988
	Pinus radiata	Mackenzie & Dick, 1984
	Pinus resinosa	Kendrick, 1962; Towers, 1977; Sinclair & Hudler, 1980; Halambek, 1981; Wingfield, 1982; Harrington, 1988; Alexander *et al.*, 1988
	Pinus strobus	Kendrick, 1962; Houston, 1969; Towers, 1977; Shaw & Dick, 1980; Sinclair & Hudler, 1980; Livingston & Wingfield, 1982; Wingfield, 1982; Lackner & Alexander, 1982; Horner & Alexander, 1983a, b; Lackner & Alexander, 1984; Mackenzie & Dick, 1984; Alexander *et al.*, 1983, 1988; Smith, 1991
	Pinus sylvestris	Wingfield & Gibbs, 1991; Wingfield, 1982; Lackner & Alexander, 1984; Horner & Alexander, 1983b; Harrington, 1988; Alexander *et al.*, 1988
	Pinus taeda	Horner & Alexander, 1983a; Alexander *et al.*, 1988
	Pinus virginia	"
	Pseudotsuga menziesii	Mielke, 1979; Morrison & Hunt, 1988; Alexander *et al.*, 1988
Leptographium pyrinum	*Pinus ponderosa*	Davidson, 1978
Leptographium reconditum	*Triticum* rhizosphere	Jooste, 1978

Table 2. cont.

Ophiostoma robustum	*Pinus ponderosa*	Robinson-Jeffrey & Davidson, 1968
Ophiostoma serpens	*Pinus monticola*	Gill *et al.*, 1951
	Pinus nigra	Morelet, 1988
	Pinus taeda	Gill *et al.*, 1951
	Pinus sylvestris	Goidanich, 1936; Kendrick, 1962; Morelet, 1988; Wingfield & Gibbs, 1991
	Pinus pinaster	Wingfield & Knox-Davies, 1980; Wingfield & Marasas, 1980; Wingfield *et al.*, 1988
	Pinus pinea	Wingfield *et al.*, 1988
	Pinus radiata	Wingfield & Knox-Davies, 1980; Wingfield & Marasas, 1980; Wingfield *et al.*, 1988
	Pseudotsuga menziesii	Mielke, 1979
Leptographium sibiricum	*Abies sibirica*	Jacobs *et al.*, 1999
Leptographium terebrantis	*Pinus sylvestris*	Highley & Tattar, 1985; Highley & Tattar, 1987; Bennet & Tattar, 1988
	Pinus thunbergiana	"
	Pinus taeda	Barras & Perry, 1971a
	Pinus banksiana	Wingfield, 1983
	Pinus ponderosa	Harrington, 1988
	Pinus resinosa	Wingfield, 1983; Bennet & Tattar, 1988; Harrington, 1988
	Pinus edulis	Harrington, 1988
	Pinus strobus	Wingfield, 1983; Harrington, 1988
	Pseudotsuga menziesii	Harrington, 1988
Ophiostoma trinacriforme	*Pinus monticola*	Parker, 1957a
Leptographium wageneri	*Abies grandis*	Mielke, 1979
	Larix occidentalis	"
	Picea glauca	Morrison & Hunt, 1988
	Picea engelmannii	"
	Pinus aristata	Mielke, 1979

Table 2. (cont.)

Leptographium wageneri (cont.)	*Pinus attenuata*	Smith & Graham, 1975
	Pinus contorta	Cobb & Platt, 1967; Smith & Graham, 1975; Goheen & Hansen, 1978; Mielke, 1979; Hunt & Morrison, 1986
	Pinus edulis	Wagener & Mielke, 1961; Kendrick, 1962; Cobb & Platt, 1967; Smith & Graham, 1975; Landis & Helburg, 1976; Walters & Walters, 1977
	Pinus jeffreyi	Wagener & Mielke, 1961; Kendrick, 1962; Cobb & Platt, 1967; Smith & Graham, 1975
	Pinus lambertiana	Smith & Graham, 1975
	Pinus monophylla	"
	Pinus monticola	Kulhavy *et al.*, 1978; Smith & Graham, 1975; Mielke, 1979
	Pinus ponderosa	Wagener & Mielke, 1961; Kendrick, 1962; Cobb & Platt, 1967; Goheen, 1976; Goheen & Cobb, 1978; Goheen & Hansen, 1978; Mielke, 1979
	Pinus strobus	Smith & Graham, 1975
	Pinus sylvestris	Mielke, 1979
	Pseudotsuga menziesii	Miller & Veirs, 1968; Mielke, 1979; Smith & Graham, 1975; Hansen, 1978
	Tsuga heterophylla	Morrison & Hunt, 1988
	Tsuga mertensiana	Leaphart, 1960; Byler *et al.*, 1983; Goheen & Hansen, 1978
Leptographium wingfieldii	*Pinus sylvestris*	Morelet, 1988; Wingfield & Gibbs, 1991; Solheim & Långström, 1993
	Pinus brutia	Morelet, 1988
	Pinus strobus	"
	Pinus densiflora	Masuya *et al.*, 1998
Leptographium yunnanensis	*Pinus yunnanensis*	Zhou *et al.*, 1999
	Pinus gaoshanensis	"
	Pinus shimaonensis	"

LABORATORY METHODS FOR *LEPTOGRAPHIUM*

Leptographium spp. can be isolated from four main sources. These include lesions associated with disease symptoms, soil around roots of diseased trees, insects such as bark beetles and from within beetle galleries, inluding blue-stained wood underneath beetle galleries. *Leptographium* spp. sporulate profusely on wood, and cultures can be obtained through direct transfer of the gloeioid conidial masses. Their presence in soil and on insects is not obvious and specialized media and techniques have been developed for their isolation. The ability of *Leptographium* spp. to tolerate high concentrations of cycloheximide provides a valuable aid in isolation (Fergus, 1956, Harrington, 1981; Marais, 1996). Most other fungi can not grow on cycloheximide, and this antibiotic is, therefore, routinely included in media for the isolation of *Leptographium* or *Ophiostoma* spp.

Some *Leptographium* spp. are conspicuous due to their large, dark, macronematous conidiophores, whereas others have small, more lightly pigmented conidiophores, which are not readily observed. Conidiophore morphology can vary, depending on the type of medium used. Malt extract agar (1-2%) normally results in good sporulation (Harrington, 1992; Wingfield & Marasas, 1980; Wingfield *et al.,* 1994; Jacobs *et al.,* 1998). Some species, such as *L. wageneri,* sporulate best when the fungus is cultured on a rich medium (MEA) before being transferred to water agar (Harrington, 1992). Some species only sporulate well in the presence of host tissue. This can be achieved by using pine twig medium (PTM) or by placing sterilized de-barked pine twigs on the surface of the growth medium. This is particularly helpful for isolates of, for example *O. huntii* and *O. piceaperdum.* In cases where teleomorphs are associated with *Leptographium* spp., using PTM or pine twigs on the medium sometimes induces the formation of perithecia. Some authors have also reported using potato dextrose agar (PDA) to grow *Leptographium* spp., but we have found that this medium leads to the formation of abundant aerial mycelium. This makes the identification and study of *Leptographium* difficult.

Culture media for *Leptographium*

Malt extract agar (MEA)

Malt extract	20 g
agar	15 g
distilled water	1000 ml

MEA (1-2%) is generally sufficient to support the growth and sporulation of most *Leptographium* spp.

Potato dextrose agar (PDA) (Singleton, Mihail & Rush, 1992).

Peeled potatoes	200 g
agar	15 g
dextrose	20 g
distilled water	1000 ml

Add the peeled potatoes to 500 ml of the water and autoclave. Strain the autoclaved potatoes through cheesecloth. Add the rest of the water to a final volume of 1 l. Add the agar and dextrose and autoclave again. It is important to note that the cultural characters differ when grown on MEA and PDA. PDA induces the formation of abundant aerial mycelium which can mask the production of conidiophores. Commercially available PDA also gives results different to those associated with laboratory prepared PDA.

Cycloheximide-streptomycin-malt-agar (CSMA) (Harrington, 1992).

Malt extract	10 g
agar	15 g
cycloheximide	200 mg
streptomycin	100 mg
distilled water	1000 ml

This medium should be used when isolations are made from natural substrates, soil or insects. Both cycloheximide and the streptomycin should be added after autoclaving (Harrington, 1992). For the isolation of *L. wageneri,* using 800 ppm (0.8 g/l) cycloheximide and 200 ppm (0.2 g/l) streptomycin sulfate in PDA (pH 4.0) has been suggested (Hicks, 1973; Hicks, Cobbs & Gersper, 1980). For the production

of perithecia in culture, Hutchison and Reid, (1988), suggested the addition of thiamine (100 µg/ml), pyridoxine (100 µg/ml) and biotin (50 µg/ml) to the medium.

Pine twig medium (PTM) (Harrington, 1992).

debarked pine twigs	
agar	15 g
distilled water	1000 ml

Debarked pine twigs are cut to 1-2 cm pieces and split longitudinally. The twigs are autoclaved for 30 minutes (or alternatively twice for 15 minutes with a 24 h interval). The autoclaved twigs are aseptically placed, facing upwards, in Petri dishes. Autoclaved water agar is poured over the twigs until they are just covered. Cycloheximide and streptomycin (see CSMA) can be added to the medium to minimize contamination during prolonged incubation (Harrington, 1992). This medium promotes sporulation and in some cases induces the formation of perithecia.

***Leptographium procerum* selective medium (LPSM)** (Swai & Hindal, 1981).

Glucose	2.0 g
Fe^{++}	0.2 mg
Zn^{++}	0.2 mg
Mn^{++}	0.1 mg
chlortetracycline hydrochloride	50 mg
cycloheximide	50 mg
streptomycin sulfate	50 mg
agar	20 g
distilled water	1000 ml

This selective medium has been used to isolate *L. procerum* from symptomatic trees as well as from soil.

Media used to produce *nit*-mutants (Zambino & Harrington, 1990).

Basal medium (BM)

Glucose	20 g
KH_2PO_4	1.0 g

$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$CaCl_2$	0.1 g
trace element solution	0.2 ml
vitamin solution	10 ml

Trace element solution

Citric acid	5.0 g
$ZnSO_4$	5.0 g
$Fe(NH_4)_2(SO)_4.6H_20$	1.0 g
$CuSO_4.5H_2O$	0.25 g
$MnSo_4.H_2O$	50 mg
H_3BO_4	50 mg
$NaMoO_4.2H_2O$	50 mg
distilled water	95 ml

Vitamin solution

Thiamin HCl	0.1 mg
pyridoxine HCL	0.075 mg
biotin	0.005 mg per 1.0 % ethanol

Complete medium (CM)

Basal medium with 1.0 g asparagine added

Nitrate minimal medium with Triton X-100 (MMT)

Basal medium with 1.0 g $NaNO_3$ and 2 ml Triton X-100 added.

Nit-mutants are obtained by growing wild type isolates on CM that contains 1.5 % $KClO_4$. Fast growing areas are hyphal tipped and incubated on malt-yeast extract medium containing chlorate. A complementation test is done by placing two mutant strains adjacent to each other on minimal medium (MMT). After a few weeks of growth, the plates can be examined for a dense band of aerial mycelium, indicating complementation (Puhalla, 1985; Zambino & Harrington, 1990).

Isolations from natural substrates

Most species of *Leptographium* occur on conifers. These species can be found associated with lesions on stems or roots, sporulating in the galleries of bark beetles or in the soil surrounding roots. Isolations from samples should be made as soon as possible after collection, because more aggressive secondary fungi tend to colonize the specimens. Samples can, however, also be stored at 4 °C for up to two weeks (Harrington, 1992).

Methods for isolation of *Leptographium* spp. have been described by several authors. Samples are taken from the canker face or blue-stained area after the bark has been removed. Small pieces of wood can be placed in moisture chambers (wet filter paper in a Petri dish) and incubated for 10 days to induce conidiophore production (Anderson & Alexander, 1979; Solheim, 1986). Conidial masses form at the apices of conidiophores and can then be transferred to agar (MEA or WA) using a sterile needle (Seifert, *et al.,* 1993).

Slivers of wood or small pieces of diseased tissue or cambium adjacent to beetle galleries can be placed directly on CSMA. The cycloheximide and streptomycin inhibit most other fungi as well as bacteria and allow *Leptographium* spp. to grow (Wingfield, 1983; Solheim & Långström, 1991; Harrington, 1992). Conidiophores develop on the host tissue, or arise from the mycelium, that has grown onto the medium. Drops of conidia can then be lifted from conidiophores and transferred onto MEA or WA. An alternative means to purify cultures is to cut hyphal tips and to transfer these to new plates (Harrington, 1992; Seifert, *et al.,* 1993). Isolates of *Leptographium* spp. can be incubated between 20 and 25 °C. Harrington (1992) noted that most species, other than *L. wageneri,* grow well at these temperatures. *Leptographium wageneri* grows best at 15 °C and temperatures above 30 °C can be lethal to isolates of this species.

When isolations are made from ascospores at the apices of perithecia, it is a good practice to make a permanent slide of the perithecium from which the isolation has been made. In this way, morphology of the teleomorph can be correlated with anamorph features. This is especially useful in isolates where the teleomorph is not readily produced in culture and might never be seen after the isolation is made (Seifert, *et al.,* 1993).

Isolations from soil

Leptographium spp. occurring in soil are generally found in close proximity to the roots of infected trees. After collection of the soil sample, a dilution series is made and plated on CSMA (Swai & Hindal, 1981; Wingfield, 1983). For isolations of certain *Leptographium* spp. such as *L. wageneri,* Hicks *et al.* (1980) proposed a medium containing 800 µg/ml cycloheximide and 200 µg/ml streptomycin sulfate. Swai and Hindal (1981) used a selective medium (LPSM) with great success to isolate *L. procerum* from the soil.

Isolations from insects

Several methods have been described for trapping of insects that carry *Leptographium* spp. and *Ophiostoma* spp. (Wingfield, 1983; Bèdard *et al.,* 1990; Krokene, 1996) and these will not be discussed in any detail here. Harrington (1992) recommended the use of "Stickem-special" sticky traps because these do not appear to be toxic to *Leptographium* spp. Other methods include pitfall traps, trap logs or freshly cut wood bolts, buried in the soil (Harrington, 1992).

After the insects have been collected, there are several techniques that can be used to isolate *Leptographium* spp. from them. Insects can be crushed and placed directly on CSMA (Gibbs, & Inman, 1991; Wingfield & Gibbs, 1991; Harrington, 1992). To minimize contamination from other sources, the insects are washed in 1% sodium hypochlorite solution containing Tween 80 for 5 min before they are placed on CSMA (Wingfield, 1983). Alternatively, the insects can be ground in a small amount of sterile distilled water. From this slurry of water and insect parts, a dilution series can then be made and plated onto CSMA. This technique is useful when quantifying the number of propagules that are transmitted by beetles (Harrington, 1992).

Insects that carry these fungi, can be place on natural media, such as logs. The fungi are then allowed to colonise the logs. Isolations can be made from these media (Furniss *et al.,* 1990; Krokene & Solheim, 1996).

Genetic studies

Mating compatibility

Leptographium spp. have *Ophiostoma* teleomorphs and typically have a heterothallic mating system. In some species where *Ophiostoma* states are known, it is possible to determine the mating compatibility between different strains of the same species or between different species. In order to do mating studies, it is necessary to work with single ascospore cultures. To make single ascospore cultures a single drop of ascospores is removed from the apex of a perithecium. The ascospores are suspended in 5 ml of sterile water and shaken vigorously. In some cases it might be necessary to use a vortex mixer to disperse spores. The spore suspension can then be transferred to plates (MEA or WA) and dispersed thoroughly using an inoculating needle or a glass rod with the basal end bent at 90° to the main axis ("hockey" stick) and incubated for 12-24 hours. After incubation, germinating ascospores can be viewed under a dissection microscope, and can be aseptically transferred, using a sterile needle, to fresh plates. After about 24 h, single ascospore cultures are usually visible. From these small colonies, hyphal tips can be aseptically transferred onto fresh plates (Fig. 12).

To test mating compatibility, single ascospore isolates can be paired in different combinations as well as with themselves. Small blocks of medium are cut from the single ascospore isolates, and placed alongside each other on fresh plates, and incubated. PTM is recommended for these studies, as most *Ophiostoma* spp. do not produce teleomorphs readily in culture. Pine twigs (or other relevant host tissue) placed alongside the inoculum can also induce the formation of perithecia. Where perithecia form in single ascospore cultures, that have not been paired with other isolates, this is usually an indication of homothallism (Jacobs *et al.,* 1998; Seifert *et al.,* 1993). Physically wounding the medium can also stimulate the formation of perithecia.

An alternative technique to test for mating compatibility, is to incubate one mating type of an *Ophiostoma* sp. until it covers the plate. A spore suspension is made from the opposite mating type culture and this is then spread onto the recipient

culture, which then results in the formation of perithecia on the plates (Seifert *et al.*, 1993). Using this technique and reciprocal paring, it is possible to determine whether isolates are female fertile (Leslie & Klein, 1996; Britz, 1997).

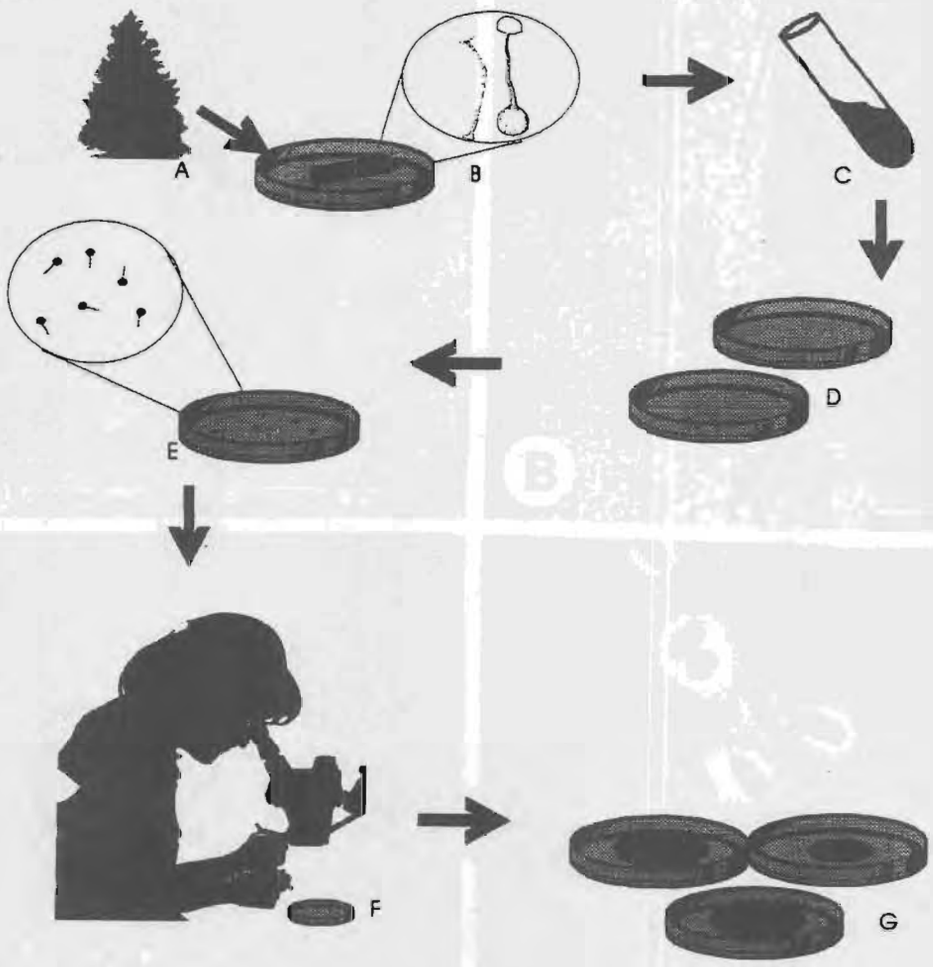

Fig. 12. Preparation of single ascospore/conidial cultures. **(A)** Plant material (pieces of bark including beetle galleries or wood pieces) are placed into moisture chambers **(B)** until the onset of sporulation. The gloeioid masses of spores at the apices of the conidiophores or perithecia are then carefully lifted from these structures with a sterile needle and suspended in sterile water **(C)**. The water is then spread over the surface of 2% MEA plates amended with 0.5 g/l cycloheximide and incubated **(D)**. The germinating spores can be lifted from plates with a needle after approximately 12 -24 hours **(E)** and transferred to clean MEA plates **(F)**.

Vegetative compatibility

In studies of vegetative compatibility, the choice of medium is important and it is necessary to test many media in order to find one in which VCG's and be visualised (Seifert *et al.,* 1993). To adequately test media, wild-type single ascospore isolates of a species can be paired against themselves and other isolates to observe interaction zones (Seifert *et al.,* 1993). Vegetative compatibility tests have not been extensively used in studies of *Leptographium.*

Zambino and Harrington (1990) used *nit*-mutants to study vegetative compatibility in *Leptographium wageneri.* This method exploits the use of nitrate non-utilizing (*nit)* mutants to indicate compatibility between isolates. Paring of complementary *nit*-mutants on minimal medium results in the development of abundant aerial mycelia. Cultures are examined for a dense band of aerial mycelia between the plugs, indicating complementation (Seifert *et al.,* 1993). This method has proved to be especially useful in *Fusarium,* as well as several other genera (Puhalla, 1985; Corell, Klittich & Leslie, 1987; 1989; Klittich & Leslie, 1988; Leslie, 1993; Hawthorne & Rees-George, 1996).

Storage of cultures

Efficient maintenance and long term storage of cultures of *Leptographium* spp. are extremely important. Cultures can be stored in the number of different ways. Generally, best results are achieved by duplicates stored using a variety of techniques, although this might not always be economically feasible. Fungi with complex conidiophores, such as *Leptographium* spp., tend to lose the capacity to produce these structures during the process of extended subculturing. To reduce this degradation, conidia, rather than mycelial plugs should be transferred to fresh plates (Seifert, *et al.,* 1993).

Most *Leptographium* spp. survive well on 2 % MEA slants, maintained at 4 °C. In our laboratory, we store all our isolates in triplicate in small McCartney bottles on MEA slants. One of each set is sealed with cigarette paper to prevent mite infestation (Snyder & Hansen, 1946). In a second bottle, an agar slant, covered

with mycelial growth, is overlaid with sterile mineral oil. A third isolate is stored in water. In the case of storage in water, the cultures are grown on MEA or PDA. Small blocks are then cut from the agar and transferred to sterile water. These are then maintained at 4 °C. Although water storage appears to be efficient, a common problem with this technique is contamination.

Lyophilisation provides an excellent method to store *Leptographium* spp. and we maintain a subset of isolates in this form. The method for storage that we recommend is described by Joubert and Britz (1987). A conidial suspension is prepared by adding 2 ml sterile, antibiotic-and endospore-free skim milk/lactose (12 %/ 5% m/v) solution to the culture. This solution is then added to sterile 6.0 mm assay disks in small ampoules. The tubes with the solution are freezed at - 20 °C and dried under vacuum. The ampoules are then sealed under vacuum and stored at -20 °C. Cultures have been shown to remain viable for up to 35 years using this method (Joubert & Britz, 1987).

SPECIES AND THEIR IDENTIFICATION

Leptographium spp. are notoriously difficult to identify. This is primarily because these fungi are morphologically similar and a comprehensive treatment of the group has not been available since the monograph of Kendrick (1962). In addition, numerous species can grow together in nature and mixed cultures are a common problem. The use of single spore cultures is, therefore, an absolute necessity (Wingfield *et al.*, 1988) (Fig. 12). This ensures that isolates are pure. In our key to *Leptographium* spp., emphasis has been placed on conidial morphology, primary branch patterns, presence and absence of rhizoids and conidiophore lengths. We have found that these characters are relatively stable and enable accurate identification of species. Correct interpretation of these characters (Figs. 13-16) is, however, crucial.

Hughes (1953) recognized the importance of conidial morphology and conidium development as taxonomic characters for Hyphomycetes including members of the *Leptographium* complex. Based on different modes of conidium development, he placed various genera of Hyphomycetes in groups. In *Leptographium* spp., conidia

are all produced through sympodial development of the conidiogenous cells but with delayed secession. Distinct scars representing the outer conidial walls, give a false appearance of percurrent proliferation. (Wingfield, 1985; Van Wyk *et al.*, 1988). Conidium development does not appear to provide a useful taxonomic characteristic in *Leptographium.*

Conidial shape

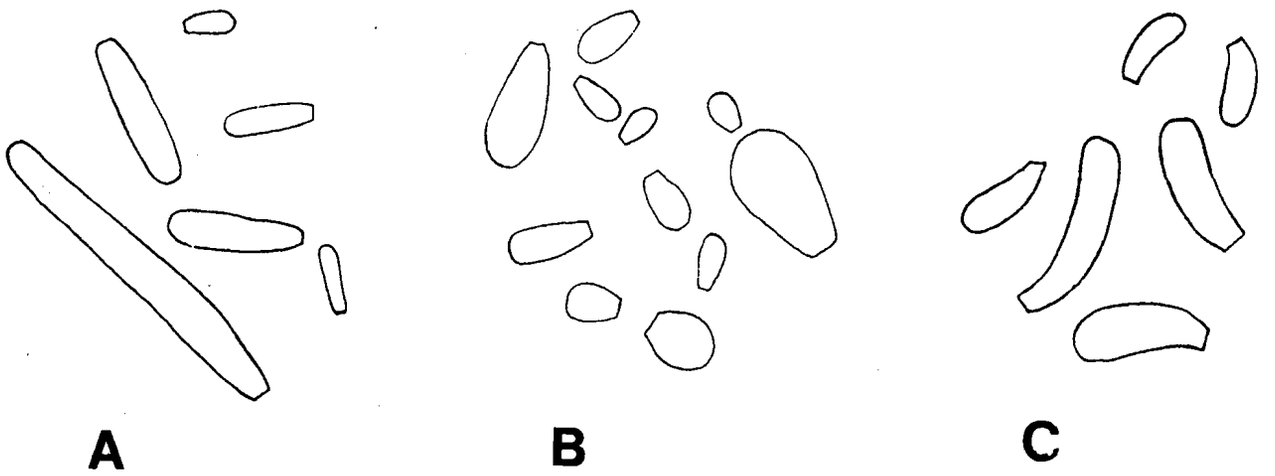

Fig. 13. Three categories of conidial shape found in *Leptographium* spp. **Type A** represents all species with long oblong to obovoid conidia. **Type B** represents species with obovoid conidia. **Type C** represents those with distinctly curved conidia.

Species of *Leptographium* can be divided into three distinct groups based on conidial shape (Fig. 13). The first of these (type A), includes all the species with oblong to obovoid conidia. This group is characterized by oblong conidia where the base of the conidium approximates the same size as the apex of the spore. In some cases, obovoid and oblong conidia are observed in the same isolate. Obovoid conidia have bases that are narrower than their apices. The second group (type B) includes those species with only obovoid conidia. No oblong conidia are observed in isolates of these species. Conidia in these species can, in most cases, also be placed in the category of *Leptographium* spp. with small conidia. The last group (type C) is characterized by species with distinctly curved conidia. Conidia in

Conidial size

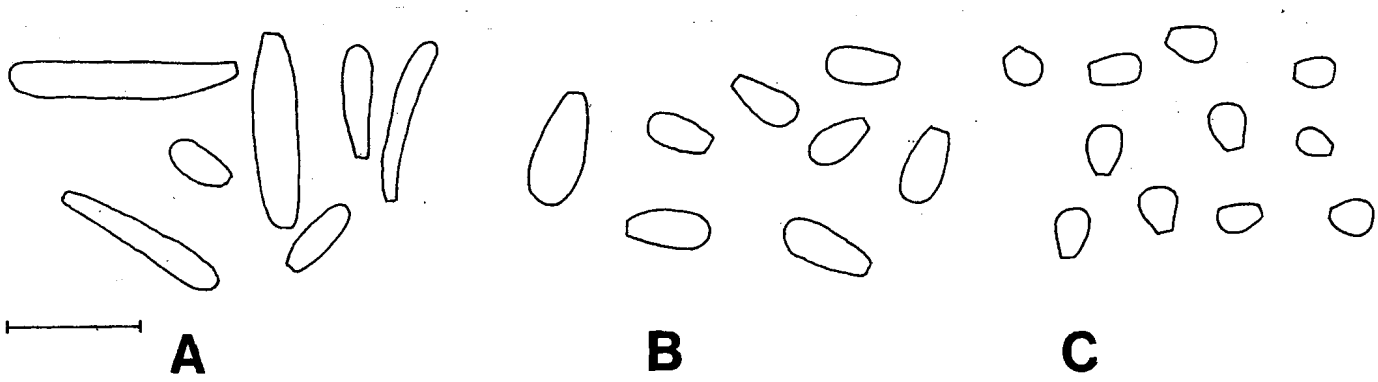

Fig. 14. Three different categories of conidial length. **A.** Long conidia are between 6 and 20 µm. **B.** Medium-sized conidia are between 5 and 12 µm. **C.** Small conidia are between 3 and 6 µm.

Conidia of *Leptographium* spp. can be divided into three groups based on conidium size. Although three distinct size groups can be distinguished, namely short, medium and long, the ranges within species can also overlap. Therefore, the sizes of the groups are given as ranges and 15-30 conidia need to be measured in order to determine the appropriate category of conidial length for an isolate (Fig. 14).

Primary branch patterns

Primary branch patterns provide a useful character for identifying *Leptographium* spp. Three distinct patterns of primary branching are found. Type A includes all species with only two primary branches. Type B includes species with two or more primary branches. Type C includes all species with more than two branches, where one of these branches is a large central branch at least twice as thick as the other primary branches. In this group, a single isolate can also display occasional

conidiophores with only two branches resembling type A or B. The majority of conidiophores should, however, be of type C (Fig. 15).

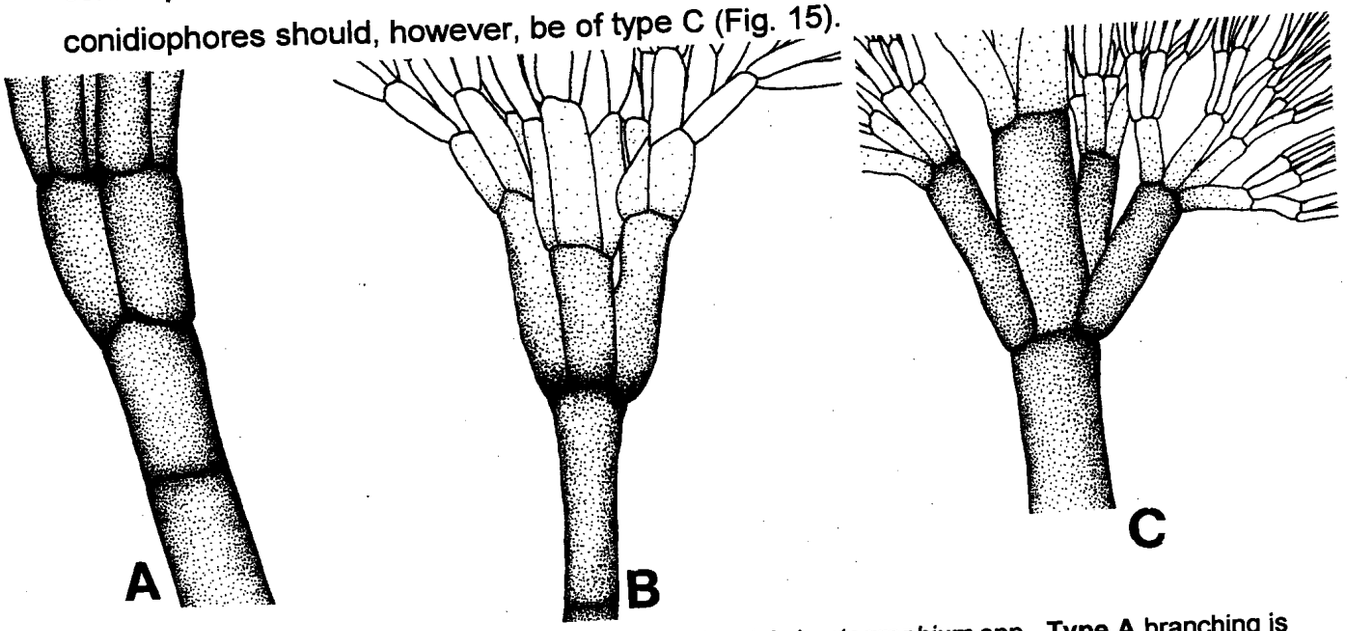

Fig. 15. Primary branch patterns can be used to distinguish *Leptographium* spp. **Type A** branching is found in species with only two branches. **Type B** branching is characterized by two or more branches. **Type C** branching is characterized by more than two branches with a single large branch in the middle.

Rhizoids

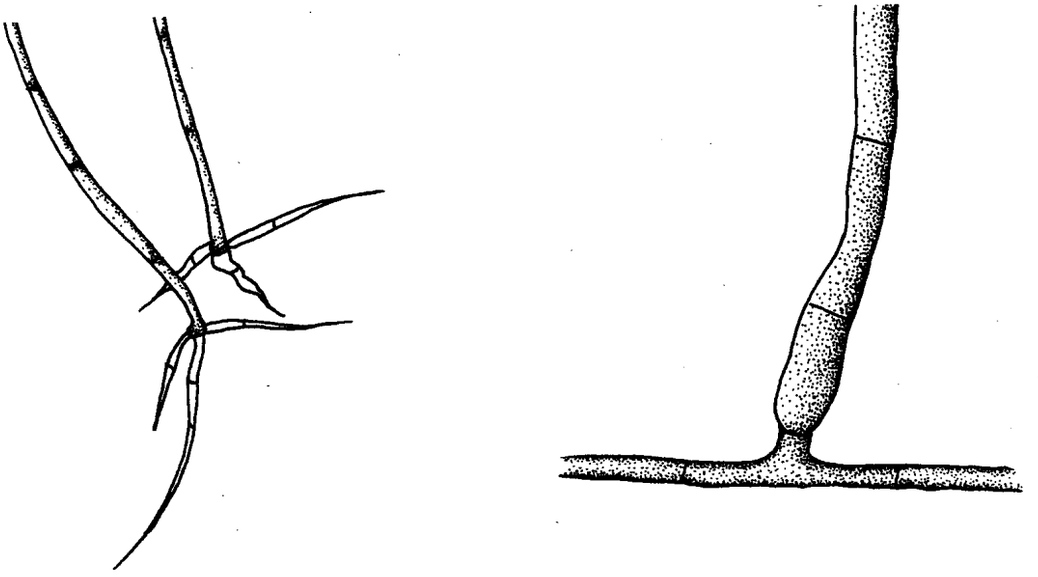

Fig. 16. Rhizoids in *Leptographium* spp. can either be present or absent.

The presence or absence of rhizoids at the base of conidiophores is a useful character in identifying *Leptographium* spp. Here, rhizoids are defined as mycelium-like outgrowths at the bases of conidiophores. Where rhizoids have been indicated as absent, the cell at the base of conidiophores grows continuos with the mycelium that gives rise to the conidiophore (Fig. 16

Cycloheximide tolerance

Species of *Ophiostoma* and *Leptographium* are able to tolerate high concentrations of cycloheximide in culture (Harrington, 1981; Marais, 1996). This antibiotic is, therefore, frequently included in selective media, when these fungi are isolated (Swai & Hindal, 1981). Tolerance to high levels of cycloheximide is a consistent character for most species of *Leptographium* although there are a small number of species that are sensitive to low concentrations of the antibiotic (e.g. *L. antibioticum, L. brachiatum* and *L. costaricense*) (Harrington, 1981, 1988; Weber *et al.,* 1996). This might suggest that these species are not appropriately placed in *Leptographium* and are not members of the Ophiostomatales. In the case of *L. costaricense,* this suggestion is strengthened by the fact that this species occurs in soil, in contrast to most other species of *Leptographium* that predominantly occur on woody substrates associated with insect activity. In this study, cycloheximide tolerance was tested at a concentration of 0.05 g/l. The tolerance of is expressed as a percentage of the control.

Cycloheximide tolerance provides a useful taxonomic characteristic for *Leptographium* spp. It also appears to be correlated with the presence of cellulose in the cell walls of most of the fungi (Horner, Alexander & Julian, 1986; Marais, 1996). *Leptographium* spp. are also characterized by the presence of rhamnose,

mannose, galactose and glucose in their cell walls. This is similar to the cell walls of *Ophiostoma* spp., and confirms the close association of these genera (Marais & Wingfield, 1999a).

Molecular characteristics

Zambino and Harrington (1992) distinguished between different species in *Leptographium* using isozyme analysis. Although this technique was shown to be valuable in distinguishing between species, variable success has been obtained in other genera of fungi. The data of Zambino and Harrington (1992) supported the synonymy of *L. serpens* and *L. alacris* as proposed by Wingfield and Marasas (1981) as well as the suggestion that *L. abietinum* and *L. engelmannii* Davidson are synonyms (Harrington, 1988; Jacobs *et al.* 1999). Furthermore, a low level of relatedness was observed among species representing the four ascospore morphology groups as defined by Olchowecki and Reid (1974). Isozyme analysis also proved useful in distinguishing between *L. douglasii, L. albopini* and *L. neomexicanum,* which are morphologically very similar (Wingfield *et al.,* 1994). This technique could also differentiate between the three varieties of *L. wageneri* (Zambino & Harrington, 1992). Similarly, Witthuhn *et al.* (1997) could distinguish between the varieties of *L. wageneri* using RAPD's.

Strydom, Wingfield & Wingfield (1997) used ribosomal DNA sequences to support the synonymy of *L. truncatum* and *L. lundbergii.* Isolates of these species had been shown to be morphologically similar and indistinguishable from each other. This similarity was confirmed through the phylogenetic analysis of sequence data for isolates of these species. Ribosomal DNA sequences have also proved to be useful in distinguishing *L. guttulatum* from *L. penicillatum.* Isolates of *L. guttulatum*

were thought to be what Mathiesen (1950) had described as a variety of *L. penicillatum,* known as *L. penicillatum* f.sp. *palliati.* DNA analysis, however, showed that *L. guttulatum* is a distinct taxon, and not related to *L. penicillatum* (Jacobs *et al.,* 1999).

Recent studies have compared a large number of *Leptographium* spp. based on sequences of the ITS2 and 28S genes of the ribosomal DNA operon (Jacobs, Wingfield & Wingfield, unpublished). Large sub-unit sequence showed that all species considered are members of the Ophiostomatales and are most likely anamorphs of *Ophiostoma.* ITS sequence data confirmed that 43 species considered, represents distinct taxa. Species previously synonomised (e.g. *L. abietinum* and *L. engelmannii*) were confirmed to be the same. No clear natural groupings emerged, although pathogenic species appeared to be most closely related to each other. There was no apparent correlation between groups defined based on sequence data and those emerging from phylogenetic analysis of morphological features.

MATERIALS AND METHODS

All available herbarium type specimens, in addition to living isolates of described *Leptographium* spp. were examined in this study. Cultures of *Leptographium* spp., included in this study have been collected over a period of approximately 20 years by M.J. Wingfield. Most of these specimens were isolated during field studies in many parts of the world and others were obtained from a variety of culture collections and colleagues. Working with herbarium specimens included the typical limitations of incomplete collections and poor specimens. *Leptographium* spp. in general do not keep well as herbarium specimens due to the fact that

conidiogenous apparatuses tend to break off, or fall apart, leaving only stipes and parts of the conidiophores intact. In a small number of instances, herbarium material could not be traced or appears not to exist, and these species have not been included in this study.

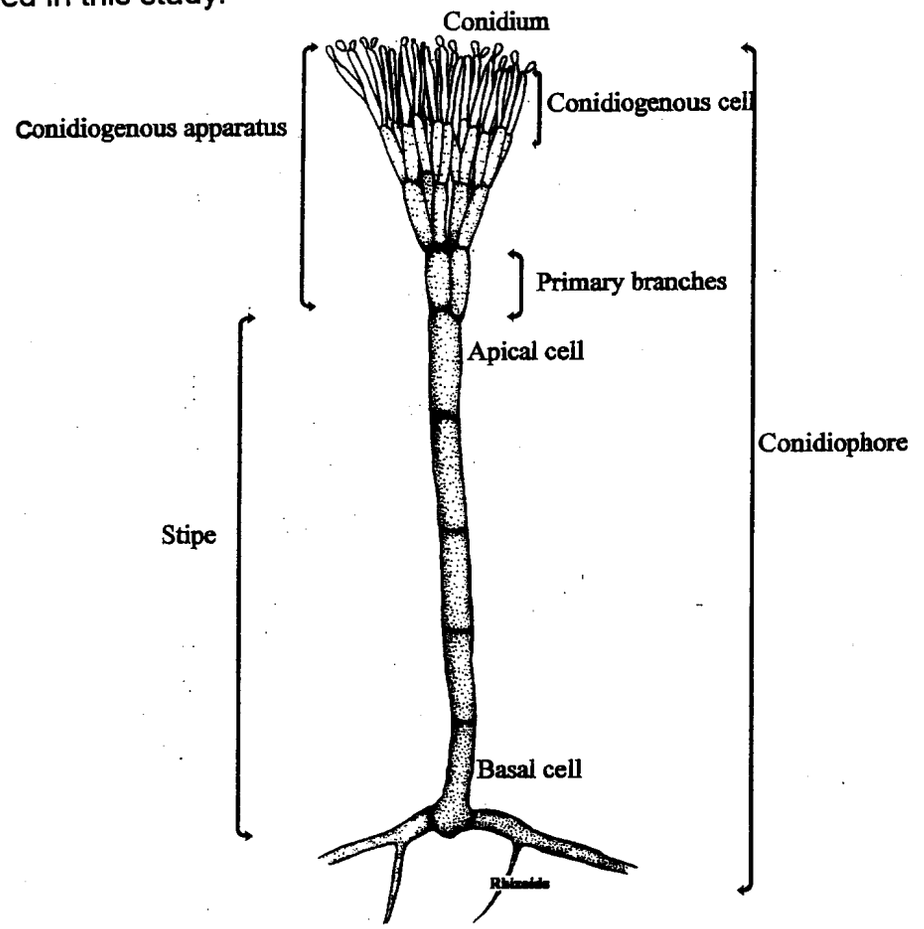

Fig. 17. Typical conidiophore of a *Leptographium* spp.

Descriptions of species were done from fungal cultures grown on 2% MEA. For microscopy, relevant structures were mounted in lactophenol, as well as in distilled water on glass slides. Herbarium specimens were examined by placing a drop of 1% KOH on the dried tissue. After five minutes, small pieces of fungal tissue were removed and mounted in lactophenol on glass slides. Fifty measurements of each relevant morphological structure were made. For some species, teleomorph structures were not produced in culture, and herbarium material included only a

small number of perithecia. In these cases, we referred back to previous studies to provide complete descriptions. Colors were determined using the colour charts of Rayner (1970). Structures that were measured and that are considered useful characteristics of *Leptographium* spp., are shown in Fig. 17.

Typical isolates of all the *Leptographium* spp. under consideration were examined using 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 JSM 840 Scanning Electron Microscope.

Isolates chosen to determine growth characteristics were those that sporulate best and are representative for the species. The optimal growth temperatures for these isolates were determined by inoculating eight MEA plates with 6.0 mm diam. agar disks taken from the actively growing margins of fresh isolates. Plates were incubated at temperatures ranging from 5 to 35 °C at 5 °C intervals. Colony diameters were measured after 4, 7 and 9 days (unless indicated otherwise) and growth was computed as an average of eight readings. Cycloheximide tolerance of these isolates was determined on MEA plates (8 per isolate) amended with 0.5g/l cycloheximide. The plates were incubated at 25°C and colony diameters were measured after 8 days.

In this study we include 46 taxa including the three varieties of *L. wageneri.* Our dichotomous key to all species includes not only morphological characteristics, but also details of hosts or substrates. This might be considered unusual but many *Leptographium* spp. are highly host or substrate specific and we argue strongly that this information is crucial to species identification. We also provide a separate dichotomous key to those species with known *Ophiostoma* states and a synoptic key to all species. We believe that the three sets of keys and detailed descriptions will make it possible for researchers to identify species of *Leptographium.*

KEY TO SPECIES BASED ON HOST AND MORPHOLOGY

1. Host/substrate non-coniferous _____ 2
   1'. Host coniferous _____ 10

2. Conidia oblong (type A) or obovoid (Type B) _____ 3
   2'. Conidia oblong, occasionally curved (Type C); colony with abundant aerial mycelia _____ *L. hughesii*

3. Conidia oblong (type A) _____ 4
   3'. Conidia obovoid (type B) _____ 5

4. Arrangement of primary branches (Type A) _____ *O. brevicolle*
   4'. Arrangement of primary branches (Type B) _____ 8

5. Arrangement of primary branches (Type B) _____ 6
   5'. Arrangement of primary branches (Type C) _____ *L. reconditum*

6. Conidiophore length: (50-)100-250(-300) µm _____ 7
   6'. Conidiophore length: (150-)250-1000(-1500) µm _____ *L. costaricense*

7. Conidiogenous cell appearing phialidic _____ *O. francke-grosmanniae*
   7'. Conidiogenous cells proliferating percurrently _____ *O. leptographioides*

8. Conidiophore length: (-50)100-300(-500) µm _____ 9
   8'. Conidiophore length: 50 - 100 µm _____ *L. calophylli*

9. Conidial size 2.5 - 5 µm; rhizoids present _____ *O. grandifoliae*
   9'. Conidial size 6 - 9 µm, rhizoids absent _____ *L. eucalyptophilum*

10. Conidia oblong to allantoid, occasionally curved (Type C) _____ 11
    10'. Conidia oblong or obovoid, occasionally ellipsoid (type A or B) _____ 12

11. Conidial size: (3-)4-6(-7) µm _____ *L. abietinum*

11'. Conidial size: (4-)6-10(-12) µm _____ *O. penicillatum*

12. Conidia obovoid to ellipsoid (type A) _____ 13

12'. Conidia obovoid (type B) _____ 28

13. Conidial size: (3-)4-8(-12) µm _____ 14

13'. Conidial size: (6-)10-20(-22) µm _____ 27

14. Conidial size: (3-)4-6(-8) µm _____ 15

14' Conidial size: (4-)6-8(-12) µm _____ 17

15. Arrangement of primary branches (Type B) _____ 16

15'. Arrangement of primary branches (Type C) _____ *L. neomexicanum*

16. Conidiophore length: (50-)100-250(-400) µm _____ 22

16'. Conidiophore length: (150-)250-1000(-1500) µm _____ *L. albopini*

17. Arrangement of primary branches (Type A) _____ *L. brachiatum*

17'. Arrangement of primary branches (Type B) _____ 18

18. Conidiophore length: (50-)100-250(-400) µm _____ 19

18'. Conidiophore length: (150-)250-1000(-1500) µm _____ 20

19. Hyphae smooth _____ *L. terebrantis*

19'. Hyphae roughened with granular appearance _____ *L. yunannensis*

20. Conidiogenous apparatus consisting of distinct series of branches, no teleomorph present _____ 21

20'. Conidiogenous apparatus consisting of a long indistinct series of branches, teleomorph in the genus *Ophiostoma* _____ *O. aureum*

21. Conidia prominently guttulate _____ *L. guttulatum*

21'. Conidia not guttulate _____ *L. wingfieldii*

22. Rhizoids present _____ 23
22'. Rhizoids absent _____ 24

23. No association with insects _____ *L. antibioticum*
23'. Associated with insects _____ 26

24. Hyphae smooth, no teleomorph present _____ 25
24'. Hyphae roughened by granular material _____ *O. crassivaginatum*

25. Prominent *Sporothrix* synanamorph present _____ *L. elegans*
25'. Prominent *Sporothrix* synanamorph absent _____ *L. sibiricum*

26. Optimal growth temperature below 20°C, colonies slow growing, associated with the conifer swift moth _____ *L. abicolens*
26'. Optimal growth temperature 25°C, associated with bark beetle activity _____ *L. euphyes*

27. Arrangement of primary branches (Type A)
Conidiophore length: (150-)250-650(-800) µm _____ *O. americanum*
27. Arrangement of primary branches (Type B)
Conidiophore length: (25-)50-250(-300) µm _____ *O. dryocoetidis*

28. Conidia 2 - 6 µm long _____ 29
28'. Conidia frequently more than 6 µm and longer _____ 38

29. Arrangement of primary branches (Type B) _____ 30
29' Arrangement of primary branches (Type C) _____ 34

30. Rhizoids present _____ 31
30'. Rhizoids absent _____ 32

31. Colonies fast growing and characterized by concentric rings in culture _____ *L. procerum*
31' Colonies slow growing, concentric rings in culture not present _____ *L. peucophilum*

32. Primary branches lower on stipes _____ *L. lundbergii*
32'. Primary branches on the apex of the stipes _____ 33

33. Conidiophore length: 50 - 100 µm,
Ophiostoma teleomorph present _____ *O. robustum*
33'. Conidiophore length: 100 - 200 µm,
Ophiostoma teleomorph absent _____ *L. pineti*

34. Isolates with distinctly serpentine hyphae _____ *L. serpens*
34'. Isolates without serpentine hyphae _____ 35

35. Only found on *Pinus* spp. _____ 36
35'. Only found on *Pseudotsuga menziesii* _____ *O. wageneri* var. *pseudotsugae*

36. Only found on *Pinus ponderosa* _____ *L. wageneri* var. *ponderosum*
36'. Only found on soft pines i.e. *Pinus*
monophylla, P. monticola and *P. sylvestris* _____ 37

37. Conidiophore length: 600 - 1000 µm _____ *L. wageneri* var. *wageneri*
37'. Conidiophore length: 100 - 600 µm _____ *L. pityophilum*

38. Conidiophores up to 400 µm long _____ 39
38'. Conidiophores frequently much longer than 400 µm _____ 42

39. *Ophiostoma* teleomorph known _____ 40
39'. No teleomorph present _____ *L. pyrinum*

40. Ascospores hat-shaped _____ 41
40'. Ascospores not hat-shaped but reniform _____ *O. laricis*

41. Perithecia with distinct neck, up to 800 µm long _____ 44
41'. Perithecia with no or very short neck _____ *O. trinacriforme*

42. Rhizoids present _____ *L. douglasii*

42'. Rhizoids absent _____ 43

43. *Ophiostoma* teleomorph present _____ *O. huntii*
43'. Teleomorph absent _____ *L. charles*

44. Hat-shaped ascospores with elongated brims, occurs on *Larix* sp. _____ *O. aenigmaticum*
44'. Hat-shaped ascospores without elongated brims, occurs on species of *Pinus* and *Picea* _____ *O. piceaperdum*

Many *Leptographium* spp. are known to have *Ophiostoma* teleomorphs. In most cases these structures are not regularly produced in culture. When the teleomorphs are present, these can aid in the identification of *Leptographium* spp. However, the absence of a teleomorph does not necessarily imply that a teleomorph does not exist.

DICHOTOMOUS KEY TO SPECIES WITH *OPHIOSTOMA* TELEOMORPHS

1. Species characterized by cucullate sheaths around the ascospores _____ 2
1'. Species characterized by curved sheaths around the ascospores _____ 11

2. Conidia of *Leptographium* state less than 5 µm long _____ 4
2'. Conidia of *Leptographium* state more than 5 µm long _____ 8

3. Perithecial necks less than 500 µm long _____ 4
3'. Perithecial necks more than 500 µm long _____ 6

4. Perithecial necks 150-500 µm in length _____ 5
4'. No obvious perithecial neck _____ *O. robustum*

5. Occurs on conifers _____ *O. brevicolle*
5'. Occurs on non-coniferous host _____ *O. francke-grosmanniae*

6. Occurs on conifers _____ 7
   6'. Occurs on non-coniferous hosts _____ *O. grandifoliae*

7. Conidia of the *Leptographium* state needle-shaped _____ *O. americanum*
   7'. Conidia of the *Leptographium* state obovoid _____ *O. serpens*

8. Perithecial necks 150-500 µm long _____ 9
   8'. Perithecial necks 500-1000 µm long _____ 10

9. Ostiolar hyphae present _____ *O. dryocoetidis*
   9'. Ostiolar hyphae absent _____ *O. penicillatum*

10. Habitat mainly on *Pinus* spp. _____ *O. wageneri*
    10'. Habitat mainly on *Larix* spp, infested with *Ips* spp. _____ *O. laricis*

11. Perithecial neck less than 500 µm long _____ 12
    11'. Perithecial necks more than 500 µm long _____ 16

12. Perithecial necks distinct and 150-500 µm long _____ 13
    12'. Perithecial neck absent _____ 15

13. Conidia of the *Leptographium* state up to 5 µm long _____ *O. aenigmaticum*
    13'. Conidia of the *Leptographium* state more than 5 µm long _____ 13

14. Habitat mostly conifers _____ *O. crassivaginatum*
    14'. Habitat non-coniferous _____ *O. leptographioides*

15. Conidiogenous apparatus with indistinct branches,
    conidial masses appearing bright yellow in culture _____ *O. aureum*
    15'. Branches of conidiogenous apparatus distinct _____ *O. trinacriforme*

16. Perithecia readily formed in culture,
    homothallic, colony with serpentine hyphae _____ *O. piceaperdum*
    16'. Perithecia not readily formed in culture, _____

heterothallic, colony with straight hyphae _____ *O. huntii*

SYNOPTIC KEY TO *LEPTOGRAPHIUM* SPECIES

Synoptic keys are not as widely used as dichotomous keys. These keys can, however, be valuable in the identification of *Leptographium* spp. Use of synoptic keys in conjunction with dichotomous keys and species descriptions, should enable the user to correctly identify species, even in the absence of the teleomorph. These keys are especially useful, where some data for important characteristics are lacking. The value of synoptic keys versus dichotomous keys was discussed in detail by Korf (1972) and the relevant arguments will not be repeated here.

The synoptic key used in this monograph has been based on those proposed and used by P.W. Leenhout (Jacobs, 1966), Korf (1972), Korf & Zhuang (1985) and Wolfaardt, Wingfield and Kendrick (1992). The key can be entered at any point. When a character has been identified, the numbers listed under the character should be noted. The user should then proceed to the next character that corresponds to the unknown species. The numbers under the second character state that do not occur in the first set of the numbers should be omitted. The user should then proceed to the next character and repeat the procedure. This should be repeated until only one or two numbers remain. The numbers correspond to species listed at the end of the key (Jacobs, 1966; Korf, 1972). The unknown species should then be compared with the description of those species

Teleomorph characters
a. Teleomorph absent: 1, 2, 4, 5, 7, 9, 11, 12, 14, 16 - 18, 21, 22, 26, 27, 29, 31 - 36, 39, 40, 43 - 46
b. Teleomorph present: 3, 6, 8, 10, 13, 15, 19, 20, 23, 24, 25, 28, 30, 37, 38, 41, 42

Perithecial characters:
Base diameter
a. 50 -100 µm: 13, 42
b. 100 -300 µm: 3, 6, 10, 15, 19, 20, 23 - 25, 28, 30, 37, 41, 42
c. 300 - 500 µm: 6, 8, 23, 24, 30, 37, 38, 41, 42

Perithecial neck
a. Absent or very short (less than 10 µm): 8, 37, 41
b. Present: 3, 6, 10, 13, 15, 19, 20, 23 - 25, 28, 30, 38, 42

Perithecial neck length
a. 50 - 100 µm: 13
b. 100 - 300 µm: 3, 10, 15, 19, 23, 25, 30
c. 300 - 500 µm: 3, 15, 23, 24, 28, 30, 38, 42
d. 500 - 700 µm: 6, 15, 20, 23, 24, 30, 38, 42
e. 700 - 900 µm: 6, 20, 23, 24, 30, 42
f. more than 900 µm: 6, 20, 24
g. no neck: 8, 37, 41

Ascospore shape
a. cucullate appearance: 3, 8, 19, 23, 30, 41
b. curved appearance: 6, 10, 13, 15, 20, 24, 25, 28, 37, 38, 42

Ascospore length
a. 2 - 4 µm: 6, 8, 19, 20, 23, 30, 37, 38, 41
b. 4 - 6 µm: 6, 3, 8, 10, 15, 20, 30, 37, 38, 41
c. 6 - 8 µm: 15, 24, 25, 28
d. more than 8 µm: 13, 24

Ascospore width
a. 1 -2 µm: 6, 10, 19, 20, 23, 38, 41
b. 2 - 3 µm: 3, 6, 8, 15, 24, 28, 30, 37, 41
c. 3 - 4 µm: 3, 8, 24, 25
d. 4 - 5 µm: 13

Anamorph characters
Hyphae
a. constricted at the septa: 3, 4, 8, 13 - 15, 18, 23, 26, 34, 38, 40, 41, 43, 45,
b. not constricted at the septa: 1 - 3, 5 - 7, 9 - 13, 16 - 46

Conidiophore length
a. less than 100 µm: 2, 9, 11, 13, 14, 15, 19, 20, 25, 27, 31, 37, 46
b. 100 - 200 µm: 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41, 45, 46
c. 200 - 400 µm: 1, 2, 3, 4, 6, 7, 8, 10, 12, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 38, 39, 40, 41, 45, 46
d. 400 - 600 µm: 2, 4 - 6, 8, 12, 14, 16, 17, 21 - 23, 26 - 29, 33, 34, 36, 38, 40 - 42, 45
e. 600 - 800 µm: 4 - 6, 8, 12, 21 - 23, 26, 27, 33, 34, 36, 38, 41 - 45
f. 800 - 1000 µm: 4, 5, 8, 22, 27, 38, 42 - 44
g. 1000 - 1500 µm: 5, 8, 22, 38, 43, 44

Stipe length
a. less than 100 µm: 1 - 3, 7 - 11, 13 - 16, 19, 20, 22, 24 - 28, 30 - 32, 35, 37, 39 - 41, 46
b. 100 - 200 µm: 1 - 4, 6 - 10, 12, 14, 15 - 36, 39 - 41, 45, 46
c. 200 - 400 µm: 1, 2, 4, 6 - 8, 10, 12, 14, 16, 17, 18, 20 - 23, 26 - 29, 33 - 36, 38, 40, 41
d. 400 - 600 µm: 2, 4 - 6, 8, 12, 14, 17, 21 - 23, 26, 27, 29, 33, 34, 36, 38, 40 - 45
e. 600 - 800 µm: 4, 5, 6, 8, 21 - 23, 34, 36, 38, 42 - 45
f. 800 - 1000 µm: 4, 5, 22, 38, 42 - 44

g. 1000 - 1500 µm: 5, 22, 38

Stipe smooth
1, 2, 4 - 12, 14 - 46

Stipe constricted at the septa
3, 13, 46

Conidiogenous apparatus length
a. 10 - 30 µm: 2, 6, 7, 9, 10, 12 - 16, 19, 20, 22, 23, 25, 27, 31, 34, 39
b. 30 - 50 µm: 1 - 20, 22, 23, 25 - 29, 31 - 34, 36 - 41, 46
c. 50 - 80 µm: 1 - 8, 11, 13 - 18, 21, 22, 24, 26 - 34, 36 - 46
d. 80 - 100 µm: 1 - 4, 8, 16, 18, 21, 22, 24, 26, 28 - 30, 33 - 36, 40 - 46
e. more than 100 µm: 4, 8, 21, 24, 26, 29, 30, 35, 40, 43, 44 - 46

Rhizoids
a. present: 1, 3, 7, 9, 14, 18, 19, 20, 22, 25, 27, 29, 31, 34 - 36, 38
b. absent: 2, 4 - 6, 8, 10 - 13, 15 - 17, 21, 23, 24, 26, 28, 30, 32, 33, 37, 39 - 46

Primary branch type
a. Type A: 6, 9, 10
b. Type B: 1 - 8, 11 - 26, 28 - 32, 34, 35, 37, 39 - 41, 45, 46
c. Type C: 27, 33, 36, 38, 42 - 44

Number of primary branches
a. 2 branches: 1 - 46
b. 2 to 3 branches: 1 - 8, 11 - 46
c. 3 to 4 branches: 4, 5, 7, 12, 21, 27, 28, 31, 33, 35, 36, 38, 42 - 45
d. 4 to 5 branches: 4, 7, 28, 33, 36, 38, 42 - 44
e. more than 5 branches: 4, 38

Primary branch length
a. less than 10 µm: 1, 2, 4, 6, 7, 9 - 11, 13 - 15, 19, 20, 22, 23, 25, 29, 31, 36, 37, 39, 42, 46
b. 10 - 15 µm: 1 - 4, 6, 7, 9 - 20, 22 - 44, 46
c. 15 - 20 µm: 1 - 4, 6, 8 - 18, 21 - 46
d. 20 - 25 µm: 1 - 4, 8, 9, 13, 14, 16 - 18, 21 - 24, 26 - 33, 34 - 36 38 - 45
e., 25 - 30 µm: 1, 3, 5, 8, 17, 18, 21 - 24, 26, 28, 30, 34, 35, 37, 40 - 44
f. 30 - 35 µm: 5, 8, 18, 21, 22, 26, 30, 34, 35, 37, 40 - 44

Secondary branch length
a. less than 10 µm: 1, 2, 4, 6, 7, 9 - 20, 22, 23, 25 - 29, 32 - 34, 36 - 40, 42 - 46
b. 10 - 15 µm: 1 - 7, 9 -11, 13 - 20, 22 - 30, 32 - 46
c. 15 - 20 µm: 2 - 6, 13, 14, 18, 21 - 24, 26 - 30, 33, 35 - 46
d. 20 - 25 µm: 3, 4, 5, 21, 24, 26, 28, 30, 35, 40 - 45
e. 25 - 30 µm: 5, 21, 24, 26, 35, 40, 43, 44
f. structure beyond primary branches long: 8

Tertiary branch length
a. less than 10 µm: 1 - 3, 6, 7, 11 - 18, 20, 22 - 24, 26 - 30, 32 -, 34, 36, 38 - 46
b. 10 - 15 µm: 1 - 7, 9, 11, 14 - 16, 18, 21 - 24, 26 - 30, 32 - 36, 38 - 46

c. 15 - 20 µm: 3 - 5, 11, 21, 23, 24, 26 - 28, 30, 35, 40, 41, 43 - 46
d. more than 20 µm: 3, 21, 24, 26, 30, 35, 40, 45, 46
e. to complex to measure: 8
f. not present: 10, 19, 25, 37

Quaternary branch length
a. less than 10 µm: 1, 2, 4 - 6, 14, 15, 18, 21 - 24, 28 - 30, 33, 34, 38, 40, 41, 45
b. 10 - 15 µm: 1, 2, 4 - 6, 15, 18, 21, 23, 24, 28 - 30, 33 - 35, 38, 41, 45, 46
c. 15 - 20 µm: 4, 5, 6, 21, 24, 30, 35, 45, 46
d. more than 20 µm: 21, 35
e. too complex: 8
f. not present: 3, 7, 9, 10 - 13, 16, 17, 19, 20, 25 - 27, 32, 36, 37, 39, 42 - 44

Conidiogenous cell length
a. less than 10 µm: 1, 6, 7, 8, 11 - 14, 17, 19, 22, 25, 27, 29, 32, 36, 37, 39, 42
b. 10 - 15 µm: 1 - 45
c. 15 - 20 µm: 1 - 9, 11, 12, 14 - 16, 18, 20 - 24, 26 - 31 33 - 42, 44 - 46
d. more than 20 µm: 1 - 6, 8, 11, 14, 16, 21, 23, 24, 26 - 28, 30, 35 - 37, 40, 41, 45, 46

Conidium shape
a. oblong to obovoid: 1, 4, 6 - 11, 13, 15, 16 - 19, 21, 22, 25, 26, 31, 34 - 39, 41 - 46
b. obovoid: 3, 5, 12, 14, 20, 23, 24, 27, 29, 30, 32, 33, 40
c. distinctly curved: 2, 22, 28

Conidial length
a. 3 - 5 µm: 1 - 7, 9 - 14, 16, 18 - 34, 36, 38 - 46
b. 5 - 7 µm: 1 - 6, 8, 10, 11, 13, 14, 17, 18, 21, 23, 24, 25, 28 - 31, 33, 35, 39 - 46
c. 7 - 10 µm: 3, 5, 6, 8, 13, 15, 17, 21, 23 - 25, 28, 30, 31, 35, 37, 40, 43, 44, 46
d. 10 - 12 µm: 6, 8, 15, 25, 31, 35, 37, 46
e. more than 12 µm: 6, 15, 37

Associated hosts/substrate
a. *Pinus* spp: 2, 4, 7, 8, 13, 18, 21, 23, 26 - 28, 30 - 35, 37, 38, 40 - 42, 44 - 46
b. *Picea* spp.: 2, 3, 9, 13, 21, 23, 26, 28, 29, 30, 34
c. *Larix* spp.: 6, 24, 26, 44
d. *Pseudotsuga* spp.: 2, 9, 14, 30, 34, 38, 40, 43
e. *Abies* spp.: 1, 5, 7, 15, 28, 34, 39, 44
f. other conifers: 7, 16
g. non-conifers: 10, 11, 17, 19, 20, 22, 25, 36

Association with insects
a. Associated with insects: 1 - 6, 8, 10, 13 - 15, 18, 19, 21, 23, 24, 26, 28, 29 - 32, 34, 35, 37 - 40, 42 - 46
b. Not associated with insects: 7, 9, 11, 12, 16, 17, 20, 22, 25, 27, 33, 36, 41

Optimum growth temperature
a. 15 °C: 1, 29
b. 20 °C: 3, 5, 6, 8, 14, 31, 33, 36, 42 - 44
c. 25 °C: 2, 4, 9, 12, 15, 16, 18 - 24, 26, 27, 30, 32, 34, 35, 37 - 41, 45, 46
d. 30 °C: 7, 10, 11, 17, 25, 28

Ratio of the conidium length: width

a. 1.5:2:35, 37
b. 2:1: 3, 12, 17 - 19, 29, 33, 34, 38 - 40, 45, 46
c. 2.5:1: 1, 4, 7 - 11, 13, 14, 20, 22 - 24, 27, 30 - 32, 36, 41 - 44
d. 3:1: 5, 21, 25, 28
e. 4:1: 6
f. 5:1: 15
g.4:3: 26

1. *L. abicolens*
2. *L. abietinum*
3. *O. aenigmaticum*
4. *L. albopini*
5. *L. alethinum*
6. *O. americanum*
7. *L. antibioticum*
8. *O. aureum*
9. *L. brachiatum*
10. *O. brevicolle*
11. *L. calophylli*
12. *L. costaricense*
13. *O. crassivaginatum*
14. *L. douglasii*
15. *O. dryocoetidis*
16. *L. elegans*
17. *L. eucalyptophilum*
18. *L. euphyes*
19. *O. francke-grosmanniae*
20. *O. grandifoliae*
21. *L. guttulatum*
22. *L. hughesii*
23. *O. huntii*
24. *O. laricis*
25. *O. leptographioides*
26. *L. lundbergii*
27. *L. neomexicanum*
28. *O. penicillatum*
29. *L. peucophilum*
30. *O. piceaperdum*
31. *L. pinidensiflorae*
32. *L. pineti*
33. *L. pityophilum*
34. *L. procerum*
35. *L. pyrinum*
36. *L. reconditum*
37. *O. robustum*
38. *O. serpens*
39. *L. sibiricum*
40. *L. terebrantis*
41. *O. trinacriforme*
42. *O. wageneri* var. *ponderosum*
43. *L. wageneri* var. *pseudotsuga*
44. *L. wageneri* var. *wageneri*
45. *L. wingfieldii*
46. *L. yunnanensis*

GENERIC DESCRIPTION FOR *LEPTOGRAPHIUM*

Leptographium Lagerb. & Melin Svenska Skogsvårdsföreningens Tidskrift. **25**, 249 1927.

= *Scopularia* Preuss. 1851.

= *Hantzschia* Auersw. 1862.

= *Verticicladiella* S. Hughes. *Canadian Journal of Botany* **31**, 653. 1953.

Teleomorph: *Ophiostoma* Sydow & P. Sydow. *Annales Mycologici* **17,** 43. 1919.

= *Rostrella* Zimmerm. *Meded's Lands Plantentuin* **37,** 24. 1900.

= *Endoconidiophora* Münch *Naturw. Zeitschrift Forst und Landw.* **6,** 34. 1908.

= *Linostoma* Von Höhnel. *Annales Mycologia* **16,** 91. 1918.

= *Grosmanniae* Goidanich. *Boll. Staz. Pat. Veg. Roma.* **16,** 26. 1936.

= *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. *Mycologia* **67,** 800. 1975.

Etymology: Lep-to-grá-phi-um: a thin, small brush. From the greek adjective, λεπτος: thin and the greek noun γραφισν: a small brush. The generic name refers to the conidiophores of the genus that resemble small brushes.

Known distribution: U.S.A., Canada, Europe, Japan, East Asia, South Africa, Central Africa, New Zealand, Australia and Mauritius.

Conidiophores occurring singly or in groups of up to eight, arising directly from the mycelium or on aerial mycelium, erect, macronematous, mononematous, 30 - 1350 µm in length, rhizoid-like structures present or absent. *Stipes* smooth or occasionally constricted at septa, cylindrical, simple, 0-18 septate, apical and basal cells occasionally swollen. *Conidiogenous apparatus* 15 - 200 µm long, excluding the conidial mass, with 2 to 4 series of cylindrical branches, 2-6 primary branches,

cylindrical or barrel shaped, 0-2 septate. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex. Conidium development occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed secession giving the false impression of sympodial proliferation (Minter *et al.*, 1982; 1983; Van Wyk *et al.*, 1988). *Conidia* hyaline, aseptate, obovoid to broadly ellipsoid with truncated ends and rounded apices occasionally prominently curved, 3 - 22 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus. *Sporothrix* synanamorph only present in *Leptographium elegans.*

Colonies with optimal growth temperatures between 15°C and 30°C on 2% MEA. Able to withstand high concentrations of cycloheximide with no more than 80% reduction in growth on 0.5 g/l cycloheximide. Colony colour ranging from cartridge buff (19'f) to olivaceous (21''m). *Colony margins* smooth, laciniate, sinuate or effuse. *Hyphae* submerged on solid medium with very sparse aerial mycelium to abundant aerial mycelium in some species, olivaceous (21''m) to hyaline, smooth or roughened by granular material, straight, in certain cases serpentine, occasionally constricted at the septa.

Perithecial bases black, globose and smooth walled, unornamented or sparsely ornamented, 143 - 420 µm in diam., necks present or absent, necks dark brown to black, cylindrical with a slight apical taper, smooth, 117 - 1700 µm long, *ostiolar hyphae* present or absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* reniform, allantoid, cucullate or pillow -shaped, aseptate, hyaline, invested in a sheath, 3 - 11 µm.

Hosts/substrate: *Abies* spp., *Calophyllum* sp., *Chamaecyparis* sp., *Eucalyptus* spp., *Fagus* spp., *Larix* spp., *Melia* spp., *Parashorea* sp., *Picea* spp., *Pinus* spp., *Populus* spp., *Pseudotsuga* spp., *Quercus* spp., *Talauma* sp., *Triticum* rhizosphere, *Tsuga* spp.

Associated animals: **Nematodes**: *Bursaphelenchus* spp. **Insects**: **Coleoptera: Scolytidae**: *Dendroctonus* spp., *Dryocoetus* spp., *Ips* spp., *Hylastes* spp., *Hylurgops*

spp., *Myelophilus* spp., *Orthotomicus* spp., *Pachylobius* spp., *Pityogenes* spp., *Pityokteines* spp., *Pityophthorus* spp., *Polygraphus* spp., *Tomicus* spp., *Trypodendron* spp., *Xyleborus* spp. **Coleoptera: Lymexylidae**: *Hylecoetus* spp. **Coleoptera: Curculionidae**: *Hylobius* spp., *Pissodes* spp., *Steremnius* spp. **Coleoptera: Cerambycidae**: *Tetropium* spp., *Monochamus* spp. **Hymenoptera: Agaonidae:** *Blastophagus* spp. **Lepidoptera: Hepialidae**: *Korscheltellus* spp.

Type: *Leptographium lundbergii* (PREM 50548). See detailed description on page 220

SPECIES DESCRIPTIONS

1. ***Leptographium abicolens*** K. Jacobs & M.J. Wingf., *MycoScience* 1999. **(Figs. 18-20).**

Teleomorph: Not known.

Etymology: a-bi-có-lens: inhabiting the fir. 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.

Conidiophores occurring singly or in groups of up to six, arising directly from the mycelium, erect, macronematous, mononematous, (120-) 160 - 196 (-360) µm in length, rhizoid-like structures present. *Stipes* dark olivaceous, smooth, cylindrical, simple, 2 - 11 septate, (72-) 92 - 239 (-264) µm long, 3.0 - 6.0 µm wide below primary branches, apical cell not swollen, 4.5 - 7.5 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (32-) 56.5 - 68 (-104) µm long, excluding the conidial mass, with 3 - 4 series of cylindrical branches, 2 - 3 primary branches, olivaceous to light olivaceous, smooth, cylindrical, aseptate, (8-) 12 - 14.5 (-31) µm long and 3.0 - 5.0 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous to hyaline, aseptate, (7.0-) 6.0 - 12 (-15) µm long, 2.0 - 4.0 µm wide, tertiary branches hyaline, aseptate, (6.0-) 7.5 - 10 (-12) µm long, 2.0 - 4.0 µm wide, quaternary branches aseptate, hyaline, (5.0-) 7.5 - 9.0 (-10) µm long, 2.0 - 4.0 µm wide. *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. *Conidia*, aseptate, broadly ellipsoidal to obovoid, (4.0-) 5.0 (-7.0) x (2.0-) 2.0 (-3.0) µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

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. Colonies dark olivaceous (19"f). *Colony margin* smooth. *Hyphae* submerged or on top of agar with abundant aerial mycelium, light olivaceous to hyaline, smooth, not constricted at the septa, 1.0 - 6.0 µm diameter.

Specimens examined: Holotype: U.S.A., Vermont, White Face Mountain, *Abies balsamea,* 30 August 1990, collected D.R. Bergdahl, PREM 56336. **Paratype**: U.S.A., Vermont, White Face Mountain, *Abies balsamea,* 30 August 1990, collected D.R. Bergdahl, PREM 56337. **Cultures:** CMW 2865; CMW 2866, CMW 2894, from *A. balsamea* roots wounded by *K. gracilus*, collected D.R. Bergdahl, White Face Mountain, New York, USA, August 1990.

Known distribution: North Western United States.

Hosts/substrate: *Abies balsamea* (Jacobs *et al*, 1999).

Associated insects: *Korscheltellus gracilus* (Jacobs *et al.*, 1999).

Notes: *Leptographium abicolens* closely resembles *L. antibioticum* (Jacobs *et al.*, 1999). These species can, however, be distinguished based on the darker stipes and more complex conidiophores of *L. abicolens. Leptographium abicolens* has 2-3 primary branches, whereas up to five primary branches have been observed in *L. antibioticum.* Furthermore, *L. antibioticum* has an optimal growth temperature of 25-30 °C compared to the 15 °C of *L. abicolens. Leptographium abicolens* also can be distinguished from *L. antibioticum* by its larger, broadly ellipsoidal conidia (4 - 7µm), in contrast to the smaller obovoid to oblong conidia (2.5 - 5 µm) in the latter species.

Leptographium abicolens occurs at high elevation sites together with *L. peucophilum.* The low optimal temperatures for growth of these fungi in culture are consistent with their habitat. This species is, furthermore, associated with the feeding activities of the larval stage of the conifer swift moth. The fungi appear to enter the roots of spruce and firs through the wounds created by the moths. This species does not appear to be pathogenic, although large areas of discoloration are associated with the wounds caused by the moths (Jacobs *et al.,* 1999).

It is not known whether *L. abicolens* is carried by the adult moths, and even if it were, these moths never enter the roots of the host plant. It is possible that this species is soil inhabiting and colonizes roots through the wounds made by the insects. Conidia

of this species may also be transmitted by phoretic mites associated with the conifer swift moth, although this is only a hypothesis (Jacobs *et al.*, 1999).

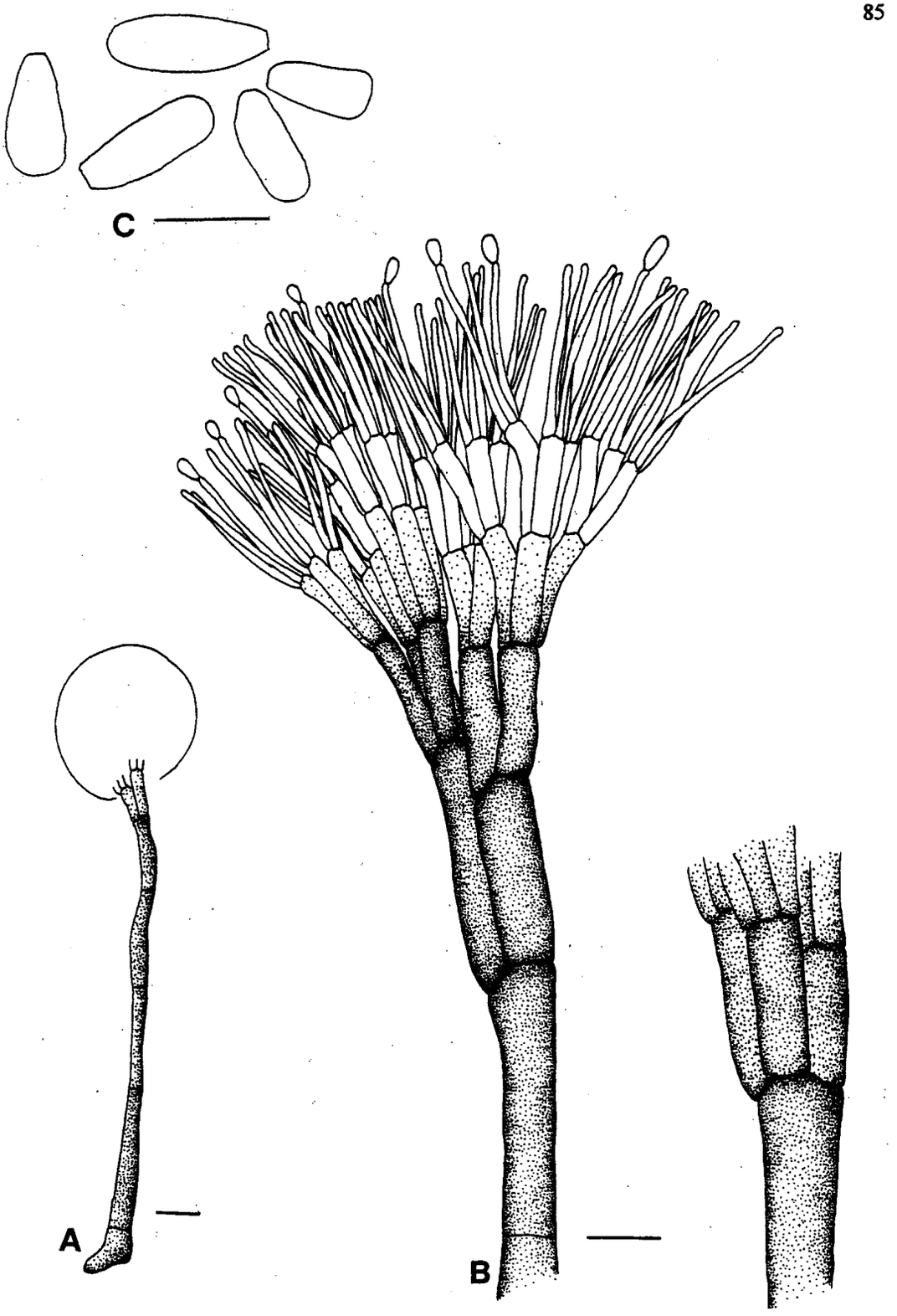

Fig. 18. Conidiophores and conidia of *L. abicolens* (PREM 56336). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 5 µm) **C.** Conidia (Bar = 5 µm).

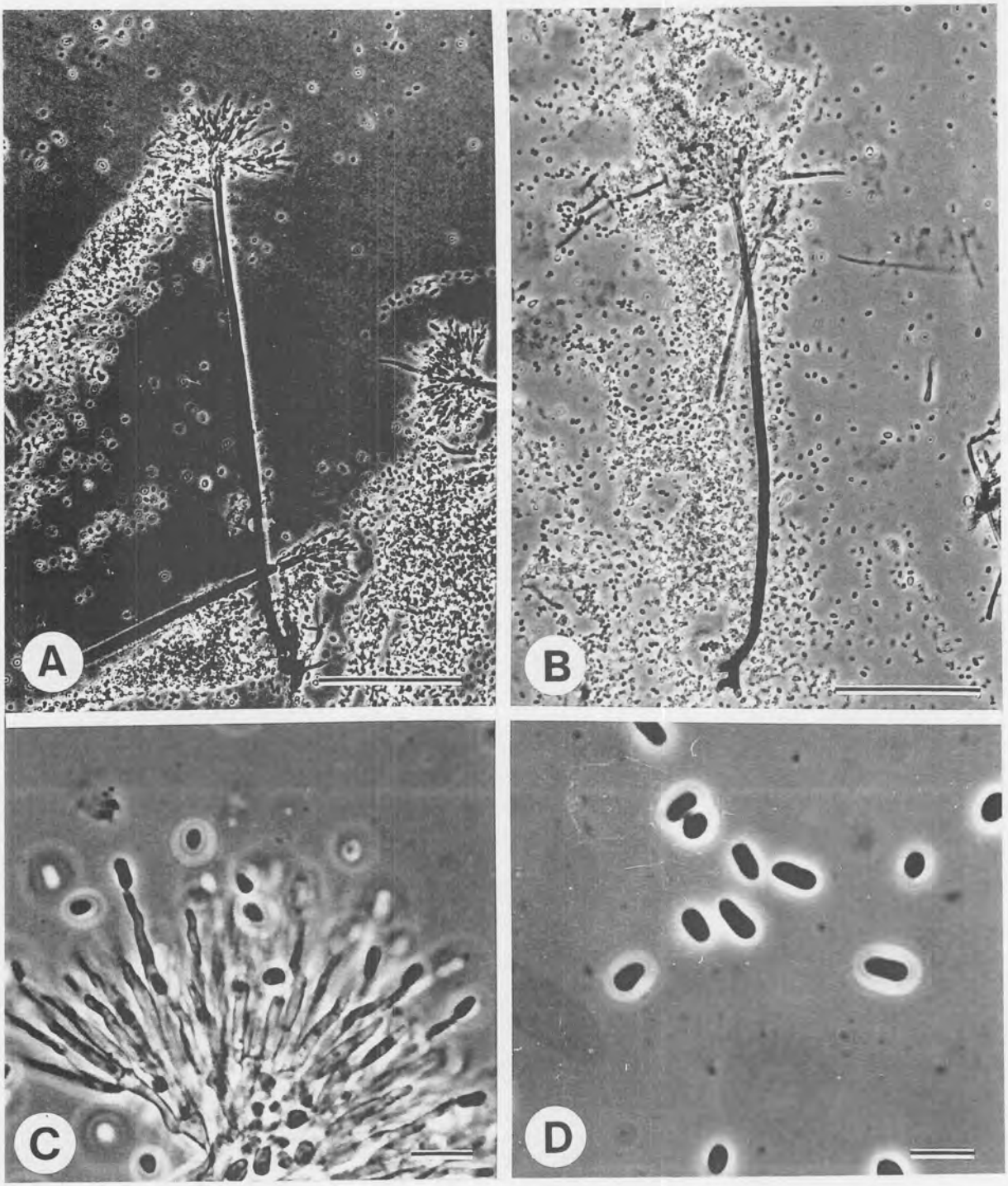

Fig 19. Light micrographs of the conidiophores and conidia of *L. abicolens* (PREM 56336). **A.** Conidiophore (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 100 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

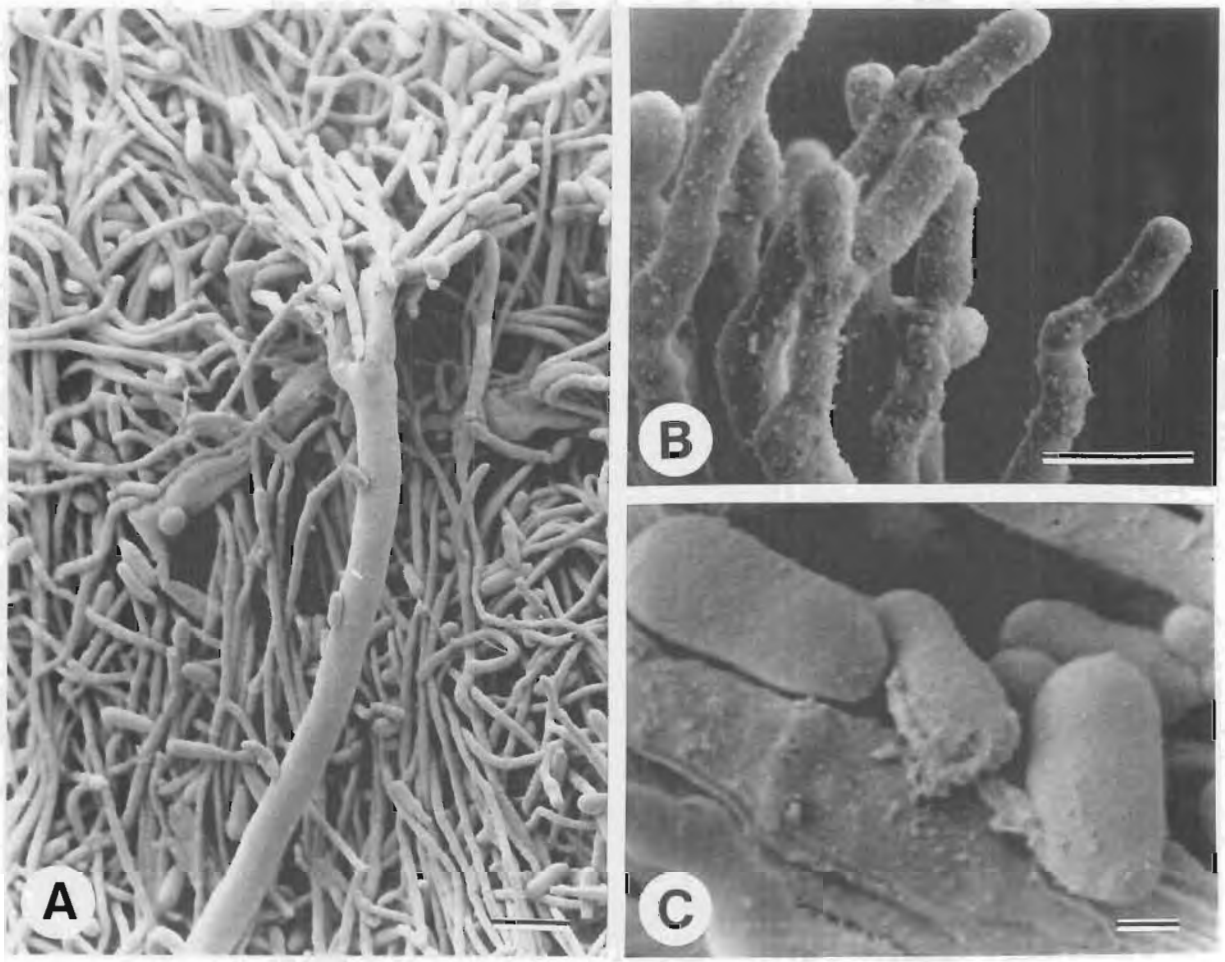

Fig. 20. Scanning electron micrographs of the conidiophores and conidia of *L. abicolens* (PREM 56336). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

2. *Leptographium abietinum* (Peck) M.J. Wingf., *Transactions of the British Mycological Society* **85**, 92. 1985. **(Figs. 21-23).**

≡ *Verticicladiella abietina* (Peck) S. Hughes, *Canadian Journal of Bot*any **31**, 653. 1953.

≡ *Sporocybe abietina* Peck, *New York State Museum Report* **31**, 45. 1879.

≡ *Periconia abietina* (Peck) Sacc., *Sylloge Fungorum*, **4**, 273. 1886.

= *Leptographium engelmannii* R.W. Davidson, *Mycologia* **47**, 59. 1955.

Teleomorph: Not known.

Etymology: a-bi-e-tí-num: belonging to the fir. From the Latin noun abies: fir. This specific epithet was intended to refer to its occurrence on *Abies*. Kendrick (1962) noted, however, that the name for this species is misleading, as this fungus has never been reported on any species of *Abies*.

Conidiophores occurring singly or in groups of up to eight, arising directly from the mycelium, erect, macronematous, mononematous, 74 - 535 (-570) µm in length, rhizoid-like structures absent. *Stipes* olive-buff (21'''b), smooth, cylindrical, simple, 2-7 septate, 37 - 442 (-471) µm long, 4.0 - 9.0 µm wide below primary branches, apical cell not swollen, 3.0 - 9.0 µm wide at base, basal cell swollen. *Conidiogenous apparatus* (25-) 45 - 50 (-99) µm long, excluding the conidial mass, with 2 - 4 series of cylindrical branches, 2-3 primary branches, olive-buff (21'''b), smooth, cylindrical, aseptate, (8-) 13.5 - 15 (-22) µm long and 3.0 - 6.5 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, aseptate, (7.5-) 10 -12.5 (-15.5) µm long, 2.5 -5.0 µm wide, tertiary branches hyaline, aseptate, (6.0-) 7 - 11 (-13) µm long, 2.0 - 4.0 µm wide, quaternary branches aseptate, (4.0-) 7.0 - 11 (-12.5) µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, tapering slightly from the base to the apex, (10.5-) 10 -23 (-25) µm long and 1.0 - 2.0 µm wide. *Conidia* hyaline, aseptate, distinctly curved at the base, (3.5-) 4.5 - 5.0 (-7.0) x 1.0 - 2.5 µm, marginal frill absent. Conidia accumulating in white slimy droplets at the apex of conidiogenous apparatus, turning cream (19'f) when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 39 mm in diam. in 8 days. Little growth at 5°C and no growth above 35°C. Able to withstand high concentrations of cycloheximide with a 17% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies cartridge buff (19"f). *Colony margin* laciniate. *Hyphae* submerged on agar with little aerial mycelium, hyaline, smooth, straight, not constricted at the septa, (2.0-) 2.0 - 3.5 (-5.0) µm diam.

Specimens examined: Holotype: Canada, Albany, on the bark and wood of spruce, *Picea mariana,* collected: Peck, DAOM 33942. **Paratypes:** Canada, Victoria, *Picea engelmanii*, 20 March 1953, collected: A. Molnar, DAOM 37980; British Colombia, McGillivray Lake, *Pseudotsuga menziesii*, 20 June 1958, collected: C. Cotrell, DAOM 64328 (DAVFP 11869). *L. engelmannii*: U.S.A., *Picea engelmanii,* collected: R. W. Davidson, US0 422466. **Cultures**: Utah, Dixie Nature Forest, *Picea engelmanii*, 1993, T.C. Harrington, C699 (same as CMW 2817; CTH 0054), Canada, *Picea* sp., Aug. 1994, collected M.J. Wingfield, CMW 3083 (PREM 56382); British Columbia, Victoria, *Picea engelmannii,* 1987; collected: A. Molnar, CMW 275, CMW 276.

Known distribution: Northern United States and Canada.

Hosts/substrate: *Picea mariana* (Kendrick, 1962), *P. engelmannii* (Davidson, 1955; Solheim, 1995b), *P. glauca* (Solheim, 1995b); *Pseudotsuga menziesii* (Mielke, 1979; Lewinsohn *et al.,* 1994; Solheim & Krokene, 1998), *Pinus contorta, P. sylvestris, P. ponderosa, P. aristata, P. mugo* (Mielke, 1979), *P. monticola* (Kulhavy *et al.*, 1978; Mielke, 1979).

Associated insects: *Dendroctonus pseudotsugae* (Harrington, 1988; Perry, 1991; Lewinsohn *et al.,* 1994; Solheim & Krokene, 1998), *D. rufipennis* (Davidson, 1955; Kendrick, 1962; Harrington, 1988; Perry, 1991; Reynolds, 1992; Solheim, 1995a,b), *Hylastes longicollis* (Harrington, 1982; 1988), *Hylurgops porosus* (Wagner, 1977), *Hylurgops planirostris* (Harrington, 1988).

Notes: Hughes (1953) named this species as the type of *Verticicladiella* based on its sympodial and apparently unique mode of conidium development. It was later

transferred to *Leptographium* by Wingfield (1985) after he found that the conidium development in the genera *Verticicladiella* and *Leptographium* could not be distinguished from each other. *Leptographium engelmannii* has similar conidia and hosts to *L. abietinum* and the two species were thought to be synonymous (Harrington, 1988). This hypothesis was supported by isozyme comparisons (Zambino & Harrington, 1992). A thorough morphological study later led to the two species being synonomised (Jacobs *et al.*, 1999).

Leptographium abietinum is morphologically similar to *L. hughesii,* but these fungi can be distinguished based on differences in colony morphology and host specificity. In addition, *L. abietinum* has conidia that are obviously curved, whereas *L. hughesii* has obovoid conidia. *Leptographium hughesii* is native to Asia, occurring on non-coniferous hosts, while *L. abietinum* is known only in North America where it occurs on spruce (Harrington, 1988).

Leptographium abietinum is not considered to be pathogenic, although a low level of pathogenicity to spruce has been demonstrated (Reynolds, 1992). The bark beetle *Dendroctonus rufipennis,* in association with this fungus can cause blue-stain of Lutz spruce (*Picea* x *lutzii*) in North America (Reynolds, 1992) as well as widespread mortality (Werner, 1995). *Leptographium abietinum* is also associated with various other bark beetles and has been shown to be weakly pathogenic to Ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga menziesii*) (Harrington & Cobb, 1983). Ross and Solheim (1995; 1996; 1997) indicated that this species might be able to kill healthy Douglas-fir trees and assist the Douglas-fir beetle (*Dendroctonus pseudotsugae*) in overcoming host defenses. It did, however, not prove to be the most pathogenic associate of *D. rufipennis* on spruce (Solheim, 1995a,b; Solheim & Safranyik, 1997).

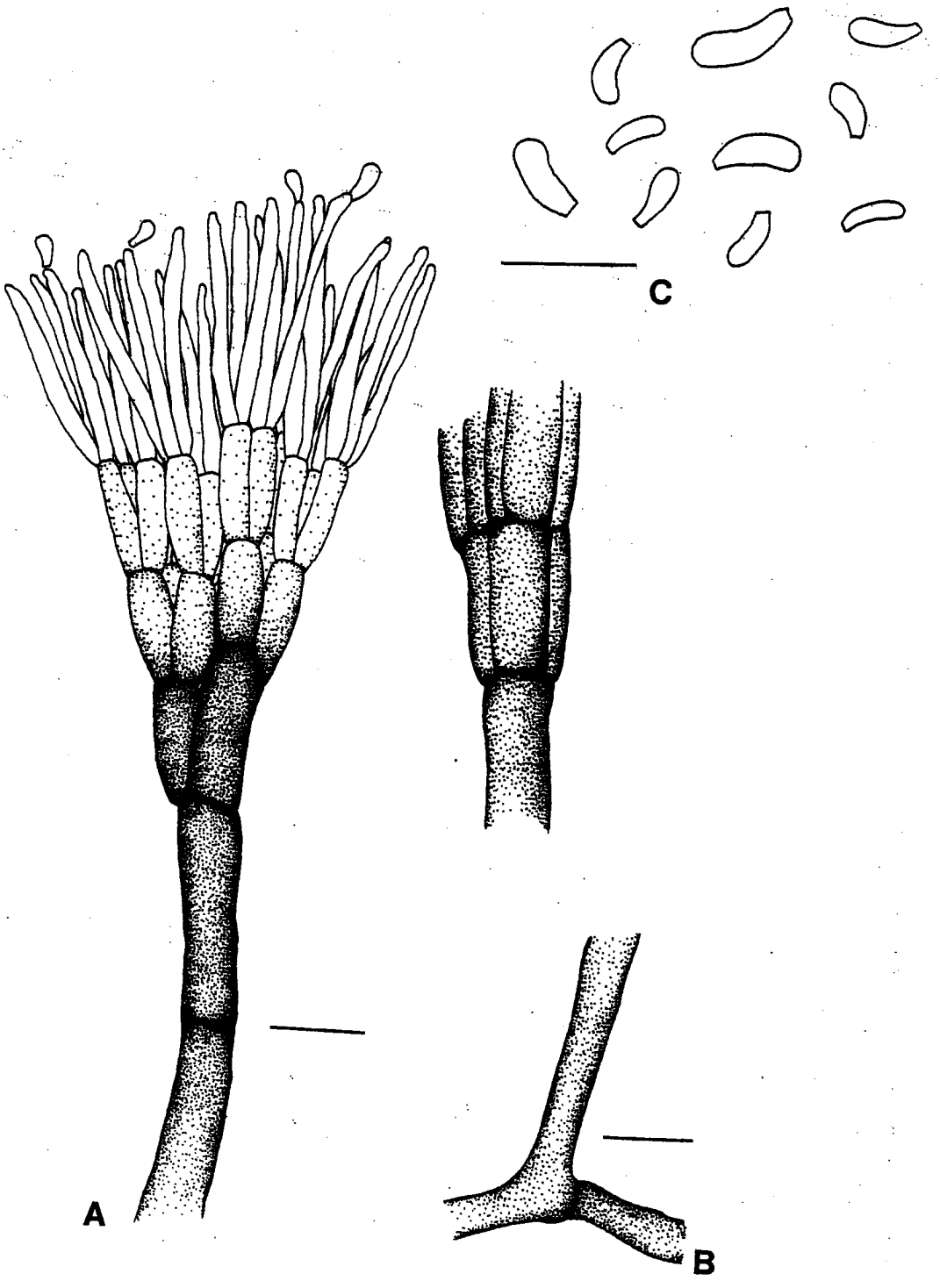

Fig. 21. Conidiophores and conidia of *L. abietinum* (CMW 2817). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

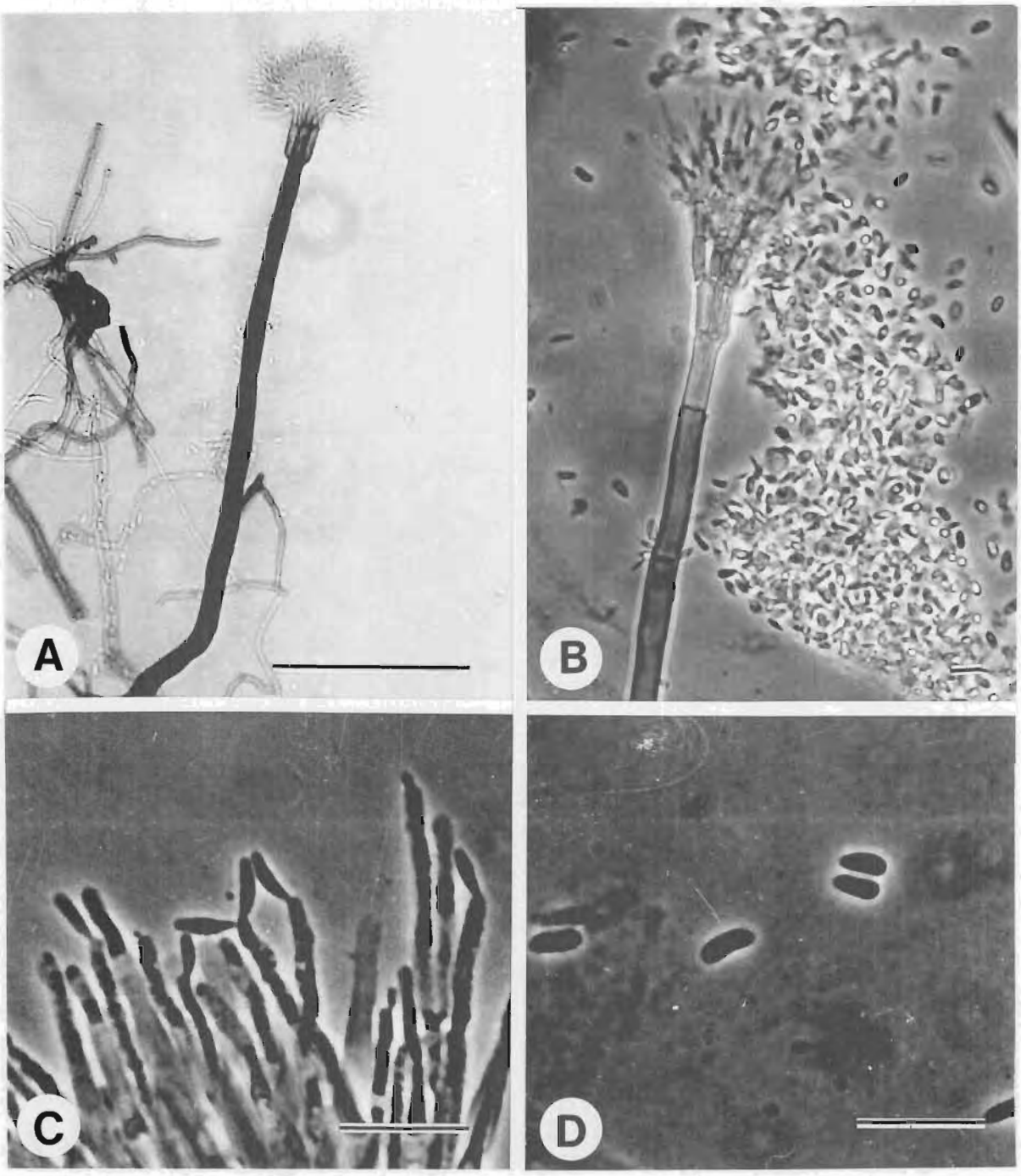

Fig. 22. Light micrographs of the conidiophores and conidia of *L. abietinum* (CMW 2817). **A.** Conidiophore (Bar = 100 µm). **B**. Conidiogenous apparatus (Bar = 10 µm). **C**. Conidiogenous cells (Bar = 10 µm). **D**. Conidia (Bar = 10 µm).

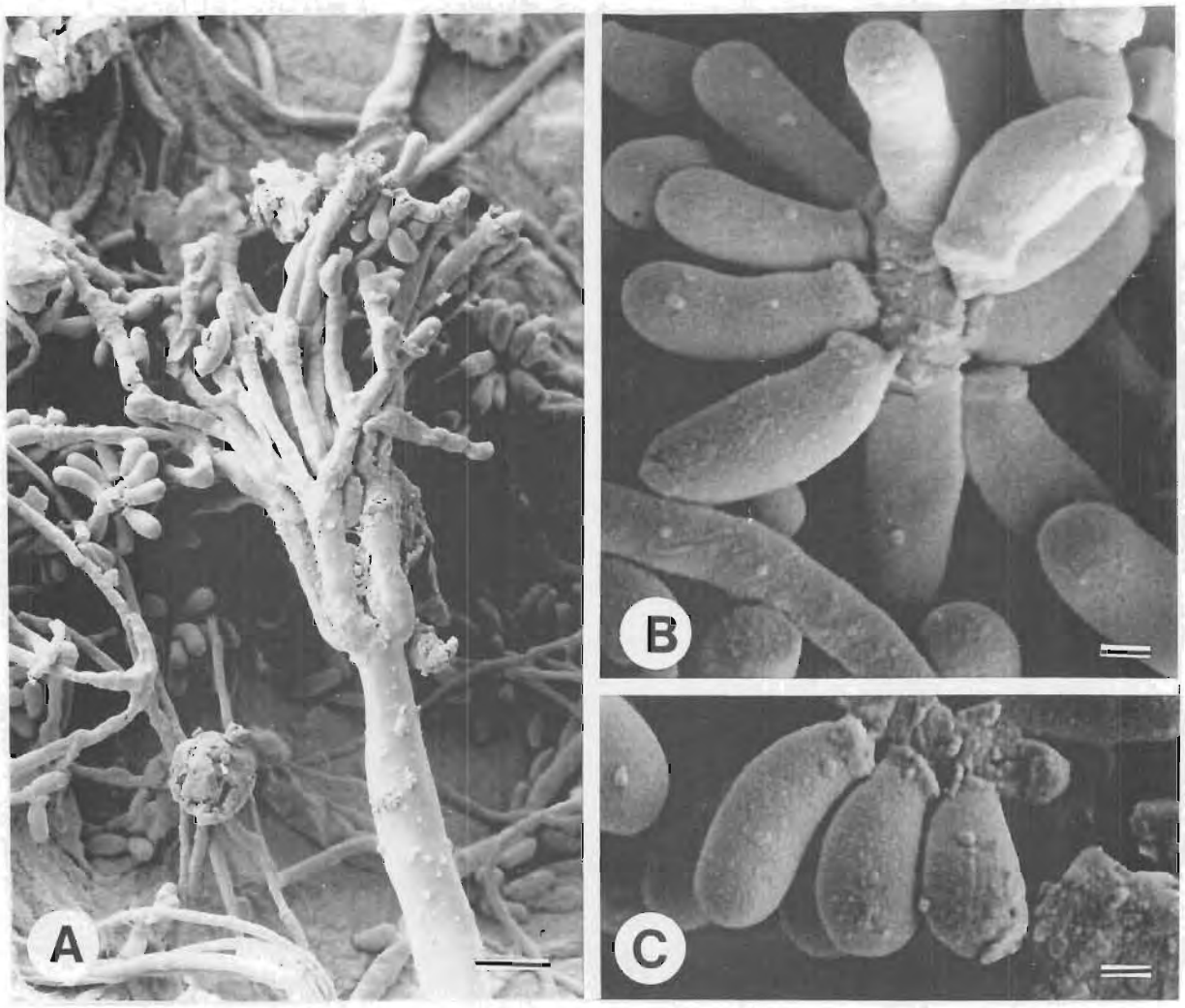

Fig. 23. Scanning electron micrographs of the conidiophores and conidia of *L. abietinum* (CMW 2817). **A.** Conidiophore (Bar = 10 µm). **B**. Conidiogenous cells (Bar = 1 µm). **C**. Conidia (Bar = 1 µm).

3. *Ophiostoma aenigmaticum* K. Jacobs *et al.*, *Mycological Research* **102**, 291. 1998. **(Figs. 24-26).**

Anamorph: *Leptographium aenigmaticum* K. Jacobs *et al.*, *Mycological Research* **102**, 291. 1998.

Etymology: ae-nig-má-ti-cum: enigmatic. From the Greek αινιγμα: a riddle. This specific epithet refers to the enigma surrounding its occurrence. While it is found alongside *O. piceaperdum* that occurs mainly in Europe, *O. aenigmaticum* is restricted to Japan.

Perithecial bases black, globose and smooth walled, with abundant hyphal ornamentation, 143 - 254 µm in diam. *Perithecial neck* dark brown to black, cylindrical with a slight apical taper, smooth, 117 - 310 µm long, 37 - 99 µm above globose base, 19 - 43 µm wide at the apex, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* cucullate in side view, aseptate, hyaline, invested in a sheath, 4 - 5 x 1.8 - 3.5 µm (Jacobs *et al.*, 1998).

Conidiophores occurring singly or in groups of up to 4, arising directly from the mycelium, erect, macronematous, mononematous, 117 - 229 µm in length, rhizoid-like structures absent. *Stipes* olivaceous (21"m), constricted at septa, cylindrical, simple, 1-6 septate, (40-) 91.5 - 113.5 (-170) µm long, 4.0 -9.0 µm wide below primary branches, apical cell not swollen, (8.0-) 10 - 12.5 (-15.5) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (34-) 54 - 76 (-95) µm long, excluding the conidial mass, with 2 to 4 series of cylindrical branches, 2 - 3 primary branches, olivaceous (21"m), smooth, cylindrical aseptate, (11-) 14.5 - 23.5 (-32.5) µm long and (2.0-) 4.0 - 5.0 (-6.0) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, 0-1 septate, 11 - 23 µm long, 2.0 - 5.0 µm wide, tertiary branches hyaline, aseptate, 9.0 - 22 µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (10-) 15.5 - 18 (-23) µm long and 2.0 - 2.5 µm wide. *Conidia* hyaline, aseptate, obovoid with truncate ends and rounded apices, 4.0 - 9.0 x 2.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at

first, becoming white with age. Conidial mass white when wet, remaining white when dry.

Colonies with optimal growth at 20°C on 2% MEA, reaching 40 mm in diam. in 9 days. No growth below 5°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 11% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies cartridge buff (19"f) to light olivaceous (19"k). *Colony margin* effuse. *Hyphae* submerged on agar with little aerial mycelium, hyaline, smooth, straight, sometimes constricted at the septa, 2.0 - 8.0 µm diam.

Specimens examined: Holotype: Japan, Hokkaido, *Picea jezoensis*, June 1990, collected: Y. Yamaoka, PREM 54680. **Paratypes**: Japan, Hokkaido, *Picea jezoensis*, June 1990, collected: Y. Yamaoka, PREM 54681, PREM 54682, PREM 54683. **Cultures**: Japan, Hokkaido, *Picea jezoensis*, June 1990, collected: Y. Yamaoka, CMW 2199, CBS 501.96, CMW 2310, CBS 502.96, CMW 2311, CBS 503.96, CMW 2200, CBS 504.96.

Known distribution: Japan.

Hosts/substrate: *Picea jezoensis* Niijima (Jacobs *et al.*, 1998).

Associated insects: *Ips typographus* f. *japonicus* (Jacobs *et al.*, 1998).

Notes: *Ophiostoma aenigmaticum* was initially thought to be similar to *O. penicillatum,* but can easily be distinguished based on conidial and ascospore morphology. *Ophiostoma aenigmaticum* is characterized by obovoid conidia and cucullate ascospores, in contrast to the allantoid conidia and curved ascospores of *O. penicillatum.* This fungus is morphologically similar to *O. piceaperdum* and *O. huntii.* It can, however be distinguished from these species based on the elongated brims of the sheath of the ascospores. The anamorph structures of *O. aenigmaticum* are also smaller than those of *O. piceaperdum* and *O. huntii* (Jacobs *et al.,* 1998).

Ophiostoma aenigmaticum has been isolated from spruce as part of a project to

describe the associated fungi of *Ips typographus* f. *japonicus* in Japan. This insect is similar to its European counterpart, *Ips typographus* and the insects share some fungal symbionts such as *O. penicillatum* and *O. piceaperdum* (Yamaoka *et al.*, 1997; Jacobs *et al.*, 1998). *Ophiostoma aenigmaticum* is, however, unique to *I. typographus* f. *japonicus* and has not been reported outside Japan.

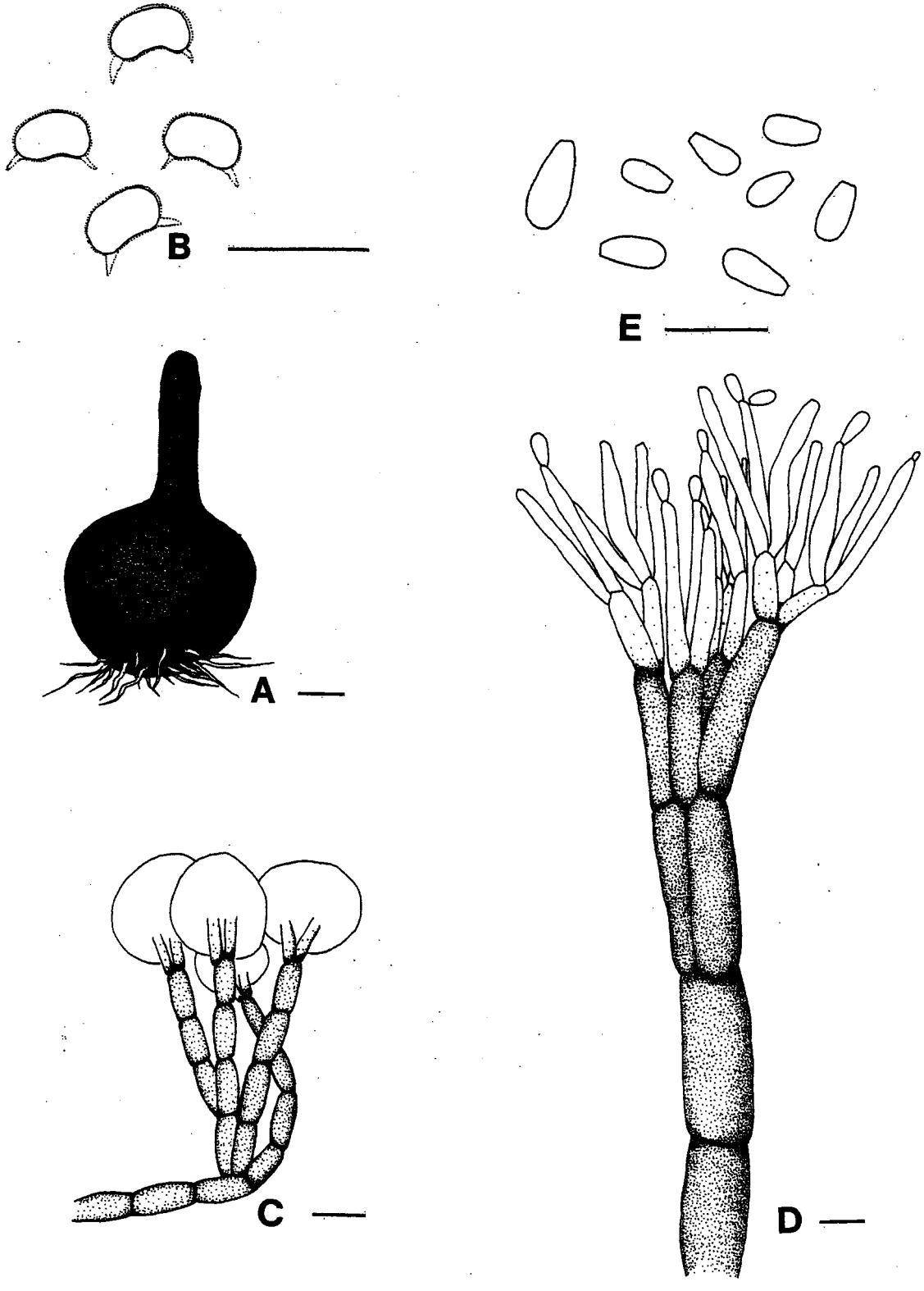

Fig. 24. Teleomorph and anamorph of *O. aenigmaticum* (PREM 54680). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

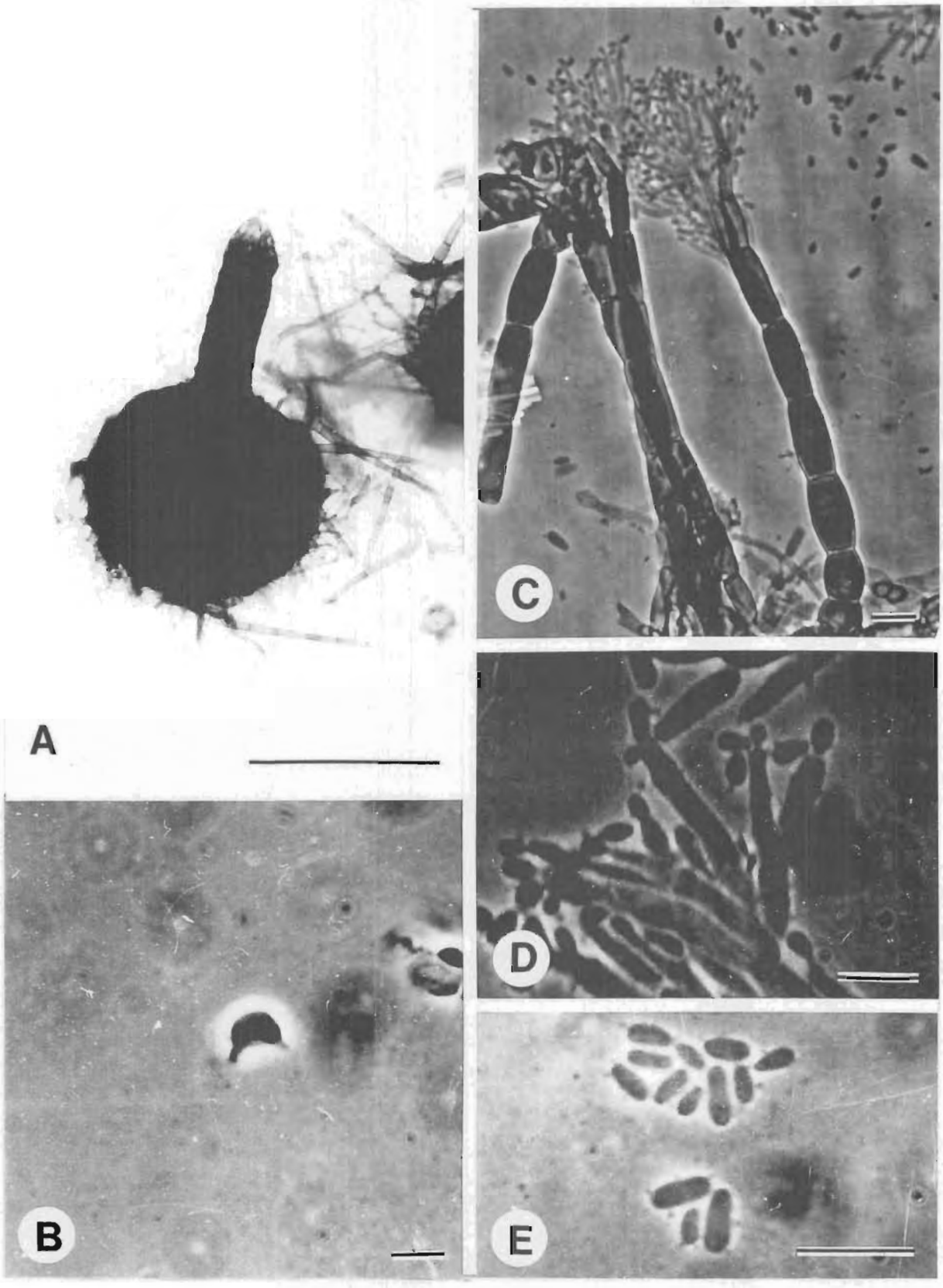

Fig. 25. Light micrographs of the teleomorph and anamorph structures of *O. aenigmaticum* (PREM 54680). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

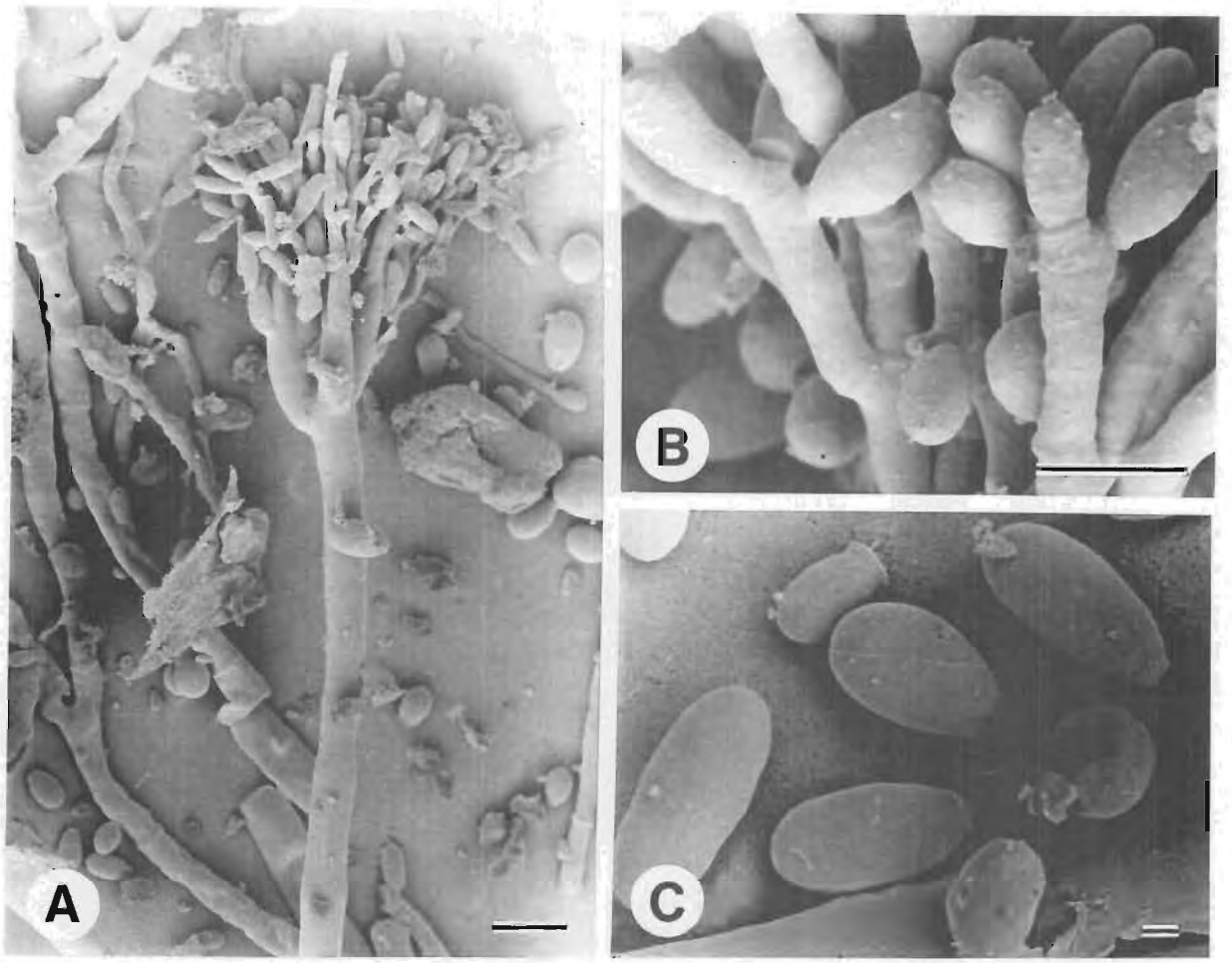

Fig. 26. Scanning electron micrographs of the conidiophores and conidia of *O. aenigmaticum* (PREM 54680). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

4. *Leptographium albopini* M.J. Wingf., T.C. Harr. & Crous *Canadian Journal of Botany* **72**, 234, 1994. **(Figs. 27-29).**

Teleomorph: Not known.

Etymology: al-bo-pí-ni: of the white pine. From the Latin adjective albus: white and Latin noun pinus: a pine tree. This specific epithet refer to *Pinus strobus* (white pine), which is the host of the fungus.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (168-) 431 - 567 (-936) µm in length, rhizoid-like structures absent. *Stipes* olivaceous (21"m), smooth, cylindrical, simple, 4 - 13 septate, (104-) 90.5 - 758 (-856) µm long, (4.5-) 7.5 - 10 (-12.5) µm wide below primary branches, apical cell not swollen, (4.5-) 7.5 - 10 (-15.5) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (32-) 70 - 81 (-152) µm long, excluding the conidial mass, with 3 - 4 series of cylindrical branches, 2-6 primary branches, olivaceous, smooth, cylindrical to barrel shape, aseptate (6.0-) 16 - 23 (-35) µm long and 4.0 - 13 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, aseptate, (7.0-) 11.5 - 17 (-25) µm long, 2.5 - 8.0 (-12) µm wide, tertiary branches hyaline, aseptate, 10 - 20 µm long, 2.0 - 5.0 µm wide, quaternary branches, hyaline, aseptate, (8.0-) 11 - 14 (-20) µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, cylindrical, 11 - 30 µm long and 1.5 - 3.0 µm wide. *Conidia* hyaline, aseptate, oblong to obovoid, 4.0 - 5.0 (-7.0) x 1.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, turning honey-yellow (19") when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 40 mm in diam. in 9 days. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 17% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15""k). *Colony margin* smooth. *Hyphae* submerged on agar with no aerial mycelium, olivaceous (21"m), rough, thick walls, straight, frequently constricted at the septa, (4.5-) 6.5 - 8.5 (-12.5) µm diam.

Specimens examined: Holotype: U.S.A., Blue Ridge Parkway, Virginia, *Pinus strobus* roots infested by insects, 1980, collected: A. Lackner, PREM 51450. **Paratypes**: U.S.A., Mesa Verde, Colorado, *Pinus edulis* roots infested with insects, 1984, collected: R. Wagener, PREM 51452, PREM 51453. U.S.A Coweeta Hydrological Experiment Station near Otto, North Carolina, *Pinus edulis* roots infested by insects, 1985, collected: T.D. Leininger, PREM 51454, PREM 51455. **Cultures**: U.S.A., Blue Ridge Parkway, Virginia, *Pinus strobus*, 1980, collected: A Lackner, CMW 2868, CMW 2065 (PREM 56383), CMW 41.

Known distribution: U.S.A.

Hosts/substrate: *Pinus strobus*, *P. edulis* (Wingfield *et al.*, 1994).

Associated insects: *Hylastes* sp. (Wingfield *et al.*, 1994).

Notes: This fungus is associated with the roots of conifers and this is considered to be a distinguishing character (Wingfield *et al.* 1994). *Leptographium albopini* superficially resembles *L. procerum,* but can be distinguished by its conidiophores that are produced singly and not in groups. The colonies of *L. procerum* are also characterized by concentric rings when grown on 2% MEA. These rings are not observed in the colonies of *L. albopini.* *Leptographium albopini* also superficially represents *L. serpens,* but can be distinguished based on the absence of serpentine hyphae, that are characteristic of the latter species (Wingfield *et al.,* 1994).

Leptographium albopini is associated with root-feeding bark beetles, and is, therefore, also isolated from the roots of conifers, especially pine (Wingfield *et al.,* 1994). Nothing is known about the pathogenicity of *L. albopini* and there is no evidence to suggest that it is pathogenic.

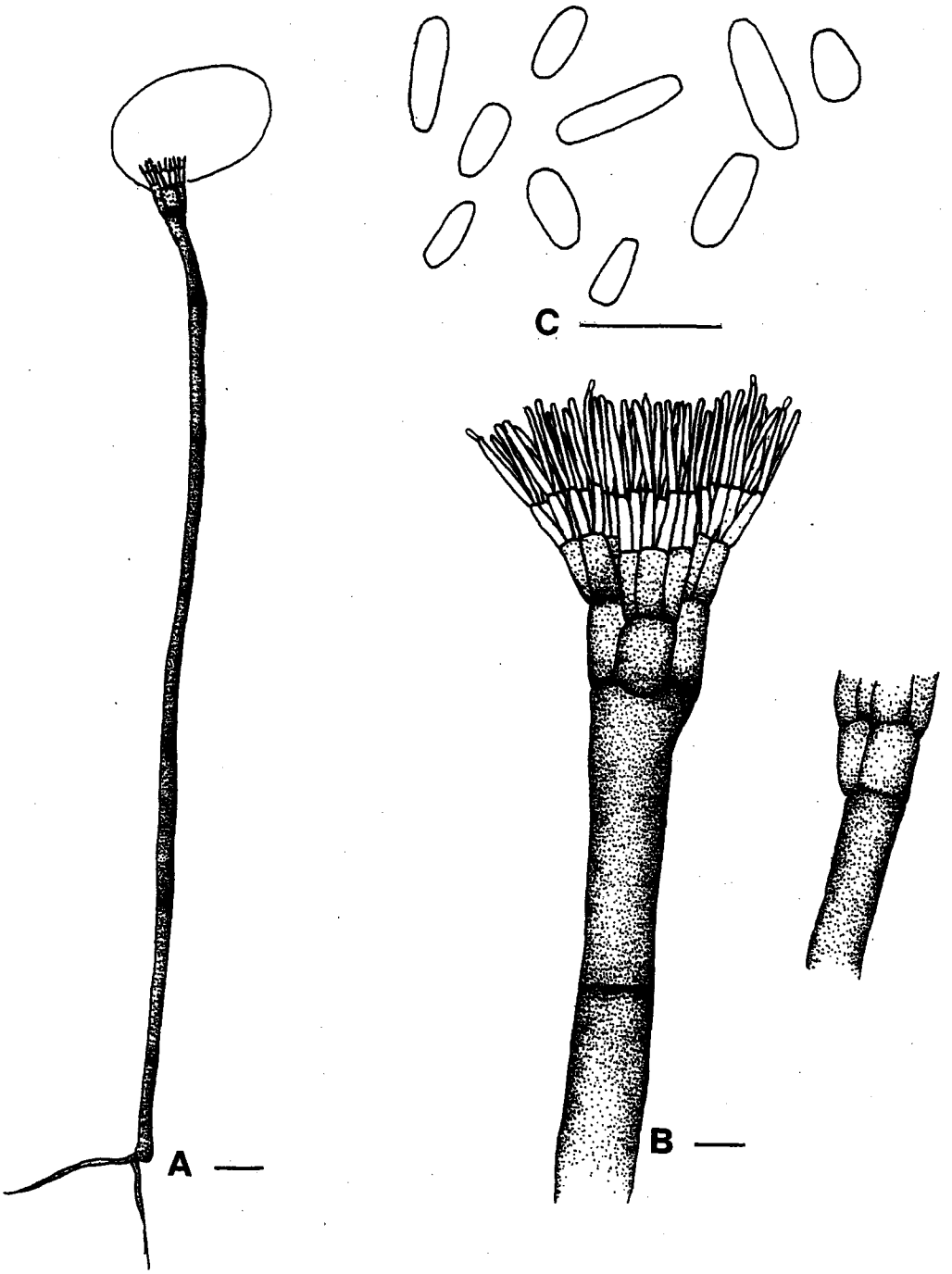

Fig. 27. Conidiophores and conidia of *L. albopini* (PREM 56383). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

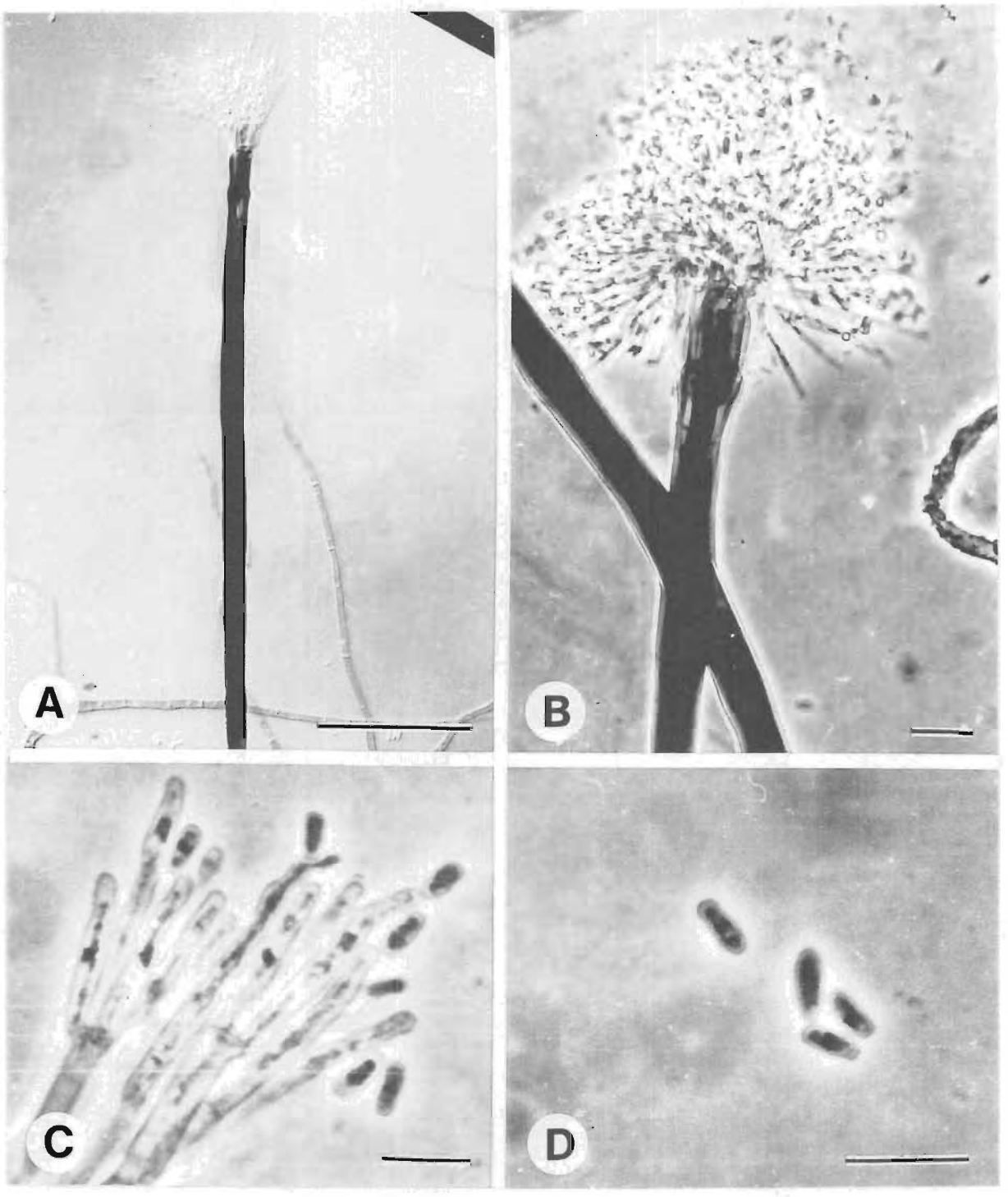

Fig. 28. Light micrographs of the conidiophores and conidia of *L. albopini* (PREM 56383). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

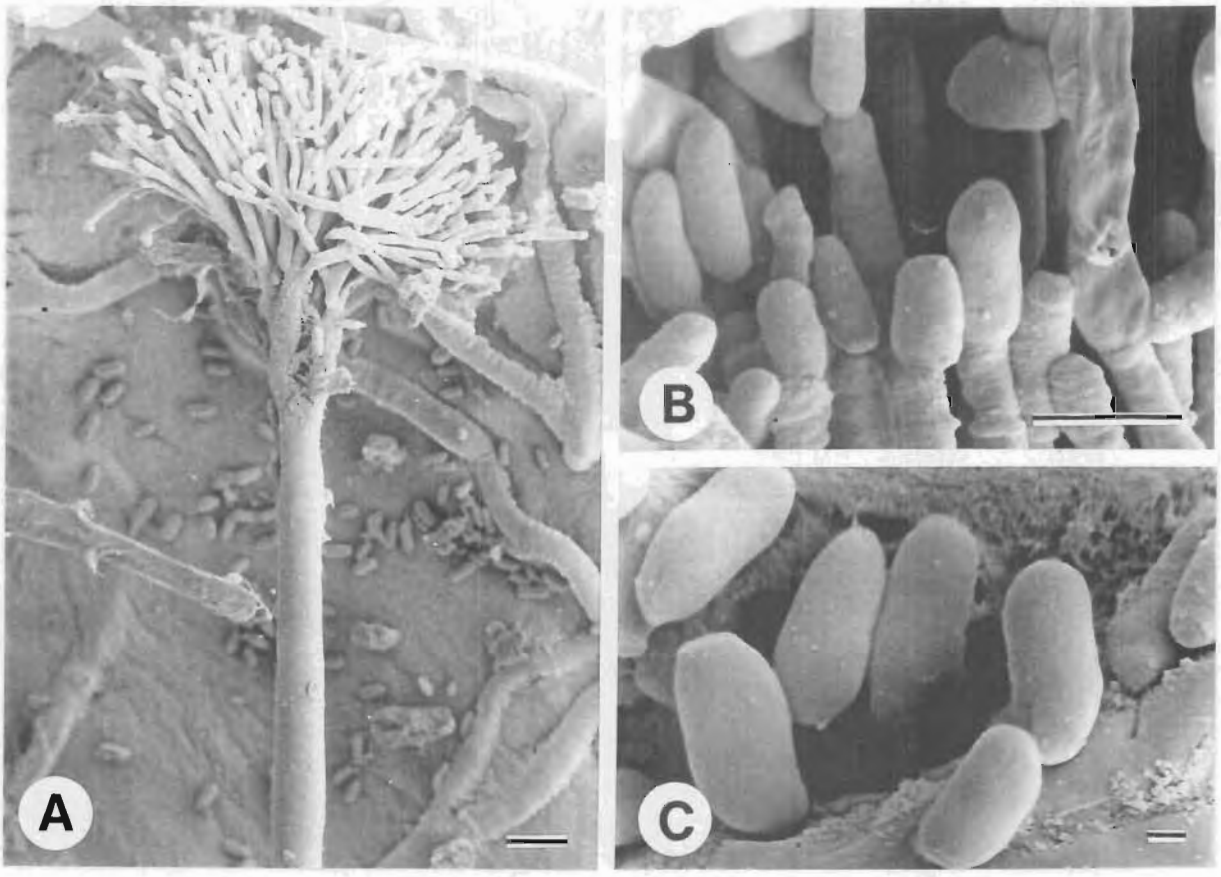

Fig. 29. Scanning electron micrographs of the conidiophores and conidia of *L. albopini* (PREM 56383). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

5. *Leptographium alethinum* K. Jacobs, M.J. Wingf. & A. Uzunovic, *Mycological Research* 1999. **(Figs. 30-32).**

Teleomorph state: Not known.

Etymology: a-le-thí-num: genuine. From the Greek adjective αληθινος: real, genuine. This specific epithet refers to the well-developed conidiophores of this fungus which is characteristic of a true *Leptographium.*

Conidiophores occurring singly or in groups of up to six, arising directly from the mycelium, erect, macronematous, mononematous, (560-) 636.5 - 1270 µm in length, rhizoid-like structures absent. *Stipes* dark olivaceous, smooth, cylindrical, simple, 6 - 10 septate, (500-) 562 - 1150 µm long, 10 - 12.5 µm wide below primary branches, apical cell not swollen, 10 - 15 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (60-) 75 - 146 (-170) µm long, excluding the conidial mass, with 3 - 4 series of cylindrical branches, 2-4 primary branches, olivaceous, smooth, cylindrical, aseptate, (25-) 42.5 - 32 (-55) µm long and (5.0-) 6.0 - 10 (-13) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches olivaceous to hyaline, aseptate, 12 - 30 (-33) µm long, 3.0 - 7.5 (-9.0) µm wide, tertiary branches hyaline, aseptate, 10 - 20 µm long, 2.0 - 5.0 µm wide, quaternary branches aseptate, hyaline, 8.0 - 17 µm long, 2.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 12 -22 (-23) µm long and 1.0 - 3.0 µm wide. *Conidia*, aseptate, obovoid with truncate ends, (4.0-) 5.0 - 7.0 (-9.0) x 2.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Colonies with optimal growth at 20°C on 2% MEA, reaching 23 mm in diameter in 6 days. Little growth below 5°C and no growth 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 20°C in the dark. Colonies olivaceous (19"f). *Colony margin* smooth. *Hyphae* submerged with no aerial mycelium, olivaceous to light olivaceous, smooth, not constricted at the septa, (2.0-) 3.0 - 9.0 (-12) µm diameter.

Specimens examined: Holotype: England, *Hylobius abietis* galleries, collected A.

Uzunovic, PREM 56349. **Paratypes:** *Hylobius abietis* galleries, England, collected A. Uzunovic, PREM 56348, PREM 56350. **Cultures:** *Hylobius abietis* galleries, England, collected A. Uzunovic; CMW 3766, CMW 3767, CMW 3765, CMW 3764, *Hylobius abietis* galleries, England, collected A. Uzunovic, Corsican pine, England, collected: J.N. Gibbs. CMW 2159.

Known distribution: England.

Hosts/substrate: *Abies* spp. (Jacobs *et al.,* 1999).

Associated insects: *Hylobius abietis* (Jacobs *et al.,* 1999).

Notes: *Leptographium alethinum* is morphologically similar to *L. procerum.* The most obvious distinguishing character in these species is the absence of the characteristic concentric rings typically formed in agar colonies of *L. procerum. Leptographium alethinum* can further be distinguished from *L. procerum* based on the absence of rhizoids, whereas these structures are prominent in isolates of *L. procerum.* Furthermore, the conidia of *L. alethinum* are obovoid, but slightly longer (4 - 9 µm) than those of *L. procerum* (3 - 5 µm).

Leptographium alethinum is morphologically also similar to *L. douglasii* (Wingfield *et al.,* 1994). *Leptographium alethinum* can be distinguished from *L. douglasii* based on its considerably longer conidiophores (560 - 1270 µm) than those found in cultures of *L. douglasii* (57 - 512 µm). *Leptographium alethinum* is also characterized by primary branches that are almost twice as long as those of *L. douglasii* and the absence of rhizoids, which are present in *L. douglasii.*

Leptographium alethinum occurs in the same habitat as *L. procerum,* and is therefore, associated with similar insects. However, nothing is known regarding the pathogenicity of *L. alethinum* although we expect that it might be mildly pathogenic or saprophytic.

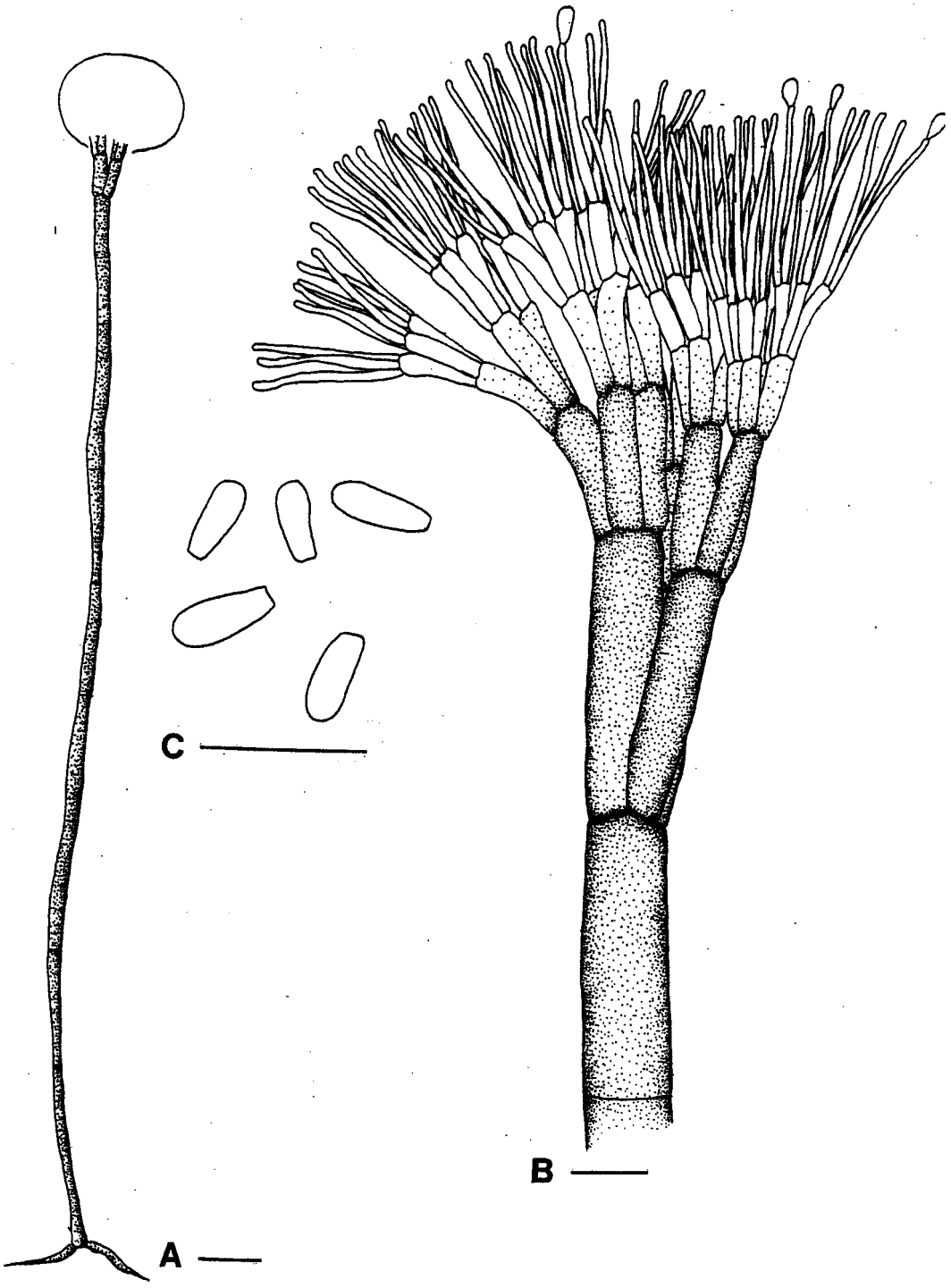

Fig. 30. Conidiophores and conidia of *L. alethinum* (PREM 56349). **A.** Habit sketch (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

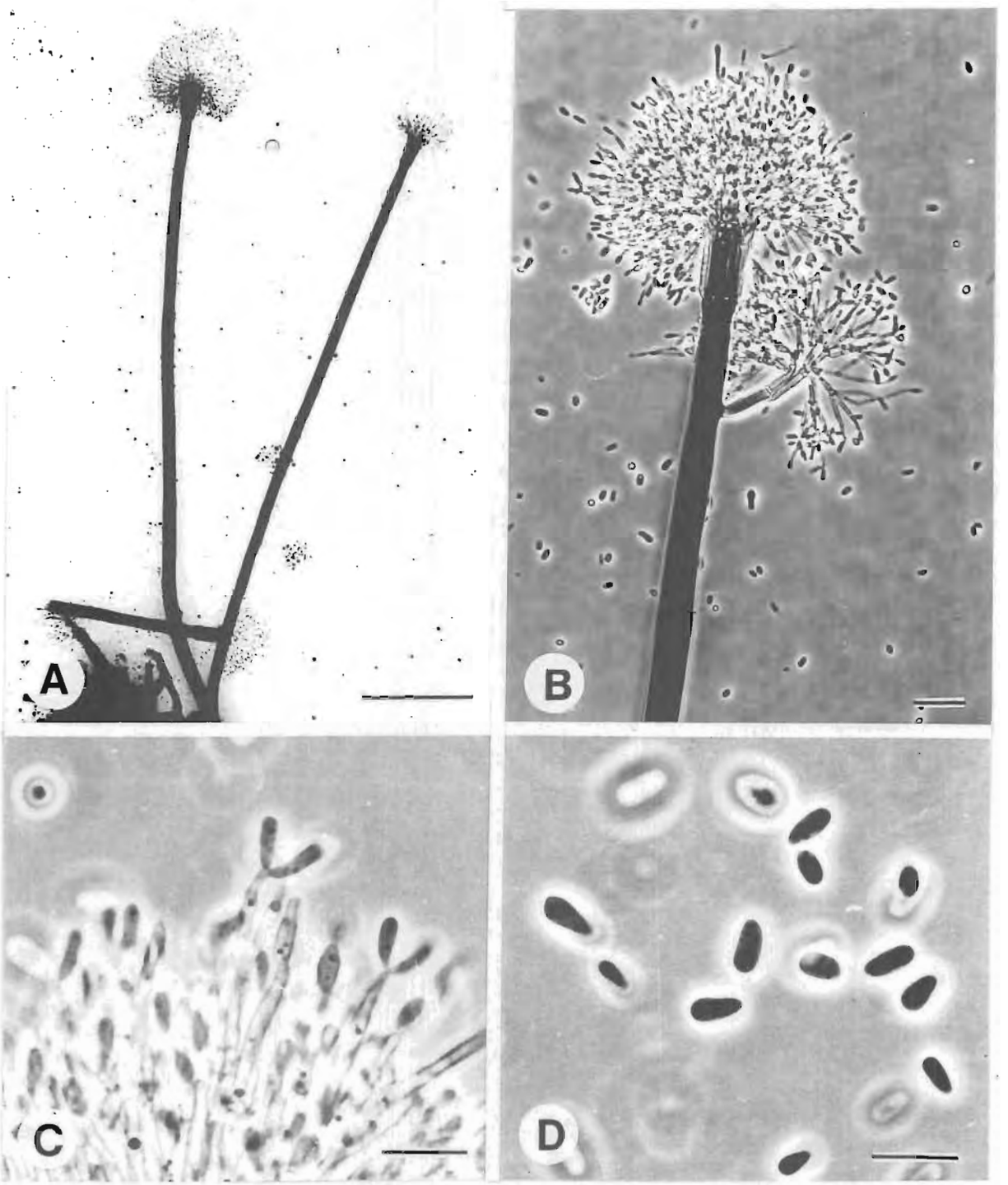

Fig 31. Light micrographs of the conidiophores and conidia of *L. alethinum* (PREM 56349). **A.** Conidiophore (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 20 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

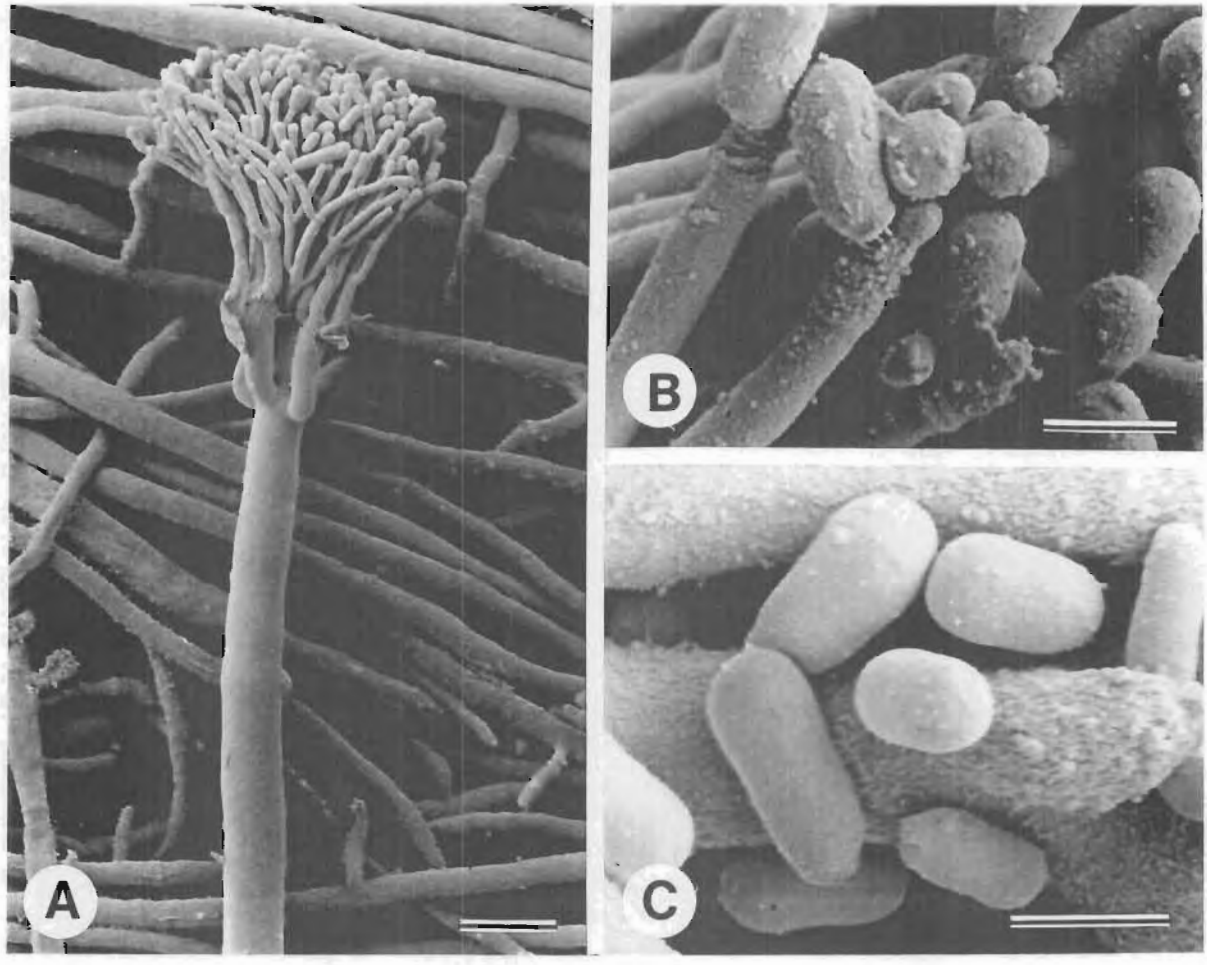

Fig. 32. Scanning electron micrographs of the conidiophores and conidia of *L. alethinum* (PREM 56349). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 5 µm).

6. *Ophiostoma americanum* K. Jacobs & M.J. Wingf. *Canadian Journal of Botany* **75,** 1316. 1997. **(Figs. 33-35).**

Anamorph: *Leptographium americanum* K. Jacobs & M.J. Wingf. *Canadian Journal of Botany* **75,** 1316. 1997.

Etymology: a-me-ri-cá-num: connected with America. This specific epithet refers to the origin of this fungus in North America.

Perithecial bases black, globose and smooth walled, sparsely ornamented, (200-) 283 (-370) µm in diam. *Perithecial neck* dark brown to black, cylindrical with a slight apical taper, smooth, (690-) 1027.5 (-1300) µm long, (50-) 60.5 (-70) µm above globose base, (20-) 25.5 (-40) µm wide at the apex, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* reniform, aseptate, hyaline, invested in a sheath, (3.0-) 4.5 (-5.5) x (1.0-) 1.5 (-2.5) µm. Sheaths not uniform, giving the ascospores a rectangular appearance (Jacobs *et al.,* 1997).

Conidiophores occurring singly or in groups of up to 9, arising directly from the mycelium, erect, macronematous, mononematous, (149-) 212 - 453 (-731.5) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), smooth, cylindrical, simple, 5 - 15 septate, (108.5-) 185 - 391 (-691) µm long, 3.0 - 6.0 µm wide below primary branches, apical cell not swollen, 4.5 - 11 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (25-) 45 - 53.5 (-77.5) µm long, excluding the conidial mass, with 3 to 5 series of cylindrical branches, 2 primary branches, light olivaceous (21"k), smooth, cylindrical to barrel shape, 0-1 septate (9.0-) 12.5 - 15 (-20) µm long and (3.0-) 4.0 - 6.0 (-8.0) µm wide, arrangement of the primary branches on the stipe - type A, secondary branches light olivaceous (21"k), aseptate, cylindrical to barrel shape 8.0 - 15 (-20) µm long, 3.0 - 6.0 µm wide, tertiary branches light olivaceous (21"k), aseptate, (6.0-) 8.0 -10.5 (-15.5) µm long, 1.5 - 6.0 µm wide, quaternary branches aseptate, (4.0-) 8.0 - 11.5 (-20) µm long, (1.0-) 2.5 -3.0 (-5.0) µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (8.0-) 8.5 - 21 (-30) µm long and 1.0 - 3.0 µm wide. *Conidia* hyaline, aseptate, oblong to obovoid, 3.5 - 22 x 1.0 - 3.0 µm. Conidia accumulating in slimy

droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 20°C on 2% MEA, reaching 45 mm in diam. in 9 days. No growth below 5°C and little growth above 35°C. Able to withstand high concentrations of cycloheximide with a 5% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies olivaceous (21''m). *Colony margin* effuse. *Hyphae* submerged on agar with abundant aerial mycelium, hyaline, smooth, straight, not constricted at the septa, 1.5 - 6.0 µm diam.

Specimens examined: Holotype: U.S.A., Vermont, *Larix laricina*, May, 1994, collected: D.R. Bergdahl and M.J. Wingfield, PREM 54866. **Paratypes**: U.S.A., Vermont, *Larix laricina*, May, 1994, collected: D.R. Bergdahl and M.J. Wingfield, PREM 54867, PREM 54868, PREM 54869. **Cultures**: U.S.A., Vermont, *Larix laricina*, May, 1994, collected: D.R. Bergdahl and M.J. Wingfield, CMW 495 (CBS 497.96), CBS 498.96, CBS 499.96; CBS 500.96.

Known distribution: Northern United States.

Hosts/substrate: *Larix decidua* (Jacobs *et al.*, 1997).

Associated insects: *Dendroctonus simplex* (Jacobs *et al.*, 1997).

Notes: This species is one of the few species of *Leptographium* that has conidia that are five times as long as they are wide. The others are *L. penicillatum* and *L. dryocoetidis. Leptographium americanum* can, however, be distinguished from these species based on the long needle-like appearance of its conidia. The conidial lengths of *L. americanum* are also distinctly variable ranging from 3.5 to 22 µm. This species has not been shown to be pathogenic and its role in its consistent association with the bark beetle *D. simplex* is not known.

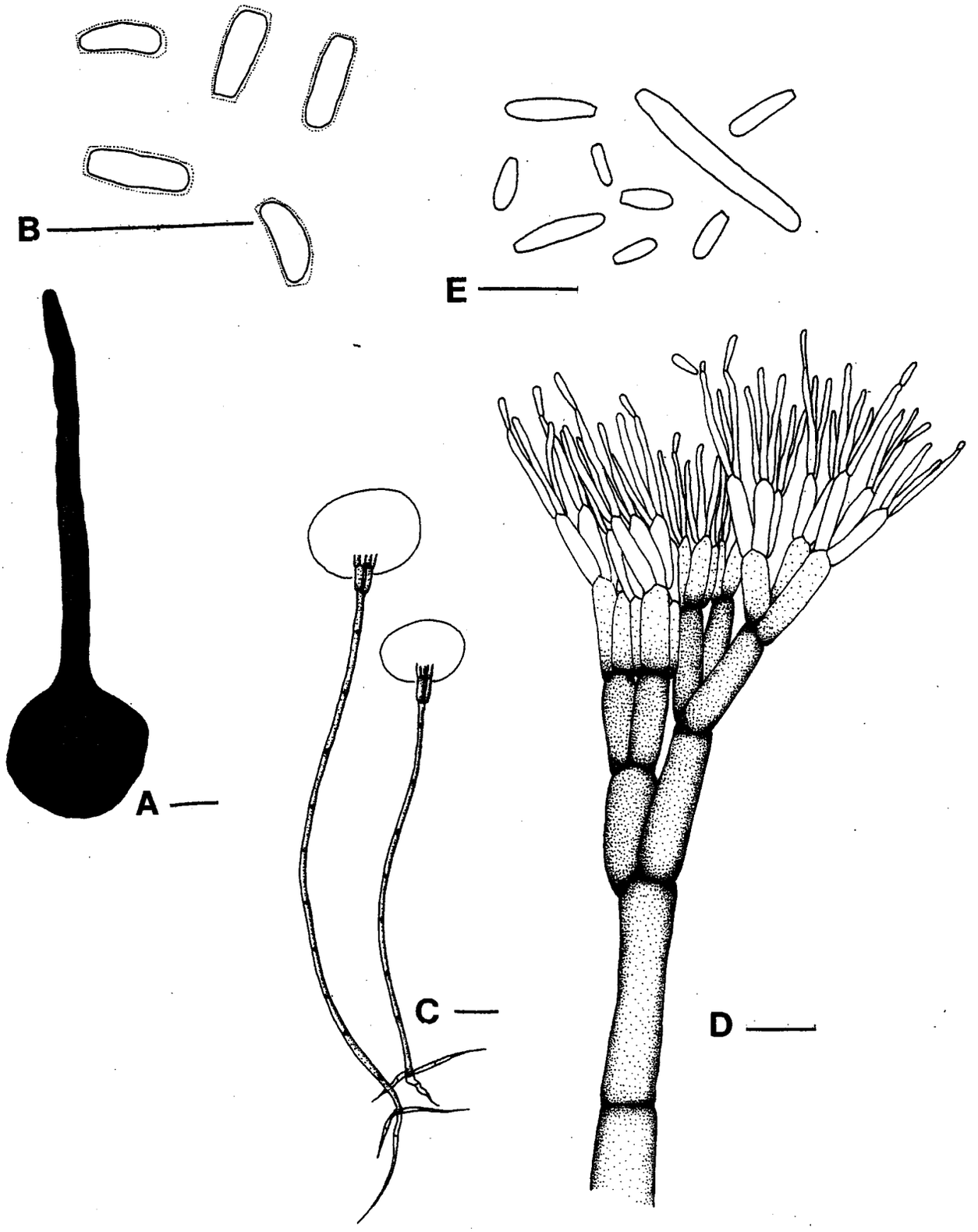

Fig. 33. Teleomorph and anamorph structures of *O. americanum* (PREM 54866). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 100 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

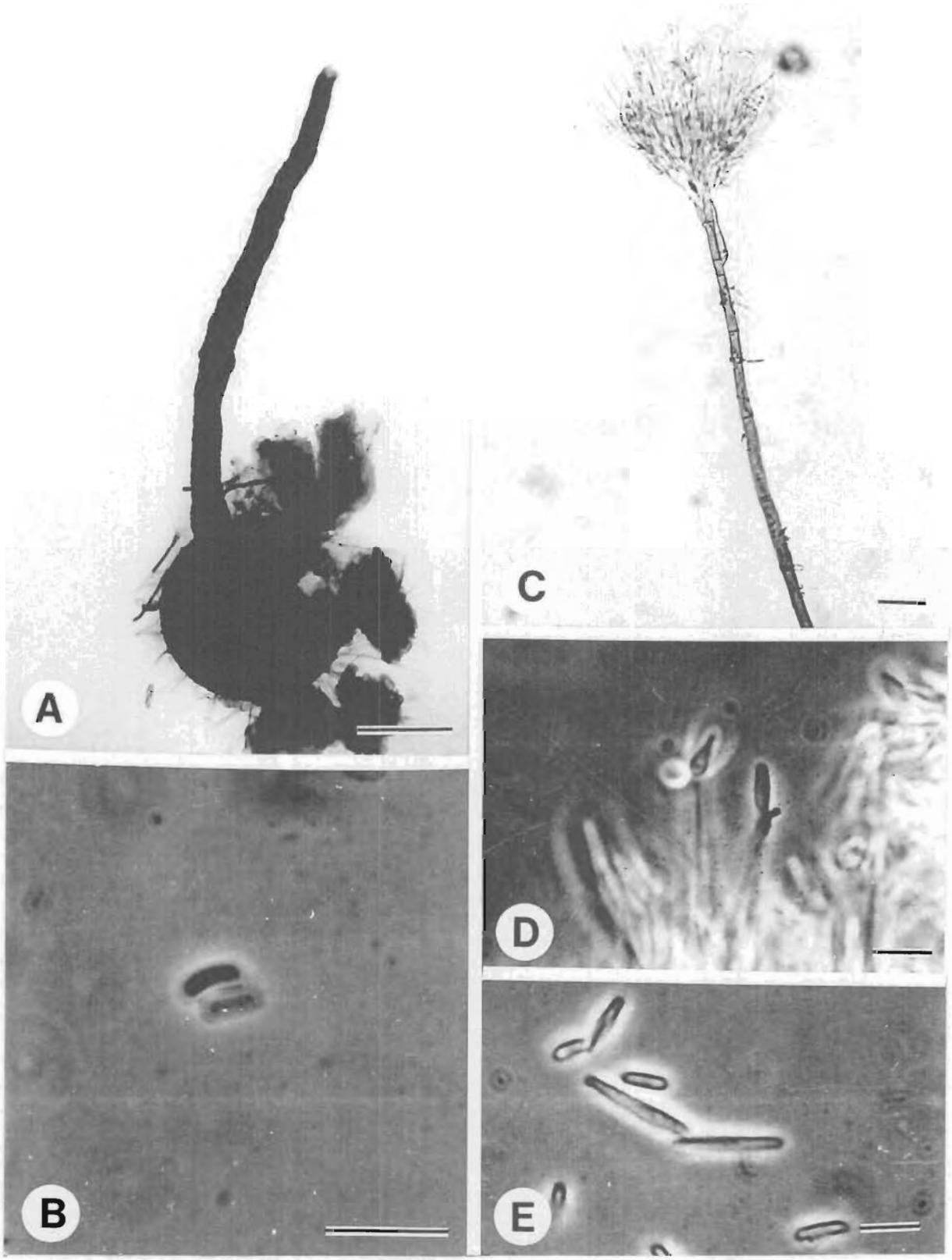

Fig 34. Light micrographs of the teleomorph and anamorph structures of *O. americanum* (PREM 54866). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 20 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

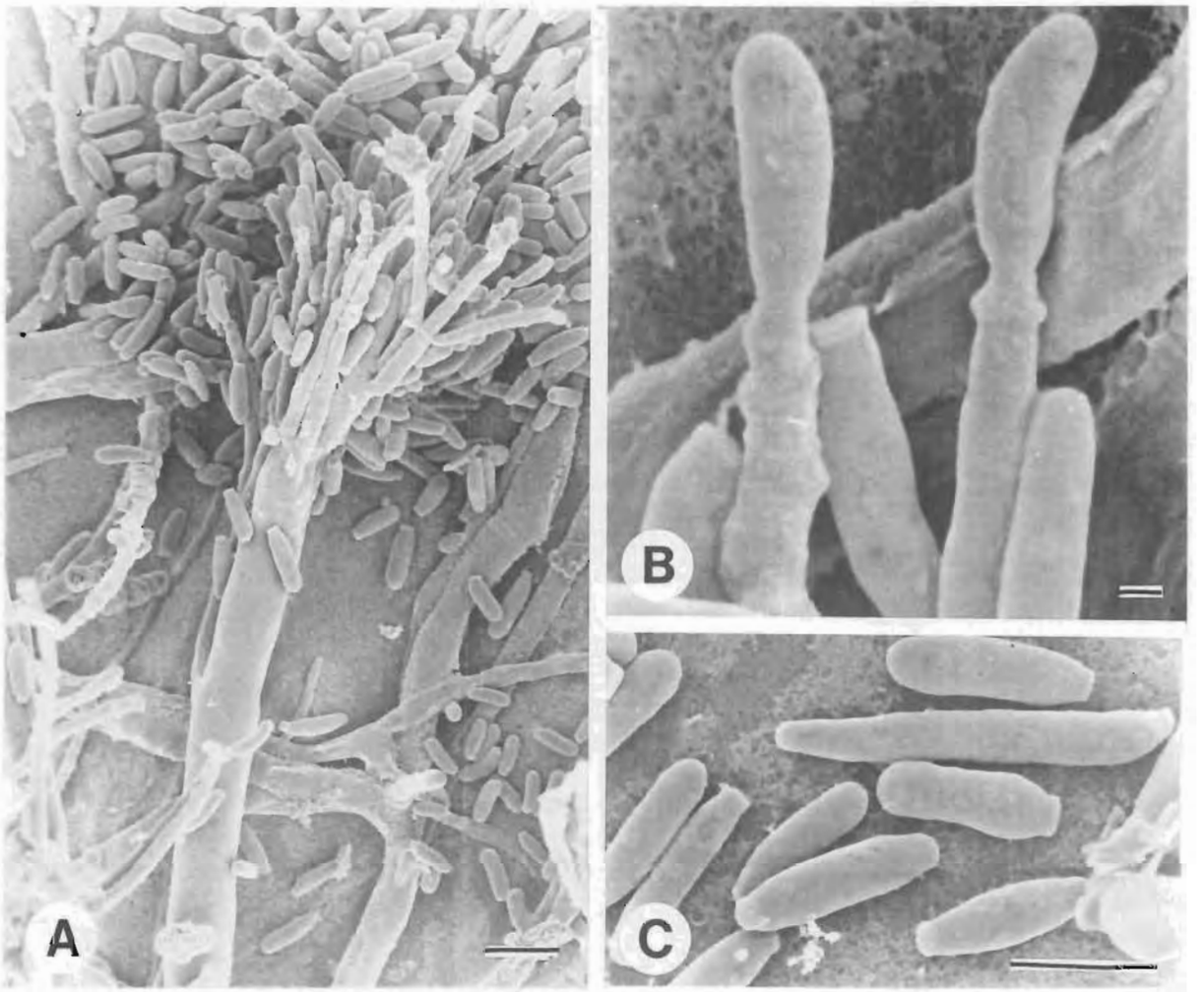

Fig. 35. Scanning electron micrographs of the conidiophores and conidia of *O. americanum* (PREM 54866). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 1 µm). **C.** Conidia (Bar = 5 µm).

7. *Leptographium antibioticum* (W.B. Kendr.) M.J. Wingf., *Transactions of the British Mycological Society* **85**, 92. 1985. **(Figs. 36-38).**

≡*Verticicladiella antibiotica* W.B. Kendr. *Canadian Journal of Botany* **40**, 789. 1962.

Teleomorph: Not known.

Etymology: an-ti-bi-ó-ti-cum: antibiotic. From the Greek αντι: against and βιοτος: life. This specific epithet refers to the fact that the fungus produces an antibiotic substance in culture.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (110-) 117 - 329 (-407) µm in length, rhizoid-like structures present. *Stipes* light olivaceous (21''k), smooth, cylindrical, simple, 3-10 septate, (65-) 76 -281 (-350) µm long, 3.0 - 4.5 µm wide below primary branches, apical cell not swollen, 3.0 - 8.0 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (22.5-) 35 -52 (-72.5) µm long, excluding the conidial mass, with 2 - 3 series of cylindrical branches, 2-5 primary branches, light olivaceous (21''k), smooth, cylindrical, aseptate (7.0-) 10 -12.5 (-14.0) µm long and 1.5 - 4.0 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21''k) to hyaline, aseptate, cylindrical, (5.0-) 7.0 - 11.5 (-13.0) µm long, 1.5 - 3.0 µm wide, tertiary branches hyaline, (5.5-) 7.5 - 8.5 (-11.0) µm long, 2.0 - 3.0 µm wide, aseptate, quaternary branches aseptate, hyaline. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 7 - 12.5 µm long and 0.8 - 1.3 µm wide (Kendrick, 1962). *Conidia* light gray olivaceous (19''''), aseptate, oblong to obovoid with truncate ends and rounded apices, 2.5 - 5.0 x 0.5 - 2.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, becoming amber (21'b) when dry.

Colonies with optimal growth at 30°C on 2% MEA, reaching 7 mm in diam. in 8 days. No growth below 10°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 5% reduction in growth on 0.5 g/l cycloheximide after 12 days

at 20°C in the dark. Colonies light yellow to light olivaceous. *Colony margin* smooth. *Hyphae* submerged on agar with no aerial mycelium, hyaline, smooth, straight, not constricted at the septa, 0.5 - 2.0 µm diam.

Specimens examined: Holotype: Canada, Big Rideau Lake near Portland, Ontario, *Pinus strobus*, 30 Aug. 1961, collected: S.J. Hughes, DAOM 84338. **Paratypes**: Canada, Banff, Alberta, *Picea* spp., 12 Aug. 1960, collected: S.J. Hughes DAOM 71293; U.S.A. **Cultures**: Jane County, G.A, *Pinus taeda*, 1989, collected: S. Alexander, CMW 2777 (same as C 393); U.S.A., Bleckley County, G.A, *Pinus taeda*, 1989, collected: Sam Alexander, CMW 2776; U.S.A. Mesa Verda, CO, *Pinus edulis*, 1984, CMW 2834 (same as C151).

Known distribution: Canada.

Hosts/substrate: worked conifer wood (Kendrick, 1962), *Pinus albicaulis* (Mielke, 1979), *Pinus contorta* (Mielke, 1979), *P. monticola* (Kulhavy *et al.*, 1978; Mielke, 1979), *Abies lasiocarpa* (Mielke, 1979), *Abies balsamea* (Harrington, 1988).

Associated insects: Not known.

Notes: This is the only species in *Leptographium* known to produce an antibiotic substance in culture (Kendrick, 1962). Slow-growing isolates of this fungus are sensitive to cycloheximide, whereas faster growing isolates have a higher degree of tolerance (Harrington, 1988). This unusual characteristic might indicate that this species does not have a close affinity to *Ophiostoma* (Harrington, 1988), or that isolates represent a species complex.

Leptographium antibioticum can be distinguished from other *Leptographium* spp. based on its colony and conidiophore colour as well as rhizoids. Cultures of *L. antibioticum* can readily be recognized by its pale, almost white colour compared to the dark olivaceous colour of other *Leptographium* spp. In addition, the conidiophore stipes of this species is also not the characteristic olivaceous green observed in other *Leptographium* spp., but rather a light olive to yellow colour. At the bases of

the conidiophores, there are short, peg-like rhizoids.

Little is known about the pathogenicity or ecological role of *L. antibioticum.* Compared to other species of *Leptographium, L. antibioticum* was found to be a saprophyte and showed no pathogenicity in trails (Bertagnole *et al.*, 1983).

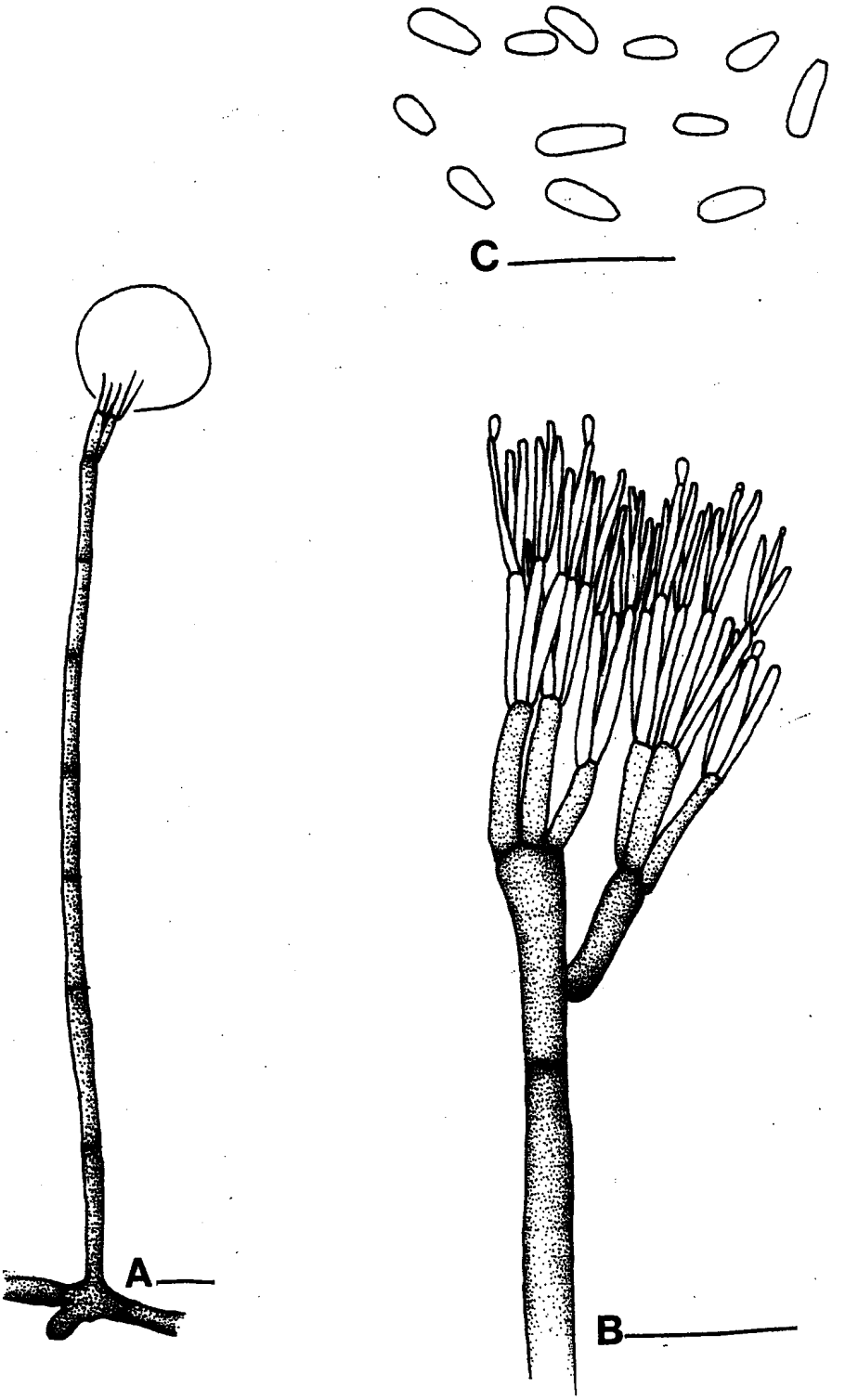

Fig. 36. Conidiophores and conidia of *L. antibioticum* (CMW 2777). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

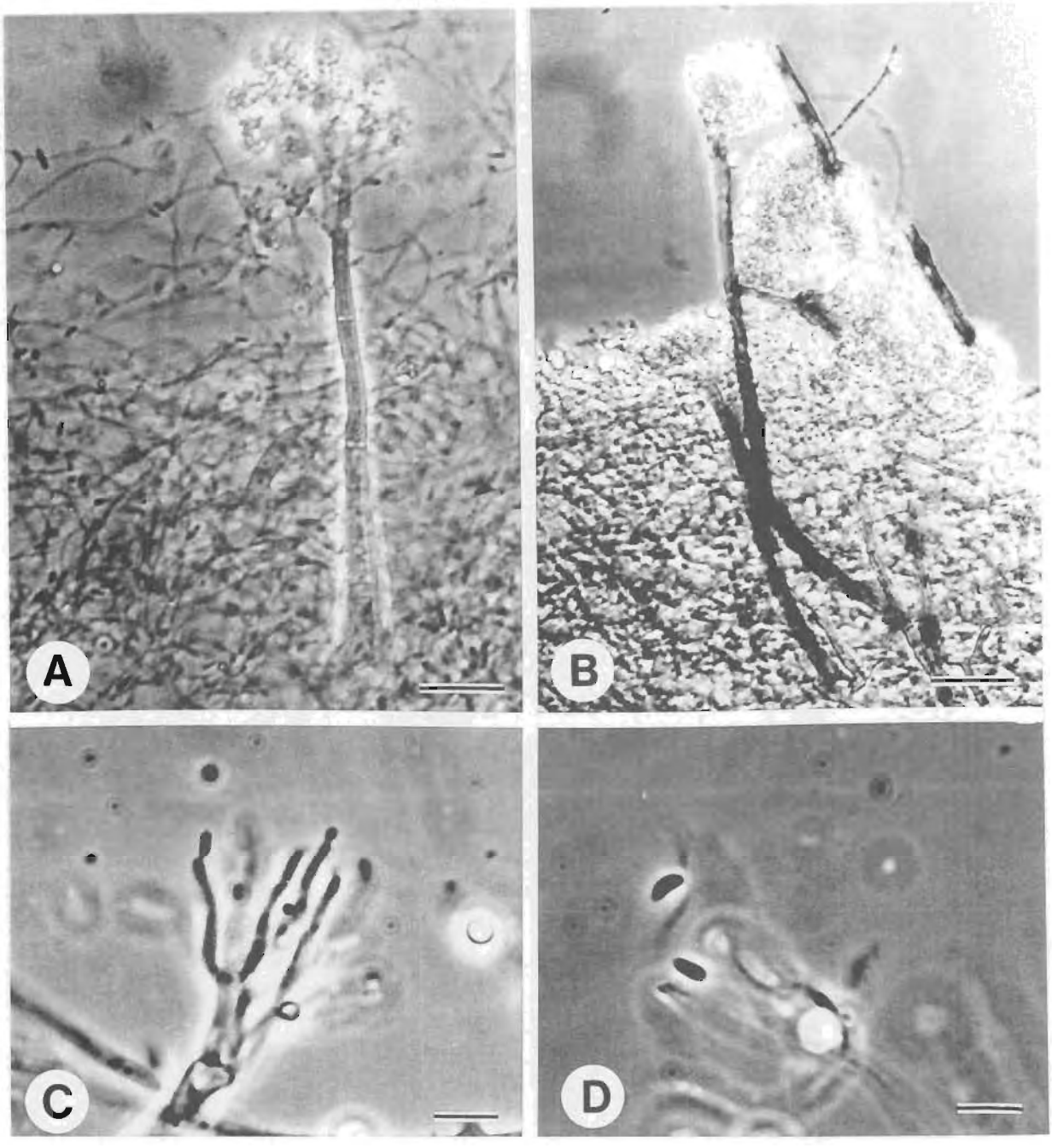

Fig 37. Light micrographs of the conidiophores and conidia of *L. antibioticum* (CMW 2777). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

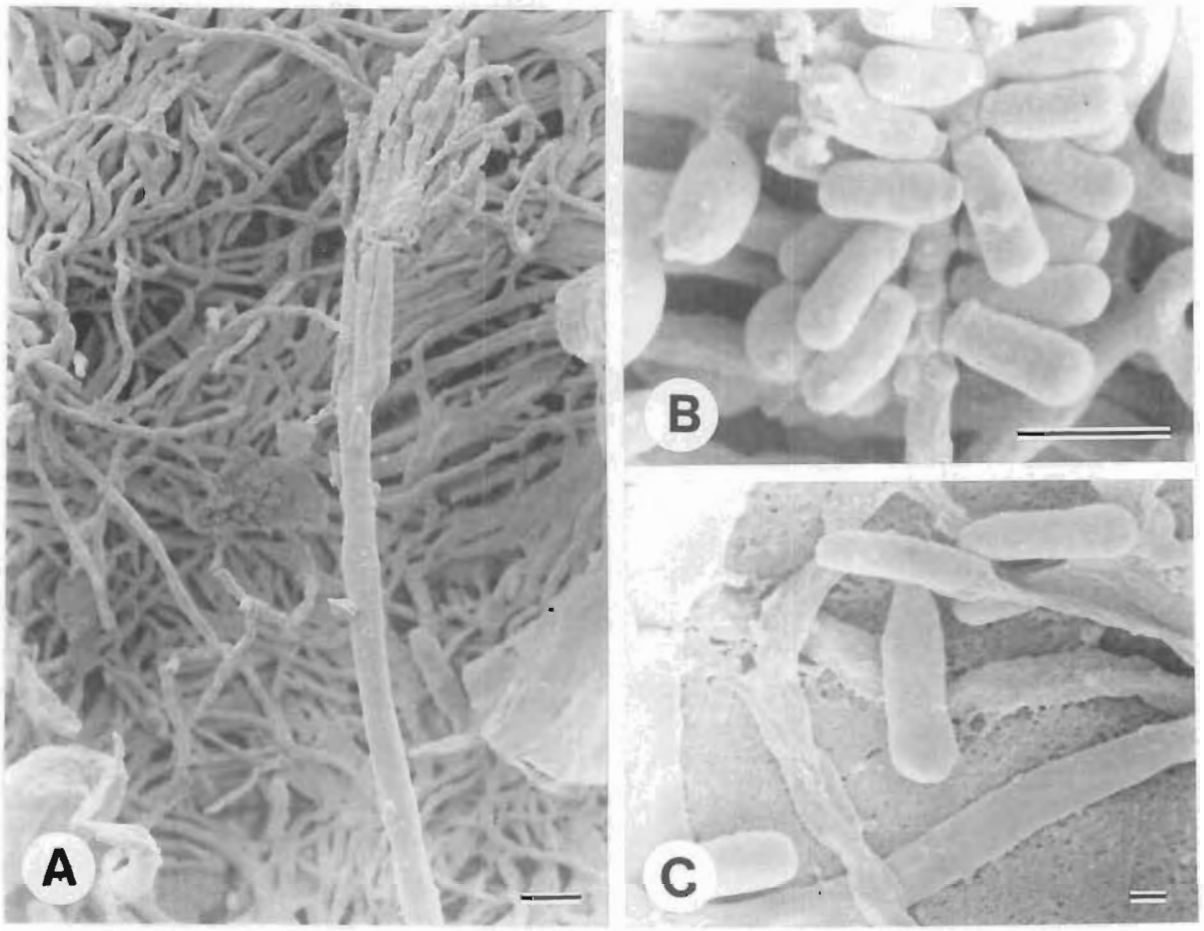

Fig. 38. Scanning electron micrographs of the conidiophores and conidia of *L. antibioticum* (CMW 2777). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

8. *Ophiostoma aureum* (Rob.-Jeffr. & R.W. Davidson) T.C. Harr. *Mycotaxon* **27**, 41. 1987. **(Figs. 39-41).**

≡ *Ceratocystis aurea* (Rob.-Jeffr. & R.W. Davidson) H.P. Upadhyay A Monograph of *Ceratocystis* and *Ceratocystiopsis*. 1981.

≡ *Europhium aureum* Rob.-Jeffr. & R.W. Davidson *Canadian Journal of Botany* **46**, 1525, 1968.

Anamorph: *Leptographium aureum* (Rob.-Jeffr. & R.W. Davidson) M.J. Wingf. *Transactions of the British Mycological Society* **85**, 92. 1985.

Etymology: aú-re-um: golden. From the Latin adjective aureus: golden. This specific epithet refers to the bright yellow masses of conidia produced by this fungus in culture (Robinson-Jeffrey & Davidson, 1968).

Perithecial bases black, globose and smooth walled, unornamented, 300 - 400 µm in diam. *Perithecial neck* dark brown to black, very short or no neck, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* cucullate, aseptate, hyaline, invested in a sheath, 3.5 - 6.5 x 2.5 - 4 µm (Robinson-Jeffrey & Davidson, 1968).

Conidiophores occurring singly, arising directly from the mycelium, occasionally on aerial mycelium, erect, macronematous, mononematous, (100-) 369 - 772 (-1350) µm in length, rhizoid-like structures absent. *Stipes* olivaceous (21"m), smooth, cylindrical, simple, 3-19 septate, (35-) 150.5 - 490 (-785) µm long, 5.0 - 15 µm wide below primary branches, apical cell occasionally swollen, 5.0- 20 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (35-) 137.5 - 349.5 (-900) µm long, excluding the conidial mass, with complex series of cylindrical branches, 2-3 primary branches, olivaceous (21"m), smooth, cylindrical or barrel shaped 0-1 septate, (8.0-) 16.5 - 36 (-46.5) µm long and 4.5 - 11 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, conidiogenous apparatus to complex to measure. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (8.0-) 15.5 - 23 (-34) µm long and 1.0 - 3.0 µm wide. *Conidia* hyaline, aseptate, oblong with truncate ends and rounded apices, (5.5-) 7.5 - 9.5 (-12.5) x 2.0 - 4.0 µm. Conidia accumulating in slimy droplets at the

apex of conidiogenous apparatus, hyaline at first, becoming amber-yellow (21'b) with age. Conidial mass white to amber yellow when wet, turning golden brown when dry.

Colonies with optimal growth at 20°C. Colonies grew uniformly well at 25°C on 2% MEA, reaching 40 mm in diam. in 9 days. No growth below 5°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 23% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15'''''k). *Colony margin* laciniate. *Hyphae* submerged on agar with very little aerial mycelium, olivaceous (21''m), thick rough walled, straight, occasionally clustered together, frequently constricted at the septa, 3.0 - 8.0 (-11) µm diam.

Specimens examined: Holotype: U.S.A., McCall, Idaho, *Pinus contorta* Dougl. var. *latifolia* Engelm., 28 October 1963, collected: R.C. Jeffrey and J.I. Ridgsay, BPI 688941. **Paratype**: U.S.A., McCall, Idaho, *Pinus contorta* Dougl. var. *latifolia* Engelm., 28 October 1963, collected: R.C. Jeffrey and J.I. Ridgsay, BPI 688943. (Note: The herbarium material for this species has deteriorated and it is not possible to observe any structures. For this description previous observations by Robinson-Jeffrey and Davidson (1968), and living cultures of the fungus were used.) **Cultures**: Canada, *Pinus contorta* var. *latifolia*, 1987, collected: R.W. Davidson, (CMW 714); Canada, *Pinus contorta* var. *latifolia*, 1987, collected: R.W. Davidson, CMW 709 (same as ATCC 16936).

Known distribution: Canada.

Hosts/substrate: *Pinus contorta var. latifolia* (Robinson-Jeffrey & Davidson, 1968), *Pinus ponderosa* (Harrington, 1988), *Pinus edulis* (Harrington, 1988).

Associated insects: *Dendroctonus sp.* (Robinson-Jeffrey & Davidson, 1968; Perry, 1991), *Hylurgops porosus* (Harrington, 1988).

Notes: This is one of four species previously accommodated in *Europhium* (Robinson-Jeffrey & Davidson, 1968). These include *E. trinacriforme, E. aureum. E. clavigerum* Robinson & Davidson and *E. robustum* Robinson & Davidson, that were

all characterized by ascocarps with no, or very short necks. This genus was later reduced to synonymy with *Ceratocystis* (Upadhyay, 1981) and Harrington (1987) transferred these species to *Ophiostoma.*

The conidiophores of *O. aureum* are characterized by a complex, brush-like conidiogenous apparatus, which is often more than half of the total conidiophore length. Two different conidial forms have been reported for this species. Robinson-Jeffrey and Davidson (1968) distinguish *O. aureum* and *O. robustum* from other species of *Europhium* based on their anamorph states, in particular, their conidia. In the case of *L. aureum,* the conidia have been described as slightly falcate, whereas, those of *L. robustum* are globose. *Ophiostoma aureum* has also been reported to have bright yellow conidial masses in culture which distinguishes it from other *Leptographium* spp.

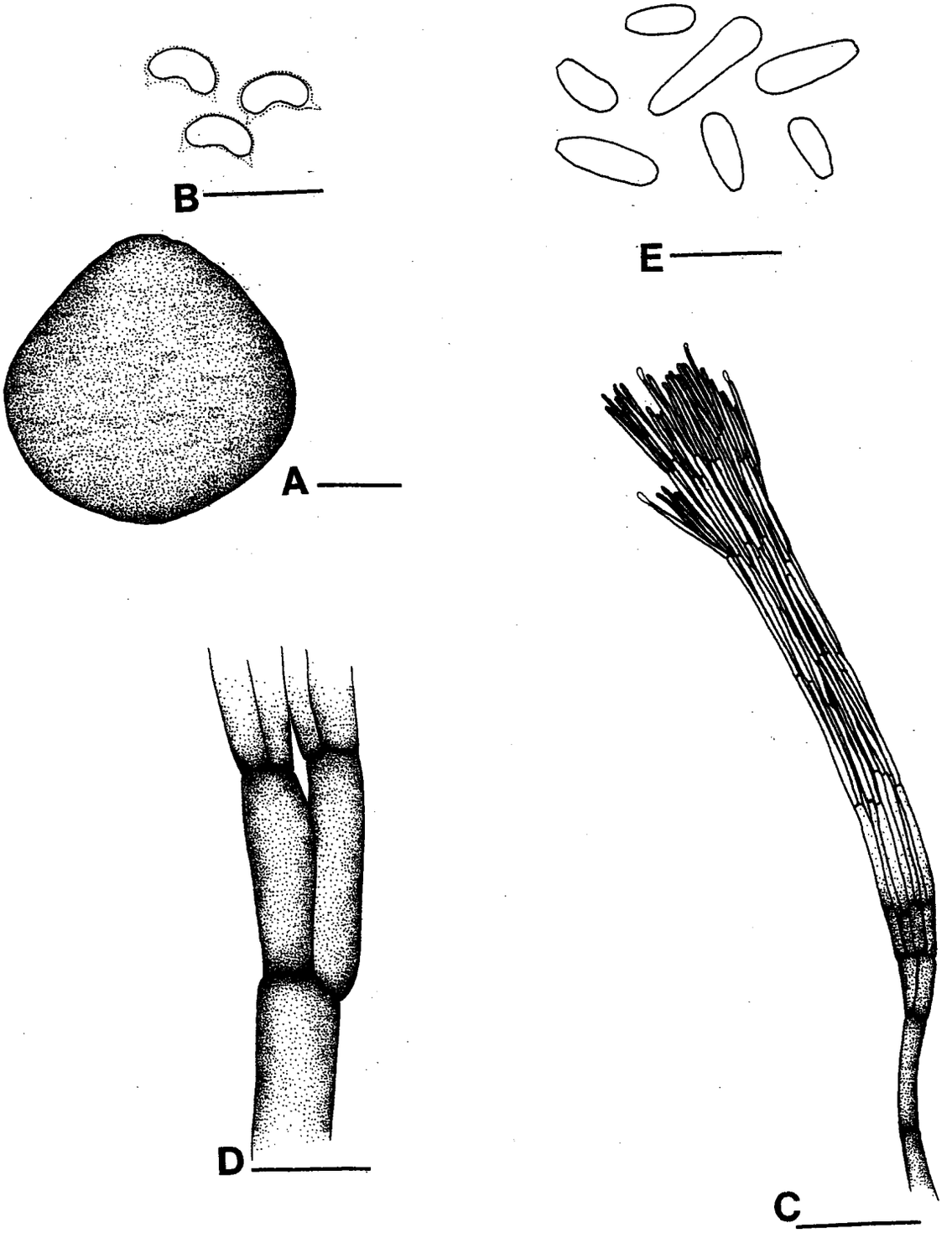

Fig. 39. Teleomorph and anamorph of *O. aureum* (CMW 714). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 50 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

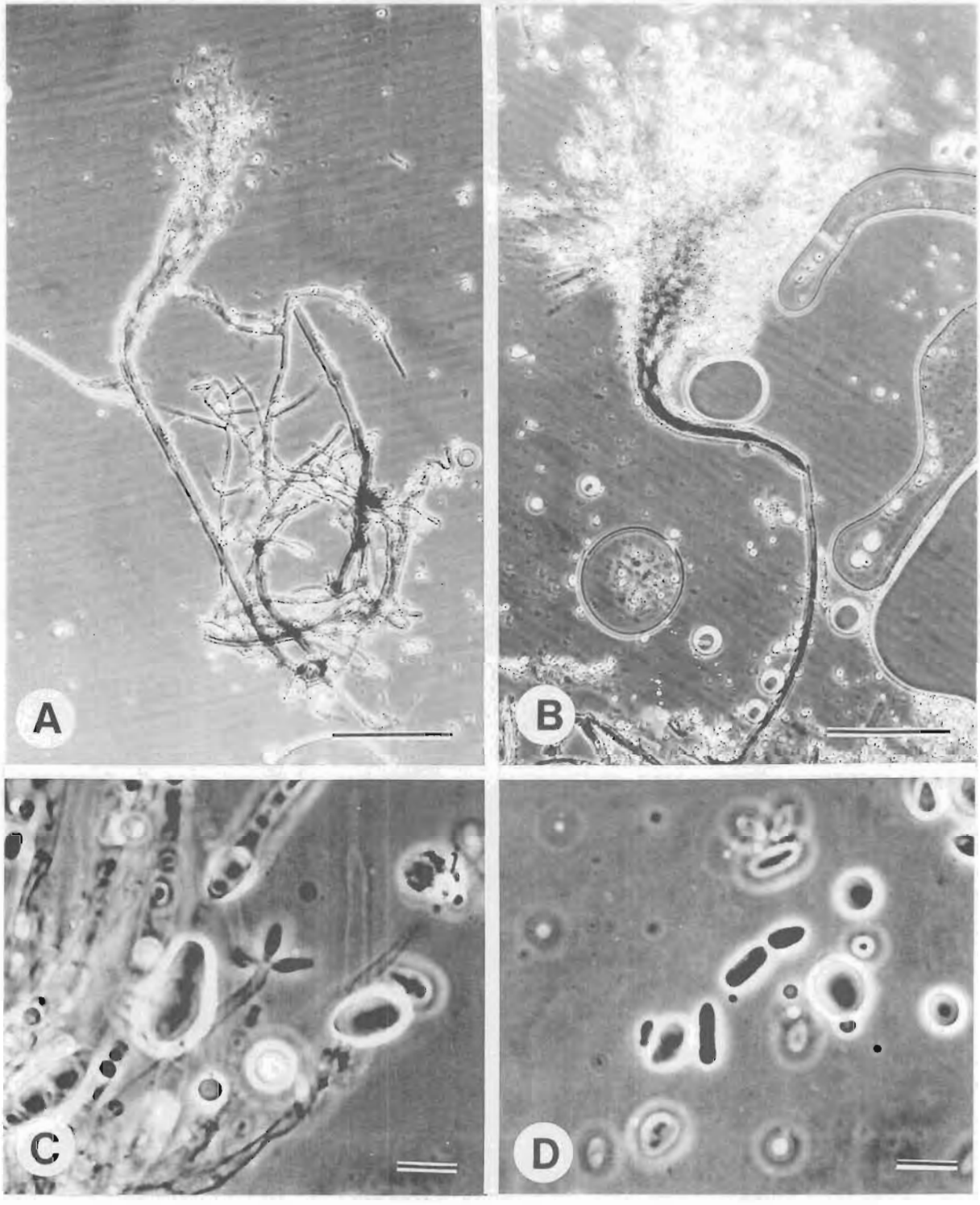

Fig 40. Light micrographs of the teleomorph and anamorph structures of *O. aureum* (CMW 714). **A.** Conidiophore (Bar = 100 µm). **B.** Conidiophore (Bar = 100 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

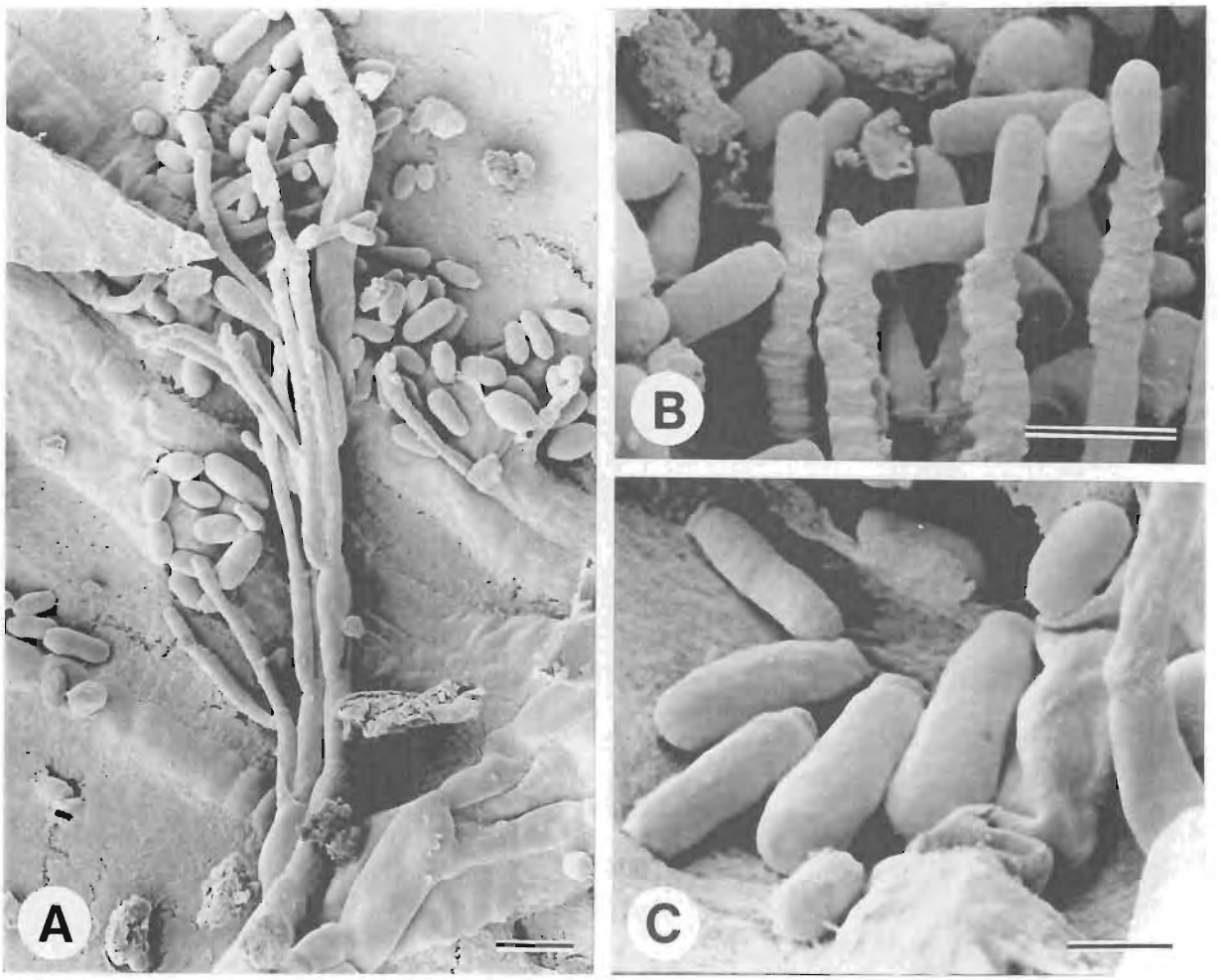

Fig. 41. Scanning electron micrographs of the conidiophores and conidia of *O. aureum* (CMW 714). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 5 µm).

9. ***Leptographium brachiatum*** (W.B. Kendr.) M.J. Wingf., *Transactions of the British Mycological Society* **85**, 1985. **(Figs. 42-44).**

≡ *Verticicladiella brachiata* W.B. Kendr., *Canadian Journal of Botany* **40**, 786, 1962.

Teleomorph: Not known.

Etymology: bra-chi-á-tum: having an arm. From the Latin noun brac(c)hium: an arm. This specific epithet refers to the characteristic side branches on the stipes of the fungus.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (73-) 112 -121 (-186) µm in length, rhizoid-like structures present. *Stipes* light olivaceous (21''k), smooth, cylindrical, simple, 2 - 5 septate, (37-) 78 - 89 (-150) µm long, 2.0 - 4.0 µm wide below primary branches, apical cells not swollen, 4.0-) 4.5 - 6.0 (-7.0) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (19-) 21.5 - 45.5 (-51) µm long, excluding the conidial mass, with 2 to 3 series of cylindrical branches, 2 primary branches, hyaline, smooth, cylindrical, aseptate, (7.0-) 10 - 19 (-22) µm long and 2.0 - 3.0 µm wide, arrangement of the primary branches on the stipe - type A, secondary branches hyaline, aseptate, (6.0-) 10 - 11 (-14.0) µm long, 1.0 - 2.5 µm wide, tertiary branches hyaline, aseptate, 10 - 13.5 µm long, 2.0 - 2.5 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 7.0 - 16 µm long and 1.0 - 2.0 µm wide. *Conidia* light gray olivaceous (19''''), aseptate, oblong to obovoid, 3.0 - 5.5 µm x 1.0 - 1.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming greenish olivaceous (23''') with age. Conidial mass hyaline to greenish olivaceous when wet, turning cream colored when dry.

Colonies with optimal growth at 25°C on 2% MEA, slow growing reaching 4 mm in diam. in 9 days. No growth below 15°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 16% reduction in growth on 0.5 g/l cycloheximide after 8 days at 25°C in the dark. Colonies greenish olivaceous (23'''), loose its color in culture after continuous transfer, colonies becoming white. *Colony*

margin smooth. *Hyphae* submerged in agar with no aerial mycelium, hyaline, smooth, straight, not constricted at the septa, 1.0 - 4.0 µm diam.

Specimens examined: Holotype: British Columbia, Copper Canyon, Vancouver Island, *Pseudotsuga menziesii*, 10 November 1949, collected: J. Short, DAOM 33961(b). **Paratypes:** 1953, collected: Rennerfelt, DAOM 34871; British Columbia, Copper Canyon, Vancouver Island, *Pseudotsuga menziesii*, 10 November 1949, collected: J. Short, DAOM 33974; Frederictor, N.B., *Picea mariana*, 24 October 1951, collected: Redmond, DAOM 34360. **Cultures**: U.S.A., Virginia, *Pinus* stump, 1987, collected: W. B. Kendrick and G.C. Bhatt, CMW 440 (same as CBS 660-70); U.S.A., New York, *Picea rubens,* 1989, collected S. Alexander, CMW 2855 (same as C388).

Known distribution: Canada.

Hosts/substrate: *Pseudotsuga menziesii*, *Picea mariana* (Kendrick, 1962).

Associated insects: Not known.

Notes: Lateral outgrowths of the stipe reported by Kendrick (1962) were observed in some of the cultures and in all the type specimens except DAOM 34871. As in the case of *L. antibioticum,* this fungus displays an unusually low level of tolerance to the antibiotic cycloheximide, suggesting that it might not be an anamorph of *Ophiostoma* (Harrington, 1988). *Leptographium brachiatum* does not appear to be pathogenic and is most probably a saprophyte (Harrington, 1988).

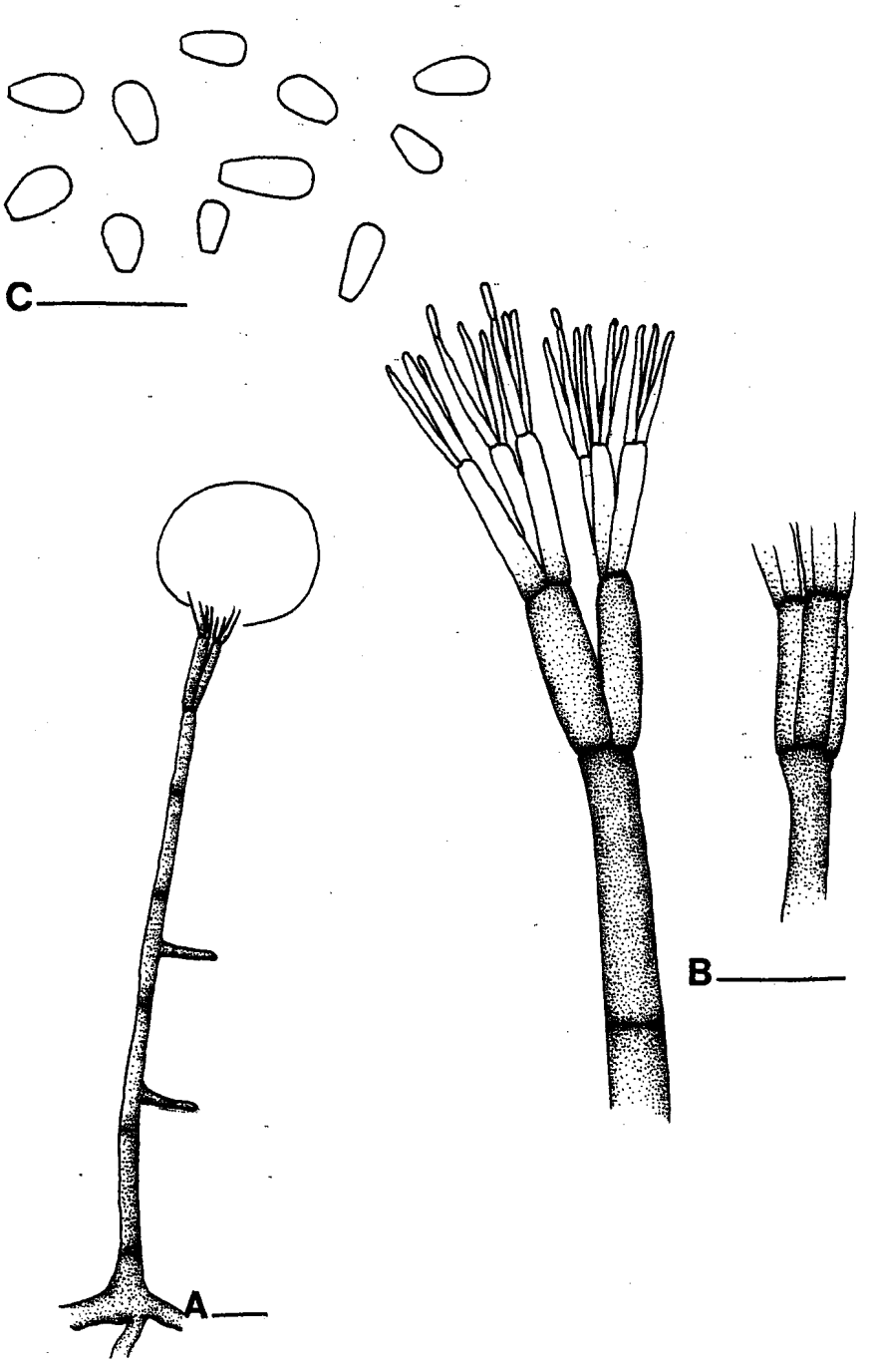

Fig. 42. Conidiophores and conidia of *L. brachiatum* (CMW 440). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

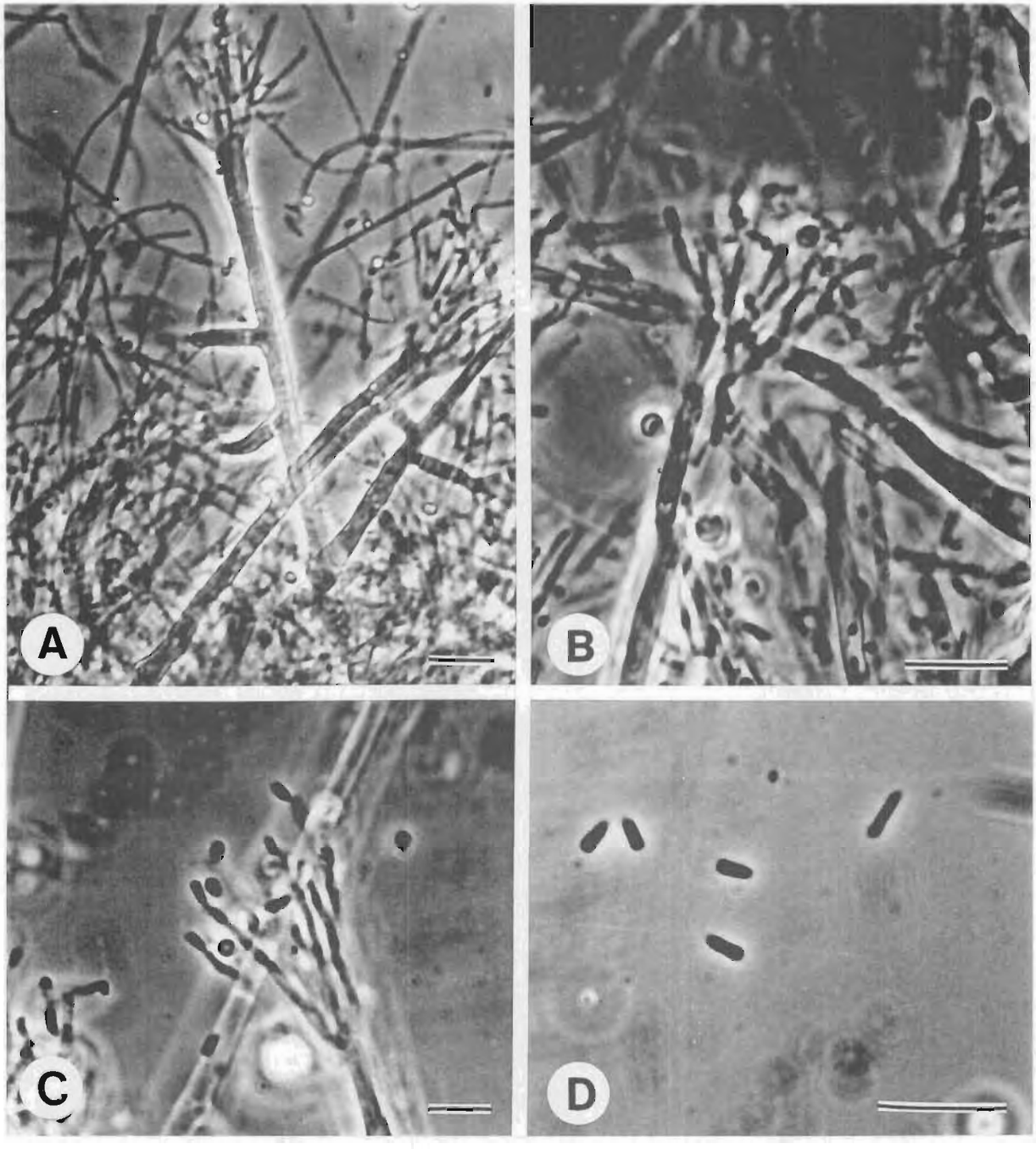

Fig 43. Light micrographs of the conidiophores and conidia of *L. brachiatum* (CMW 440). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

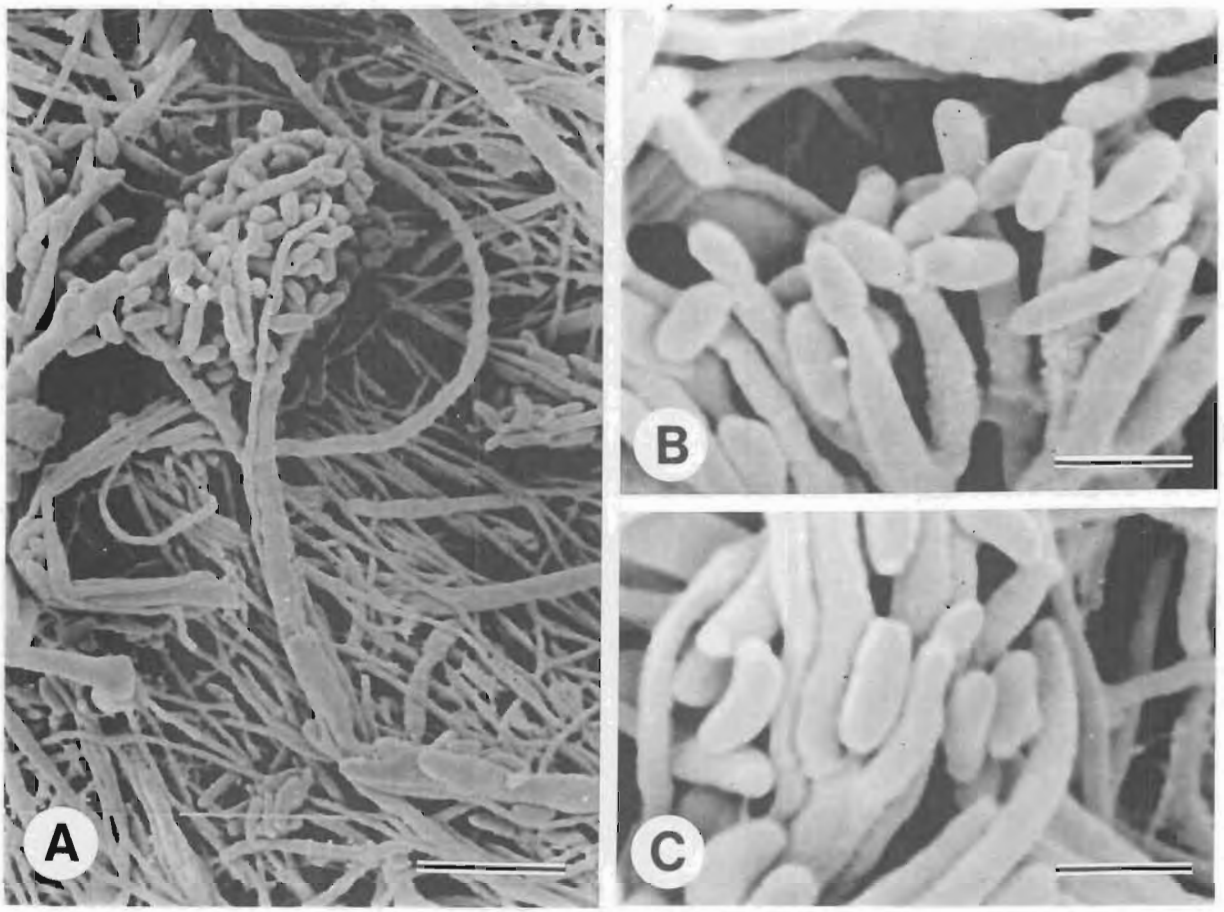

Fig. 44. Scanning electron micrographs of the conidiophores and conidia of *L. brachiatum* (CMW 440). **A**. Conidiophore (Bar = 10 µm). **B**. Conidiogenous cells (Bar = 5 µm). **C**. Conidia (Bar = 5 µm).

10. *Ophiostoma brevicolle* (R.W. Davidson) De Hoog & R.J. Scheff., *Mycologia* **76**, 297. 1984. **(Figs. 45-47).**

≡ *Ceratocystis brevicollis* R.W. Davidson, *Mycologia* **50**, 667. 1958.

Anamorph: *Leptographium brevicolle* Jacobs & Wingfield sp. nov.

Etymology: bre-vi-cól-lum: of the short neck. From the Latin adjective brevis: short and collum: a neck. This specific epithet refers to the short necks of the perithecia of the *Ophiostoma* state.

Perithecial bases olivaceous becoming black, globose and smooth walled, unornamented, 150 - 180 µm in diam. *Perithecial neck* dark brown to black, cylindrical with a slight apical taper, smooth, 200 - 300 µm long, 25 - 45 µm above globose base, 18 - 32 µm wide at the apex, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* allantoid, aseptate, hyaline, invested in a uniform sheath, 4.0 - 5.5 x 1.3 - 2.0 µm (Davidson, 1958, Upadhyay, 1981).

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (112.5-) 148.5 - 173.5 (-265) µm in length, rhizoid-like structures absent. *Stipes* olivaceous buff (21'"b), becoming hyaline towards the apex, constricted at septa, cylindrical, simple, 4 - 13 septate, (77.5-) 112 - 150 (-232.5) µm long, 5.0 - 7.5 µm wide below primary branches, apical cell not swollen, (7.5-) 8.5 - 12.5 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (22.5-) 23 - 44 (-50) µm long, excluding the conidial mass, with 1 to 2 series of cylindrical branches, 2 primary branches, hyaline, smooth, cylindrical, aseptate (8.5-) 10.5 - 13.5 (-18.5) µm long and (2.5-) 4.0 - 4.5 (-7.5) µm wide, arrangement of the primary branches on the stipe - type A, secondary branches hyaline, aseptate, (5.5-) 9.5 - 11 (-14) µm long, (2.0-) 2.5 - 3.5 (-5.0) µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 9.0 - 15.5 µm long and 1.0 - 2.0 µm wide. *Conidia* olivaceous gray (21""), aseptate, oblong with truncate ends and rounded apices, (3.0-) 4.0 - 4.5 (-6.0) x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming white with age. Conidial mass white when wet, remaining white when dry.

Colonies with optimal growth at 30°C on 2% MEA, reaching 14 mm in diam. in 9 days. No growth below 10°C or above 35°C, little growth at 35°C. Able to withstand high concentrations of cycloheximide with a 10% reduction in growth on 0.5 g/l cycloheximide after 12 days at 20°C in the dark. Colonies olivaceous (21"m) with white aerial mycelium. *Colony margin* smooth. *Hyphae* submerged on agar with little aerial mycelium, hyaline, smooth, straight, not constricted at the septa, 1.0 - 3.0 µm diam.

Specimens examined: Holotype: U.S.A. Colorado Fort Collins, *Populus tremuloides*, Sept. 1954, collected: T.O. Thatcher, BPI 70810 (received from NFC). **Cultures**: collected: R.W. Davidson, CMW 447 (same as CBS 795-73 and ATCC 129771); U.S.A., Colorado, *Populus tremuloides,* 1970, collected: R.W. Davidson, CMW 474 (CBS 150.78).

Known distribution: U.S.A. (Colorado).

Hosts/substrate: *Populus tremuloides* (Davidson, 1958).

Associated insects: *Trypodendron retusus* (Davidson, 1958).

Notes: Davidson (1958) described only the teleomorph of this fungus and mentioned the presence of a *Leptographium* anamorph. A description of the anamorph was later provided by Upadhyay (1981). Based on ascospore morphology, this species was placed in the "fimbriata" - group by Olchowecki and Reid (1974). *Ophiostoma brevicolle* is similar to *O. francke-grosmannniae.* These species can, however, be distinguished based on the apparent phialidic appearance of the conidiogenous cells in *O. francke-grosmanniae.* In *O. brevicolle,* the conidiogenous cells are clearly annellidic. The conidia of *O. brevicolle* are also more oblong, compared to the short obovoid conidia of *O. francke-grosmanniae.*

Ophiostoma brevicolle was reported to be associated with *Trypodendron retusus.* However, no survey was conducted to determine whether the fungus is consistently associated with the bark beetle, or its distribution in the area (Davidson, 1958).

Hinds & Davidson (1972) also reported a loss of viability in the fungus (Hinds & Davidson, 1972).

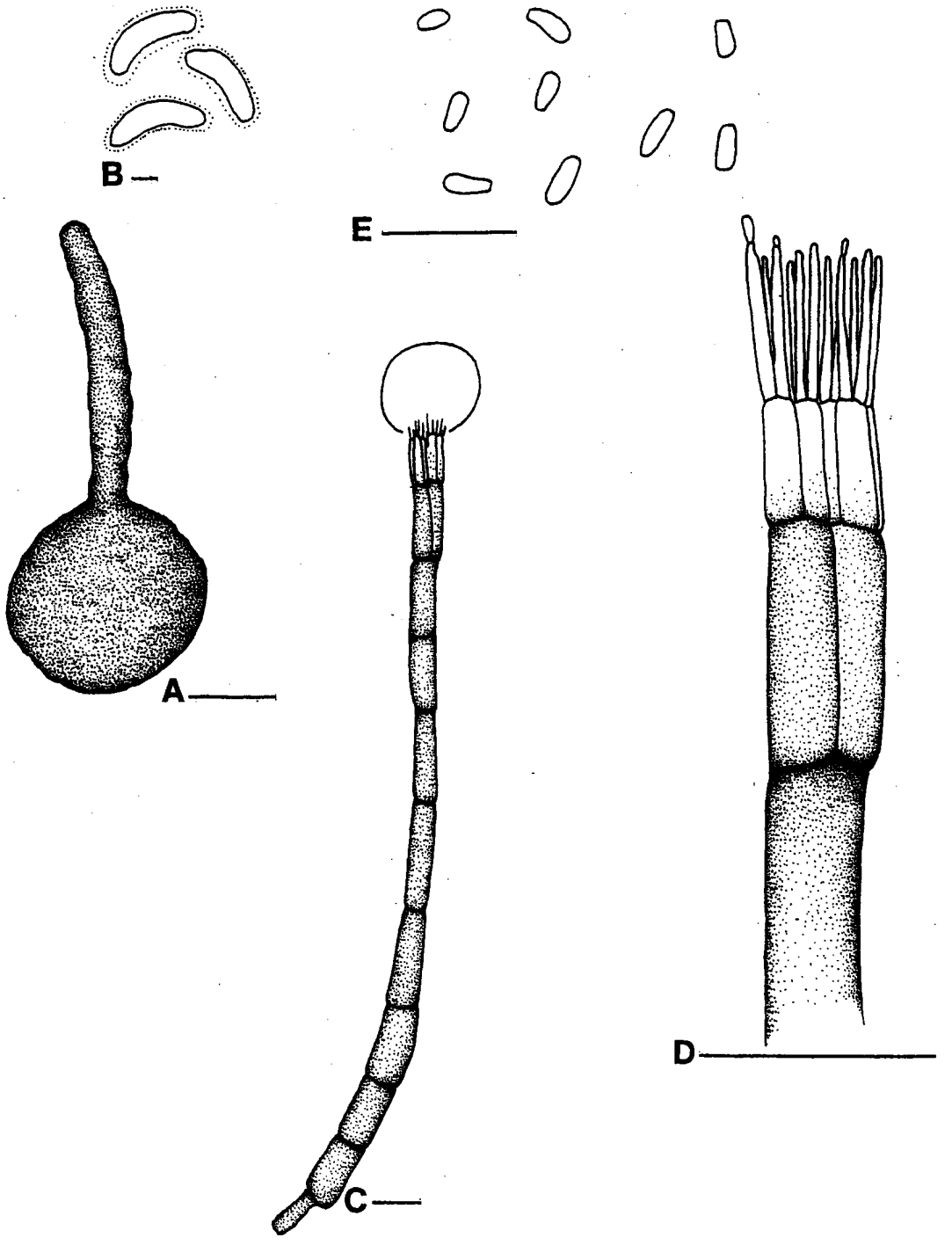

Fig. 45. Teleomorph and anamorph structures of *O. brevicolle* (CMW 447). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 1 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

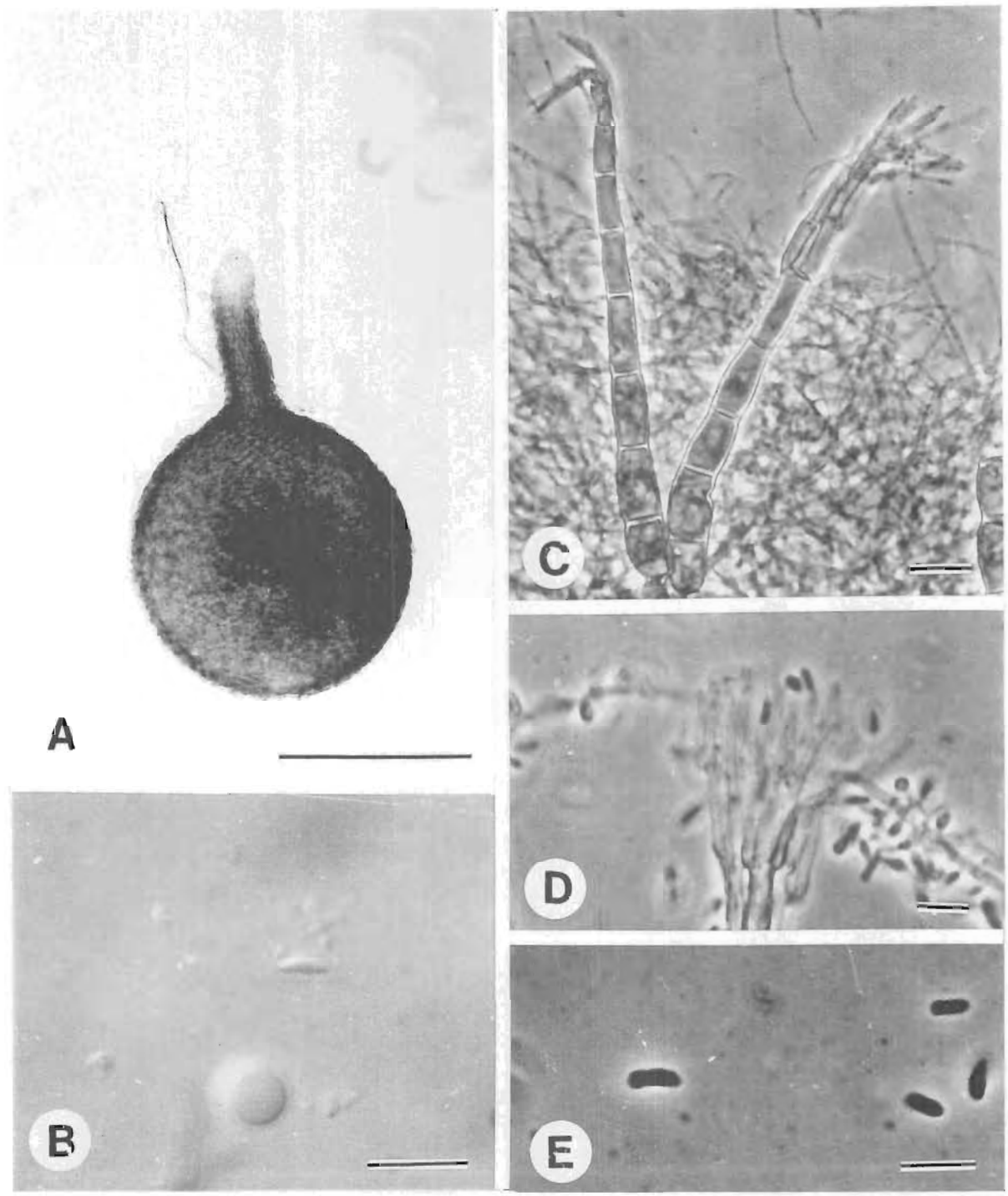

Fig 46. Light micrographs of the teleomorph and anamorph structures of *O. brevicolla* (CMW 447). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

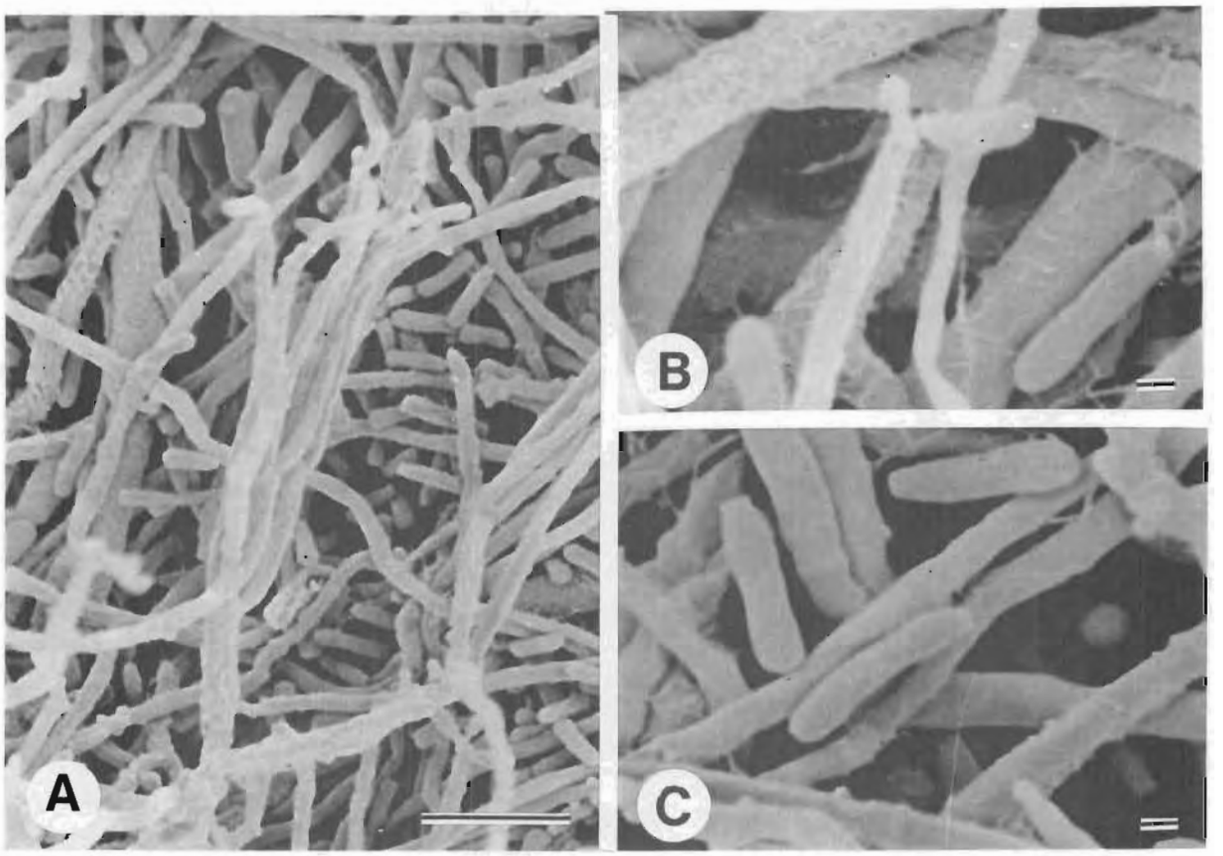

Fig. 47. Scanning electron micrographs of the conidiophores and conidia of *O. brevicolla* (CMW 447). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 1 µm). **C.** Conidia (Bar = 1 µm).

11. ***Leptographium calophylli*** (Wiehe) J. Webber, K. Jacobs & M.J. Wingf. *Mycological Research*, **103,** 1580. 1999. **(Figs. 48-50).**

= *Haplographium calophylli* Wiehe *Mycological Papers* **29**, 5. 1949.

= *Verticillium calophylli* (Wiehe) W. Gams. 1971.

Teleomorph: Not known.

Etymology: ca-lo-phý-lli: of dry leaves. From the Greek adjective καλον: dry and latinised Greek noun φυλλον: made of leaves. This specific epithet refers to *Calophyllum inophyllum* var. *tacamaha* which is the only known host of this fungus.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (41-) 46 - 89 (-100) µm in length, rhizoid-like structures absent. *Stipes* hyaline, smooth, cylindrical, simple, 0-1 septate, (5.0-) 12.5 - 18 (-30) µm long, 2.0 - 4.0 µm wide below primary branches, apical cell not swollen, 2.0 - 4.0 µm wide at base, basal cell swollen. *Conidiogenous apparatus* (30-) 43 - 58.5 (-80) µm long, excluding the conidial mass, with 2 - 3 series of cylindrical branches, arrangement of the primary branches on the stipe - type B, 2-3 primary branches, hyaline, smooth, cylindrical, 0-1 septate, (7.0-) 11 - 12.5 (-18) µm long and 1.5 -- 4.0 µm wide, secondary branches hyaline, aseptate, (7.0-) 9.5 - 11 (-15) µm long, 1.5 - 5.0 µm wide, tertiary branches hyaline, aseptate, (6.0-) 8.0 - 11 (-16) µm long, 1.0 - 2.0 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, tapering slightly from the base to the apex, (8.0-) 13 - 20.5 (-25) µm long and 1.0 - 2.0 µm wide. *Conidia* hyaline, aseptate, oblong to obovoid with truncate ends, (3.0-) 4.0 - 5.5 (-7.0) x 1.2 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Colonies with optimal growth at 30°C on 2% MEA, reaching 20 mm in diam. in 8 days. Little growth at 15°C and no growth above 40°C. Able to withstand high concentrations of cycloheximide with a 80% reduction in growth on 5 g/l cycloheximide after 8 days at 30°C in the dark. Colonies olivaceous (21"m) (Rayner, 1970). *Colony margin* smooth. *Hyphae* submerged on agar with little aerial

mycelium, hyaline to light olivaceous (19"k), smooth, straight, not constricted at the septa, 1.0 - 3.0 µm diam.

Specimens examined: Holotype: Mauritius, Plaine Sophie, isolated from tracheids and medullary rays of *Calophyllum inophyllum* var. *tacamaha* wood, March 1945, collected: P.O. Wiehe, IMI 28925. **Cultures**: Seychelles, Mahe, *Calophyllum* sp., 24 April 1997, collected: J. Webber, CMW 4257 (B2), CMW 4256 (B1), CMW 4260 (B5), CMW 4262 (B7), CMW 4263 (B9).

Known distribution: Seychelles, Mauritius.

Hosts/substrate: *Calophyllum sp.* (Webber *et al.*, 1999).

Associated insects: *Cryphalus trypanus* (Webber *et al.*, 1999).

Notes: *Leptographium calophylli* can easily be recognized by its short conidiophore stipes and large conidiogenous apparatus. Conidiophores of *L. calophylli* occur mainly on aerial mycelia, in contrast to other species where these structures occur directly on the substrate. Furthermore, this fungus is characterized by an optimal growth temperature of 30 °C, which is unusual for species in *Leptographium* (Webber *et al.,* 1999), but is consistent with its occurrence on tropical islands.

This species is unusual among *Leptographium* spp. in that it does not occur on conifers. *Leptographium calophylli* is thought to be the casual agent of a wilting disease of *Calophyllum* trees in the Seychelles (Ivory & Andre, 1995). The spread of the disease is probably the result of an insect vector (*C. trypanus*), although this has not been proven. It might also have an alternative host, which can explain the absence of the disease subsequent to its first report in 1949 by Wiehe (Ivory & Andre, 1995).

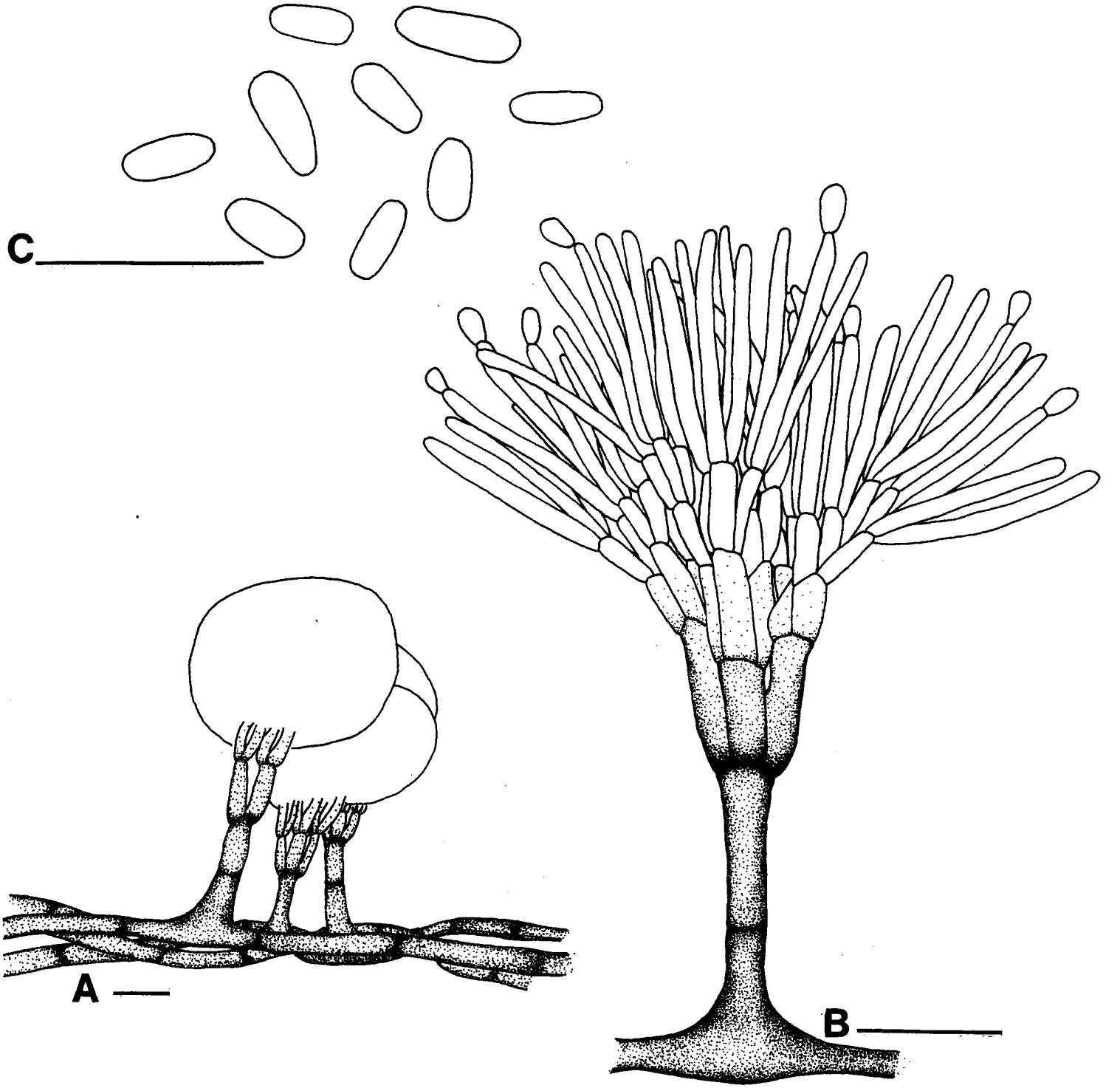

Fig. 48. Conidiophores and conidia of *L. calophylli* (CMW 4257). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

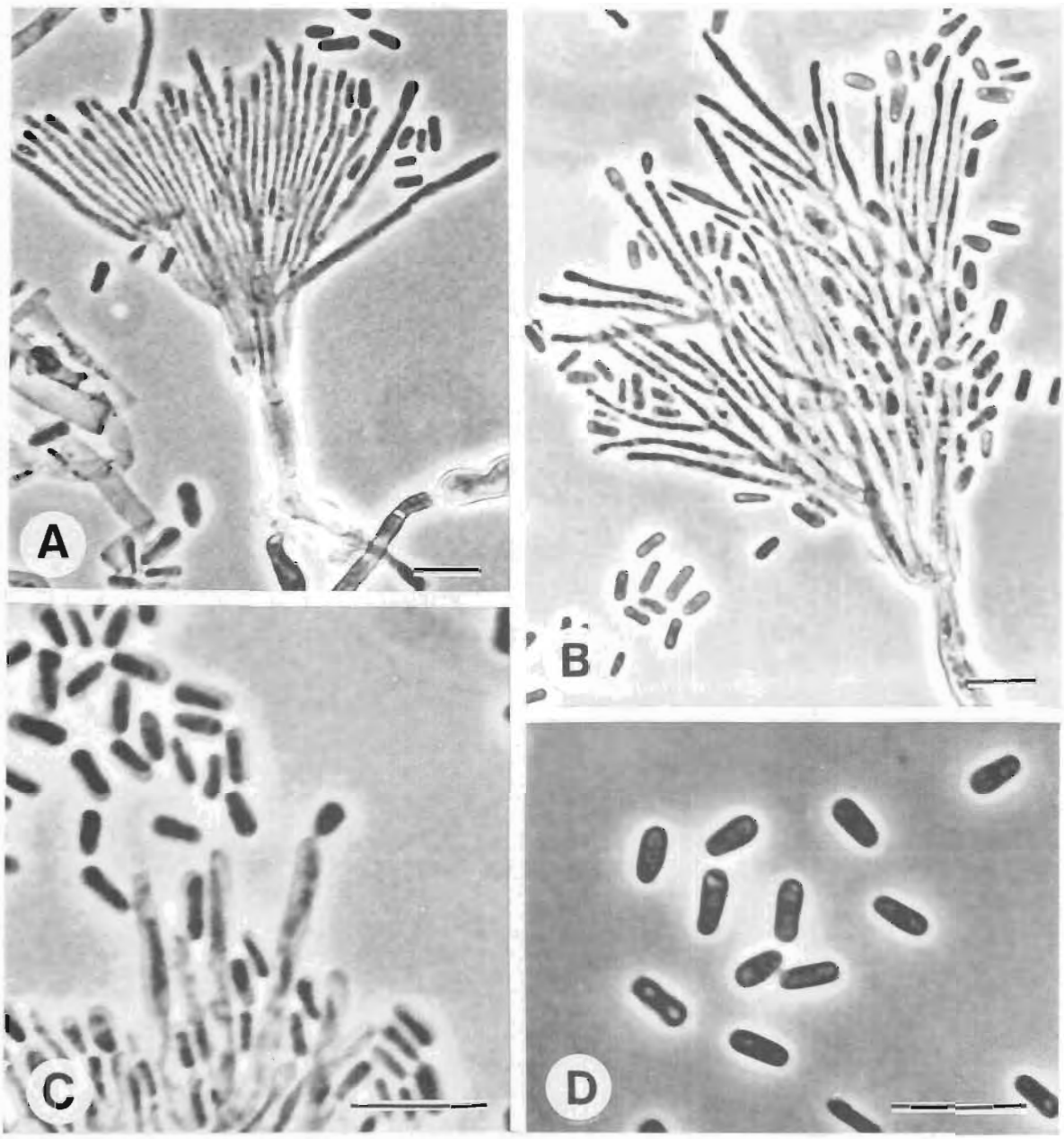

Fig. 49. Light micrographs of the conidiophores and conidia of *L. calophylli* (CMW 4257). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

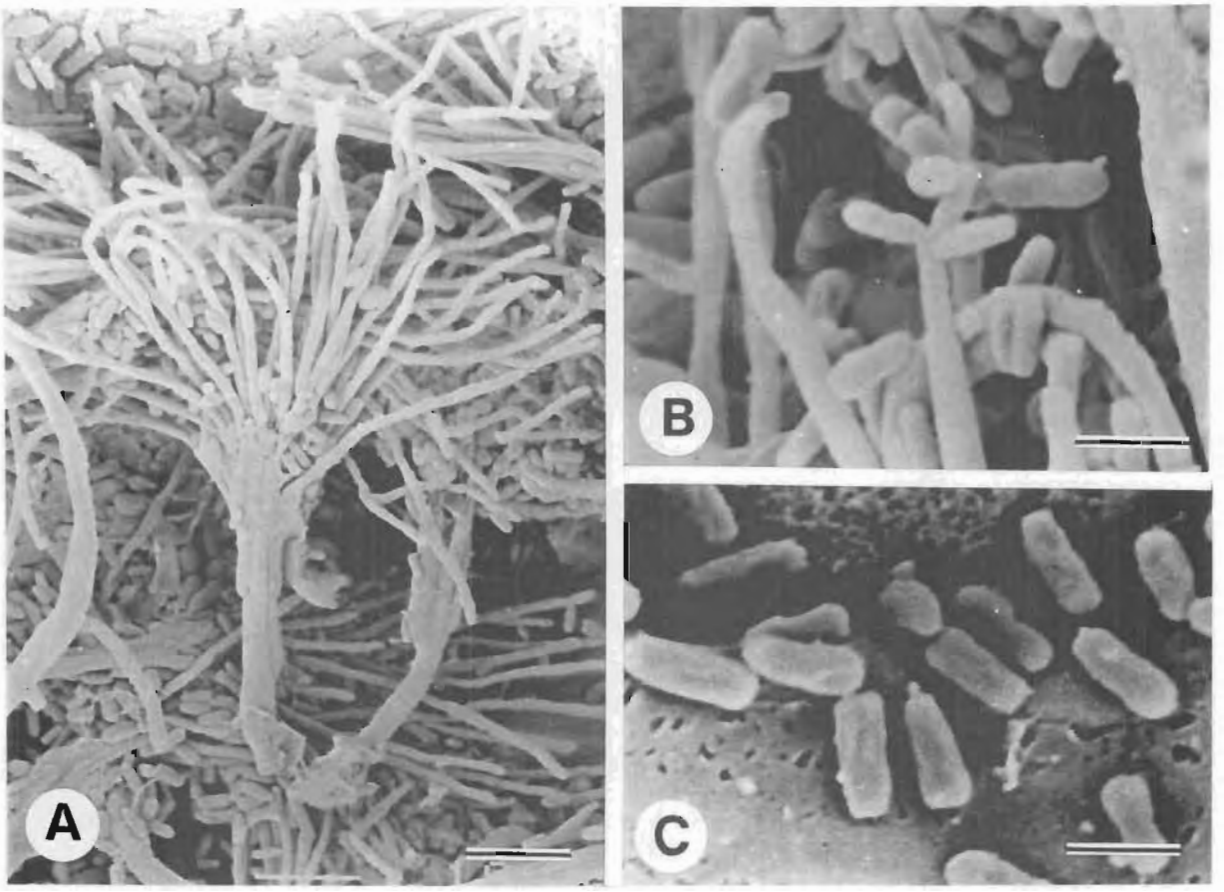

Fig. 50. Scanning electron micrographs of the conidiophores and conidia of *Leptographium calophylli* (CMW 4257). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 5 µm).

12. *Leptographium costaricense* G. Weber, Spaaij & M.J. Wingf. *Mycological Research* **100**, 733. 1996. **(Fig. 51-53).**

Teleomorph: Not known.

Etymology: cos-ta-ri-cénse: growing in Costa Rica. This specific epithet refers to the origin of this fungus in Costa Rica.

Conidiophores occur mostly singly, arising directly from the mycelium, erect, macronematous, mononematous, (150-) 221 - 256 (-625) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), not constricted at septa, cylindrical, simple, 3-11 septate, (112-) 193.5 - 215 (-585) µm long, 2.0 - 4.0 µm wide below primary branches, apical cell not swollen, 2.0 - 4.5 µm wide at base, basal cell sometimes swollen. *Conidiogenous apparatus* (25-) 30 - 37 (-50) µm long, excluding the conidial mass, with 1 - 3 series of cylindrical branches, 2-4 primary branches, light olivaceous (21"k), smooth, cylindrical, aseptate 10 - 18 (-20) µm long and 2.0 - 4.0 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline to light olivaceous (21"m), aseptate, (5.0-) 8.0 - 9.0 (-12) µm long, 2.0 - 3.0 µm wide, occasionally hyaline tertiary branches 6.0 µm long and 2.0-3.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 8.0 - 15 (-16) µm long and 1.0 - 2.0 µm wide. *Conidia* hyaline, aseptate, obovoid with truncate ends, 3.0 - 5.0 x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming amber (21'b) with age. Conidial mass amber when wet, remaining amber when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 9 mm in diam. in 8 days. No growth below 10°C with little growth at 35°C. Not able to withstand high concentrations of cycloheximide with a 100% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies olivaceous (21"m) edges cartridge buff (19"f) becoming pale cinnamon pink (13"f) with age. *Colony margin* smooth. *Hyphae* submerged on agar with no aerial mycelium, hyaline, smooth, straight, not constricted at the septa, 2.0 - 4.0 µm diam.

Specimens examined: Holotype: CMW 3041, isolated from roots of *Talauma*

sambuensis, Biological station of 'La Selva', Sarapiqui, Costa Rica, collected G. Weber, March 1992. **Cultures**: CMW 3066, isolated from roots of *Talauma sambuensis,* Biological station of 'La Selva', Sarapiqui, Costa Rica, collected G. Weber, March 1992.

Known distribution: Costa Rica.

Hosts/substrate: Rhizosphere of *Talauma sambuensis* (Weber *et al.*, 1996).

Associated insects: Not known.

Notes: This species is unusual in that it was isolated from soil, which is an unusual substrate for species of *Leptographium*. *Leptographium reconditum* is the only other *Leptographium* sp. that has been isolated from soil (Jooste, 1978). Weber *et al.* (1996) found that *L. costaricense* has a low tolerance to cycloheximide, which implies a lack of relatedness to *Ophiostoma*. This was confirmed in the present study. As a result of this, they suggested that the species deserved further study. Weber *et al.,* (1996) further suggested that although *L. costaricense* and *L. reconditum* are morphologically similar to other *Leptographium* species, their generic affinities might fall outside those of *Leptographium*.

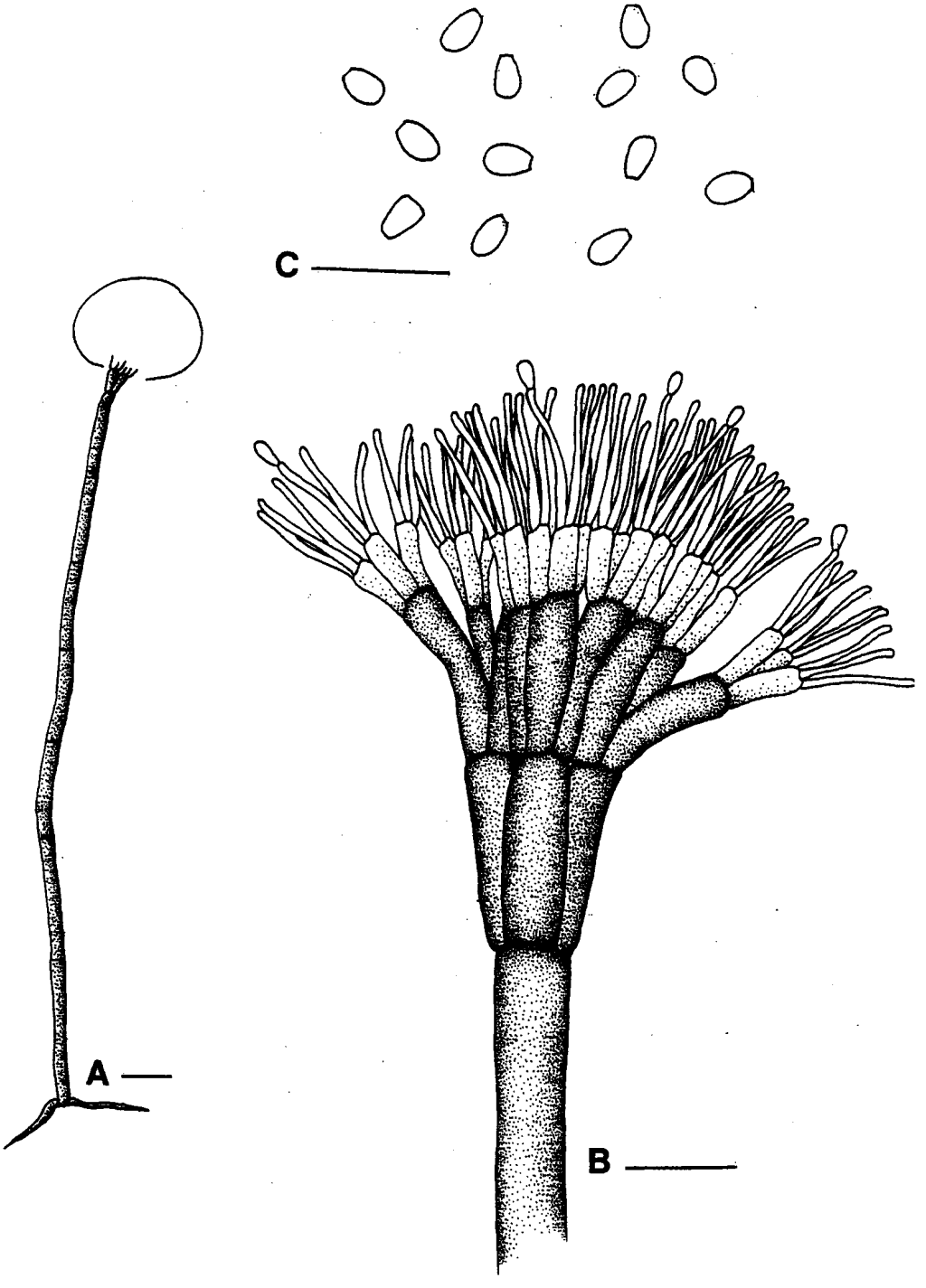

Fig. 51. Conidiophores and conidia of *L. costaricense* (CMW 3041). **A.** Habit sketch (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

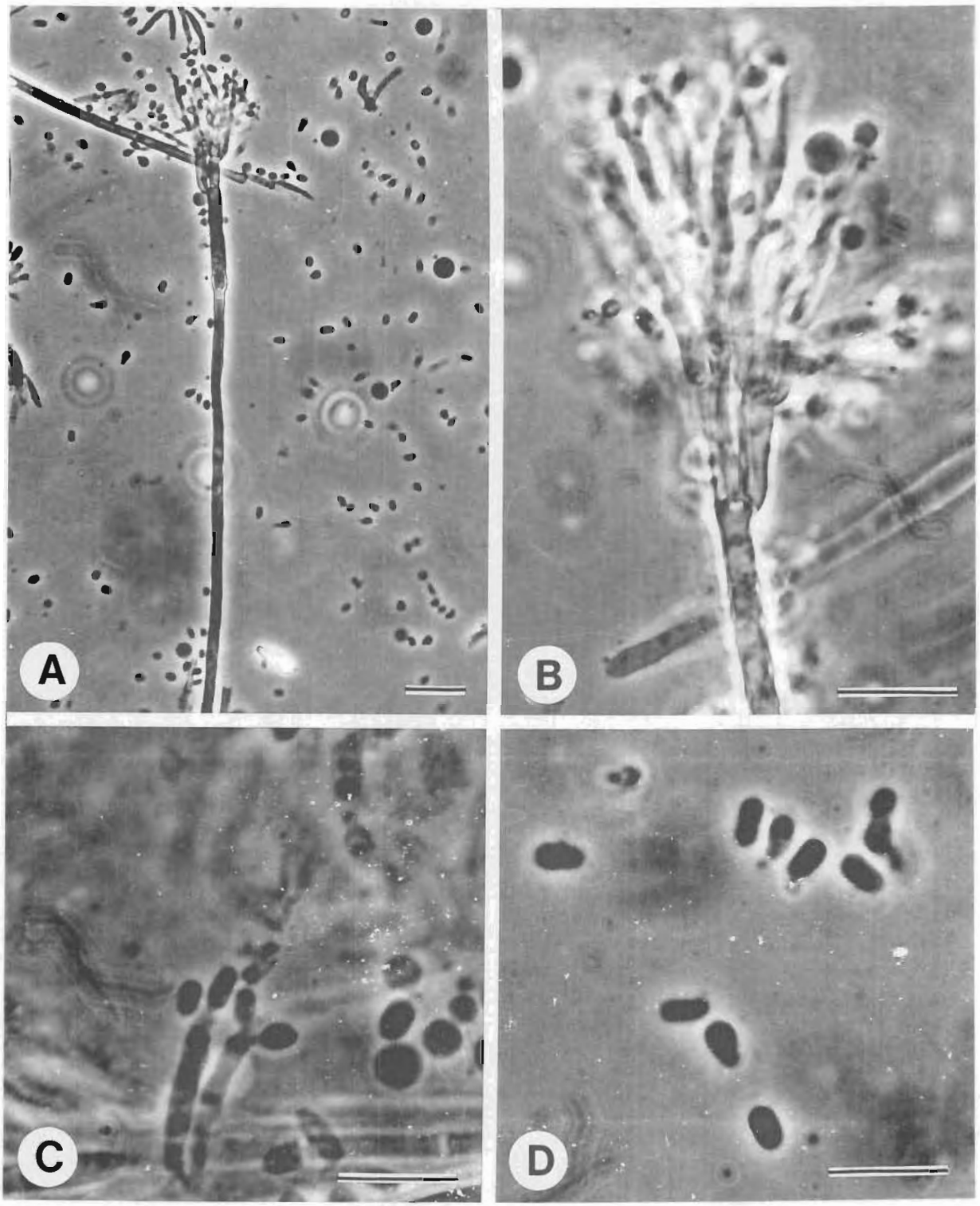

Fig. 52. Light micrographs of the conidiophores and conidia of *L. costaricense* (CMW 3041). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

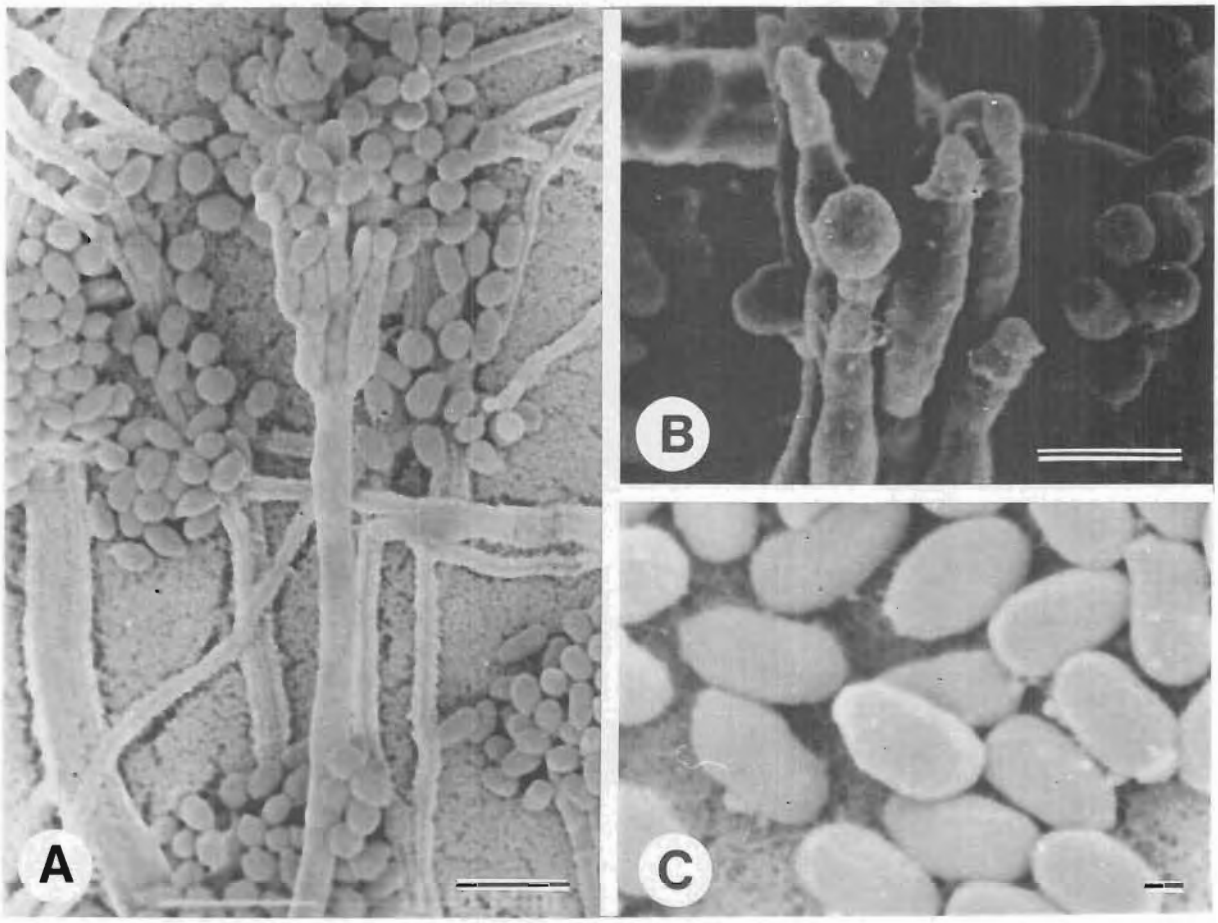

Fig. 53. Scanning electron micrographs of the conidiophores and conidia of *L. costaricense* (CMW 3041). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

13. ***Ophiostoma crassivaginatum*** (H.D. Griffin) T.C. Harr., *Mycotaxon* **27**, 41. 1987. **(Figs. 54 - 56).**

≡ *Ceratocystis crassivaginata* H.D. Griffin, *Canadian Journal of Botany* **46**, 701. 1968.

≡ *Ceratocystiopsis crassivaginata* (H.D. Griffin) H.P. Upadhyay, Monograph of *Ceratocystis* and *Ceratocystiopsis*, **123**. 1981.

Anamorph: *Leptographium crassivaginatum* (H.D. Griffin) M.J. Wingf. *Transactions of the British Mycological Society* **85**, 92. 1985.

Etymology: cras-si-va-gi-ná-tum: possessing a thick sheath. From the Latin adjective crassus: thick and Latin noun vagina: a sheath. This specific epithet refers to the characteristic rough, granular sheath around the hyphae.

Perithecial bases black, globose and rough walled, unornamented, 40 - 90 µm in diam. *Perithecial neck* black at the base, turning dark brown towards the apex, 40 - 60 µm long, 15 - 30 µm above globose base, 12 - 15 µm wide at the apex, *ostiolar hyphae* present. *Asci* prototunicate, hyaline, evanescent. *Ascospores* boat-shaped, aseptate, hyaline, invested in a sheath, 10.0 - 11.5 x 5.0 - 6.0 µm (Griffin, 1968).

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, 25 - 106 (-118) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), constricted at septa, cylindrical, simple, 0 - 5 septate, 8.0 - 60 (-85) µm long, (3.0-) 4.0 - 8.0 (-11) µm wide below primary branches, apical cell sometimes swollen, (3.0-) 4.0 - 8.0 (-11) µm wide at base, basal cell sometimes swollen. *Conidiogenous apparatus* 15.5 - 56.5 (-62) µm long, excluding the conidial mass, with 1 to 3 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical or barrel shape, 0-2 septate (8-) 12.5 - 14 (-23.5) µm long and 2.5 - 9.0 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous, 0-1 septate, (7.5-) 11 - 15.5 (-22) µm long, (3.0-) 4.0 - 7.0 (-7.5) µm wide, occasionally hyaline

tertiary branches. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (7.0-) 8.5 - 10.5 (-12.5) µm long and 2.0 - 3.0 µm wide. *Conidia* hyaline, aseptate, oblong to obovoid, (4.0-) 4.5 - 5.5 (-10) x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming amber (21'b) with age. Conidial mass amber when wet, remaining amber when dry.

Colonies with optimal growth at 30°C on 2% MEA, reaching 32 mm in diam. in 9 days. No growth below 5°C with little growth at 35°C. Able to withstand high concentrations of cycloheximide with a no reduction in growth on 0.5 g/l cycloheximide after 12 days at 20°C in the dark. Colonies olivaceous (21"m). *Colony margin* laciniate. *Hyphae* submerged on agar with little aerial mycelium, hyaline to light olivaceous (21"k), smooth, sometimes roughened with granular material, straight, occasionally constricted at the septa, (3.0-) 4.0 - 8.0 (-11) µm diam.

Specimens examined: Holotype: Canada, Ontario, Geraldton District near Stevens Highway 625, *Picea mariana*, June 1963, collected: H.D. Griffin, DAOM 110144. **Cultures**: collected: T. Hinds, 1986, CMW 90, CMW 134; Canada, *Populus* sp., 1986, collected: K.A. Seifert, CMW 884.

Known distribution: Canada.

Hosts/substrate: *Picea mariana* (Griffin, 1968; Olchowecki & Reid, 1974), *Picea glauca* (Olchowecki & Reid, 1974), *Pinus resinosa* (Olchowecki & Reid, 1974), *Pinus strobus* (Olchowecki & Reid, 1974), *Pinus sylvestris* (Olchowecki & Reid, 1974), *Populus grandidentata* (Griffin, 1968), *Populus tremuloides* (Griffin, 1968; Hinds, 1972), *Fraxinus nigra* (Olchowecki & Reid, 1974).

Associated insects: *Trypodendron retusus* (Harrington, 1988).

Notes: *Leptographium crassivaginatum* can easily be distinguished from other *Leptographium* spp. based on the small, robust conidiophores and granulated sheath

material around the hyphae. *Leptographium pyrinum* and *L. yunnanensis* are also characterized by granular sheath material around the hyphae. However, the conidiophores and conidia of *L. pyrinum* are considerably larger than those of *L. crassivaginatum*. *Leptographium yunnanensis* can be distinguished from *L. crassivaginatum* based on the narrower conidia in the latter species. The hyaline pear-shaped cells in the mycelium reported by Griffin (1968) were observed in the isolates examined. This is also the only species of *Leptographium* with a teleomorph that has falcate ascospores (Harrington, 1988) and was placed in the minuta - group by Olchowecki and Reid (1974) based on this character. The teleomorph was transferred to *Ceratocystiopsis* by Upadhyay (1981). Later, Harrington (1987) transferred this species to *Ophiostoma*, where it currently resides. Nothing is known about pathogenicity of *Ophiostoma crassivaginatum*.

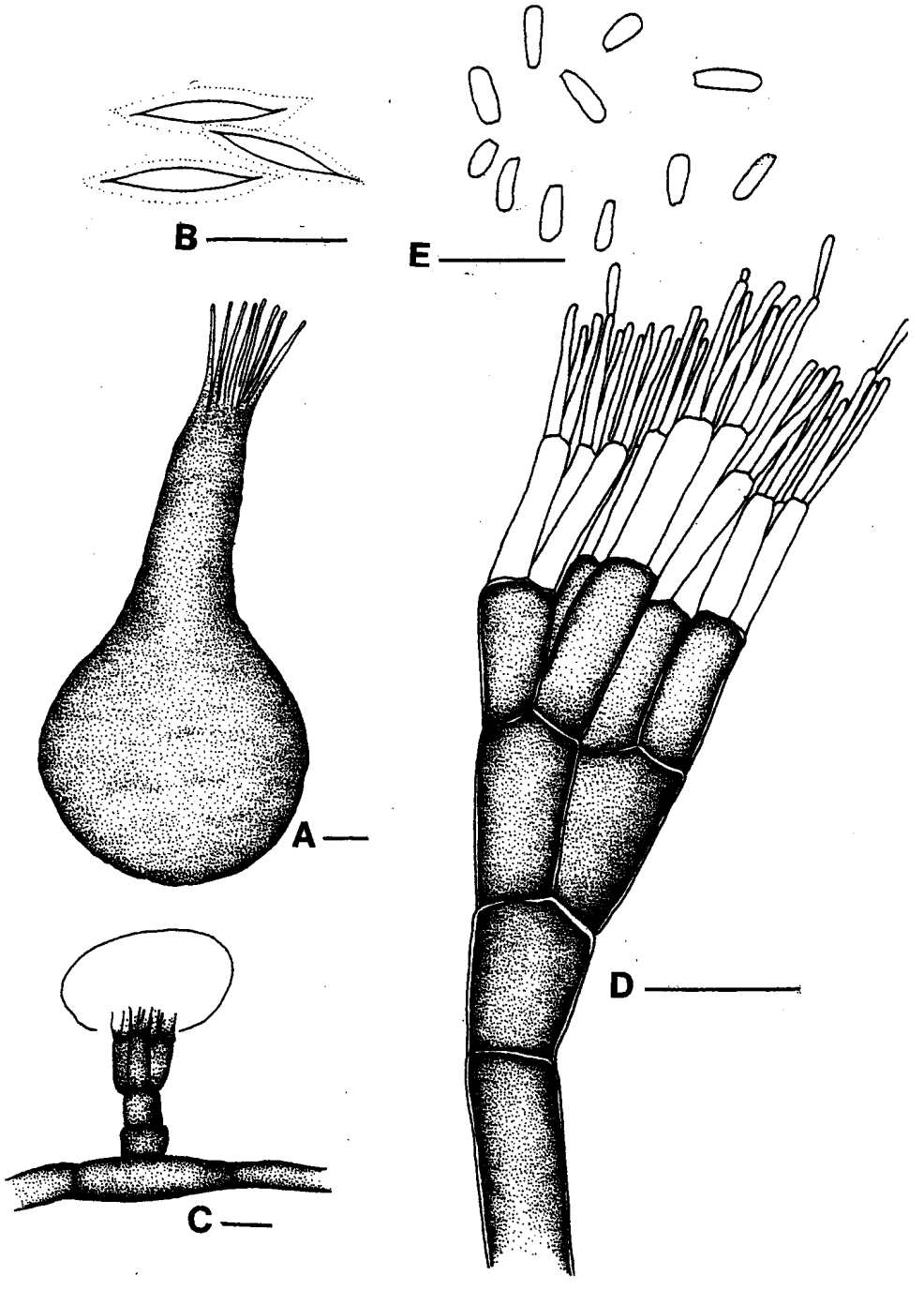

Fig. 54. Teleomorph and anamorph structures of *O. crassivaginatum* (CMW 90). **A.** Perithecium (Bar = 10 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

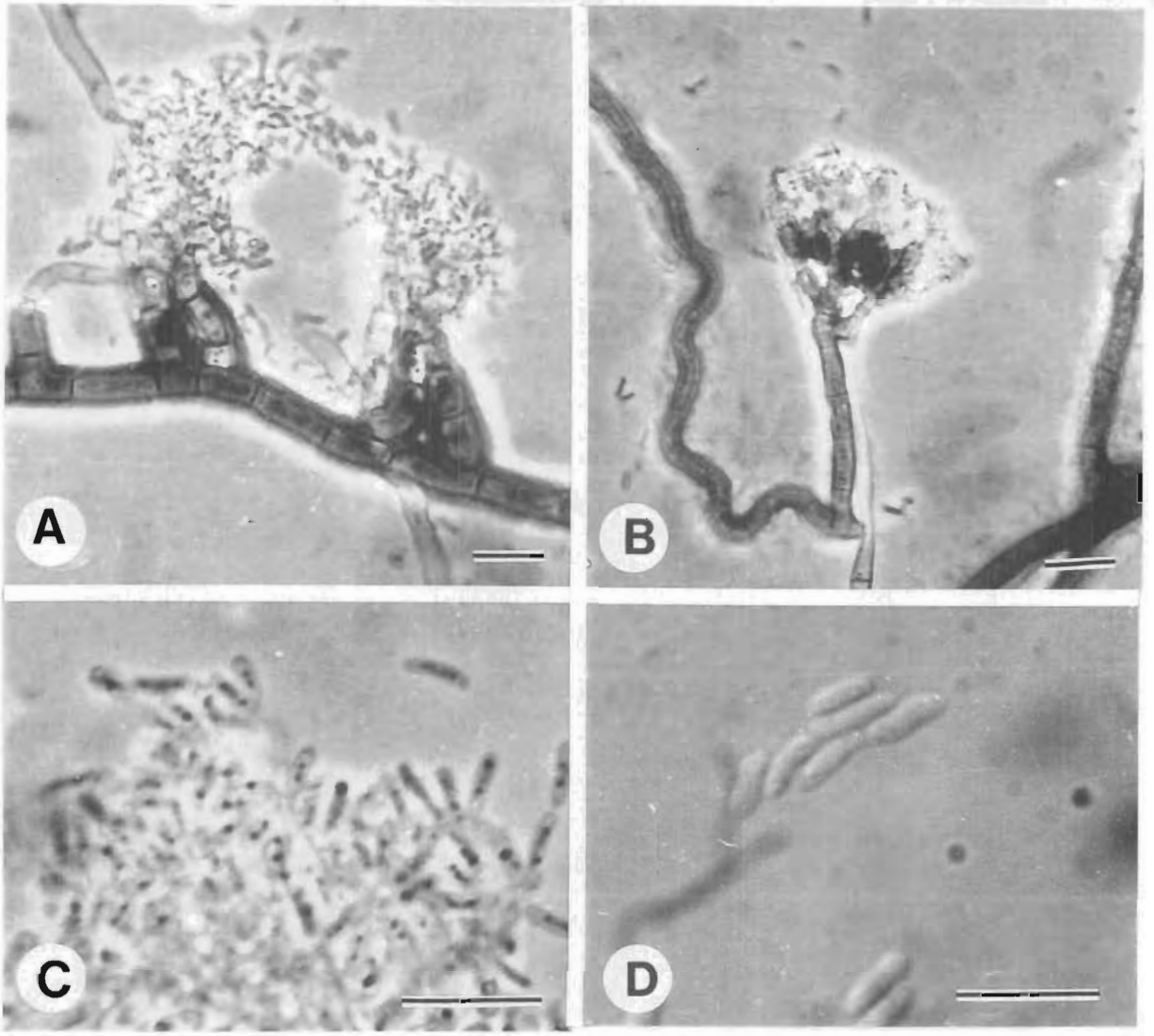

Fig 55. Light micrographs of anamorph structures of *O. crassivaginatum* (CMW 90). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiophore (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

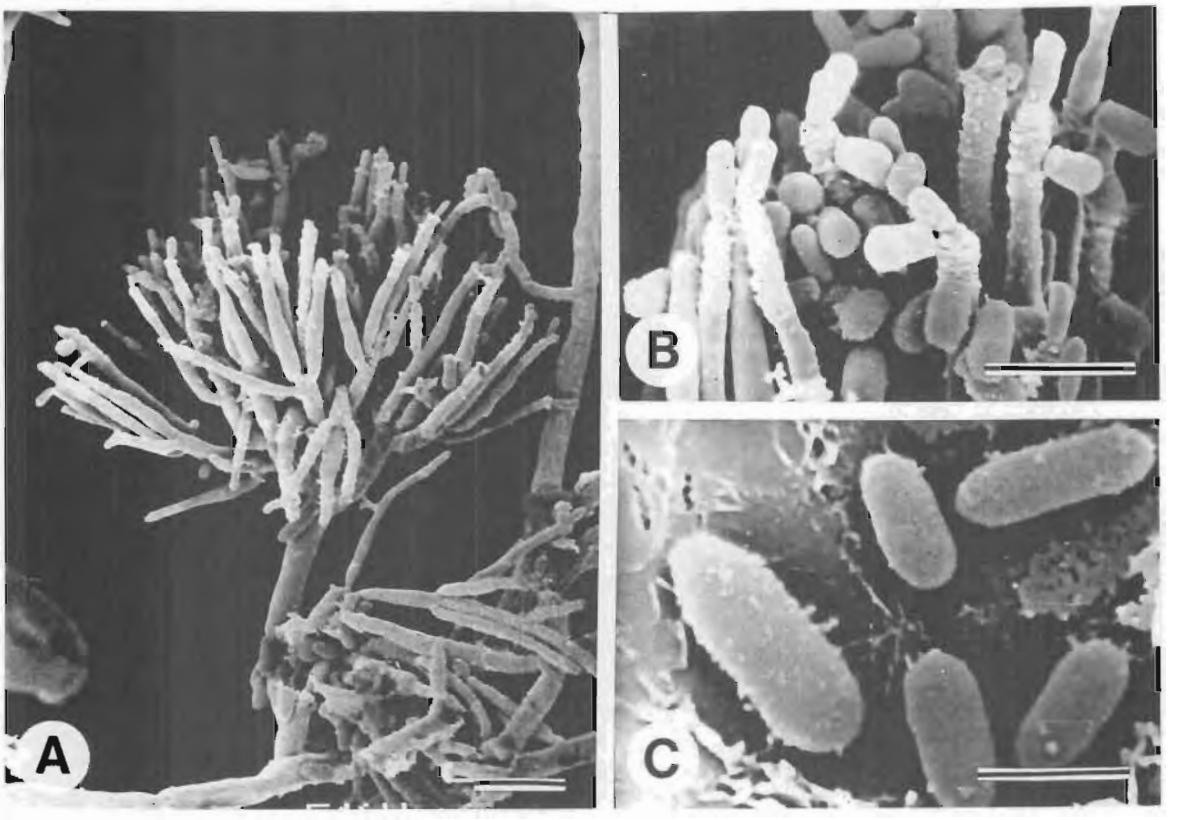

Fig. 56. Scanning electron micrographs of the conidiophores and conidia of *O. crassivaginatum* (CMW 90). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 5 µm).

14. *Leptographium douglasii* M.J. Wingf., T.C. Harr. & Crous, *Canadian Journal of Botany* **72**,231. 1994. **(Figs. 57 - 58).**

Teleomorph: Not known.

Etymology: dóug-la-sii: of Douglas (-fir). This specific epithet refers to the occurrence of this fungus on Douglas-fir.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (57.5-) 107 - 320 (-512.5) µm in length, rhizoid-like structures present. *Stipes* olivaceous (21"m), smooth, cylindrical, simple, 3 - 11 septate, (42.5-) 52.5 - 282.5 (-475) µm long, (5.0-) 7.0 - 8.0 (-15) µm wide below primary branches, apical cell not swollen, (5.0-) 6.0 - 9.5 (-12.5) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (20-) 33.5 - 58.5 (-80) µm long, excluding the conidial mass, with 2 - 4 series of cylindrical branches, 2-3 primary branches, light olivaceous (21'k), smooth, cylindrical, aseptate, (6.0-) 12 - 14 (-22) µm long and (3.0-) 4.0 - 6.5 (-9.0) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21"k), aseptate, (7.0-) 9.0 - 11 (-16) µm long, 2.0 - 5.0 µm wide, tertiary branches hyaline, aseptate, (4.0-) 7.5 - 9.0 (-12) µm long, 1.0 - 2.5 µm wide, quaternary branches aseptate, hyaline, 8.0 - 9.0 µm long, 1.0 - 2.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (6.0-) 9.5 - 20 (-21) µm long and 1.0 - 2.0 µm wide. *Conidia* light gray olivaceous (19""), aseptate, obovoid, 4.0 - 6.0 (-7.0) x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 20°C on 2% MEA, reaching 16 mm in diam. in 9 days. No growth below 10°C or above 25°C. Able to withstand high concentrations of cycloheximide with a 22% reduction in growth on 0.5 g/l cycloheximide after 12 days at 20°C in the dark. Colonies olivaceous black (21""m). *Colony margin* sinuate. *Hyphae* submerged on agar with little aerial mycelium, hyaline to light olivaceous (21"k), smooth, slightly serpentine, occasionally constricted at the septa,

(2.5-) 5.0 - 8.0 (-15) µm diam.

Specimens examined: Holotype: U.S.A., Oregon Prospect, *Pseudotsuga menziesii* roots, 1980, collected: D. Goheen, PREM 51446. **Paratypes**: U.S.A., Clouderof Indian Reserve near Mescalero, *Pseudotsuga menziesii* roots, 1980, collected: M. Mielke, PREM 51447; U.S.A. California, *Pseudotsuga menziesii* roots, 1980, collected: T.C. Harrington, PREM 51448; U.S.A., Colorado San Juan National Forest, *Pseudotsuga menziesii* roots, 1980, collected: R. James, PREM 51449. **Cultures**: U.S.A., Prospect Oregon, *Pseudotsuga menziesii*, 1980, collected: D. Goheen, CMW 2078, CMW 2782 (PREM 56386), CMW 725 (PREM 56370).

Known distribution: U.S.A.

Hosts/substrate: *Pseudotsuga menziesii* (Wingfield *et al.*, 1994).

Associated insects: *Hylastes nigrinus* (Wingfield *et al.*, 1994).

Notes: *Leptographium douglasii* is restricted to douglas-fir (*Pseudotsuga menziesii*) and is consistently associated with a root-feeding insect (*Hylastes nigrinus*) (Wingfield *et al.*, 1994). This is the same insect that occasionally carries *L. wageneri* var. *pseudotsugae*. *Leptographium douglasii* can, however, be distinguished from *L. wageneri* var. *pseudotsugae* based on a more robust conidiogenous apparatus and longer narrower conidia in the former species as well as differences in their optimal growth temperatures. This fungus was also shown to have a low level of virulence towards *P. menziesii* (Harrington & Cobb, 1983).

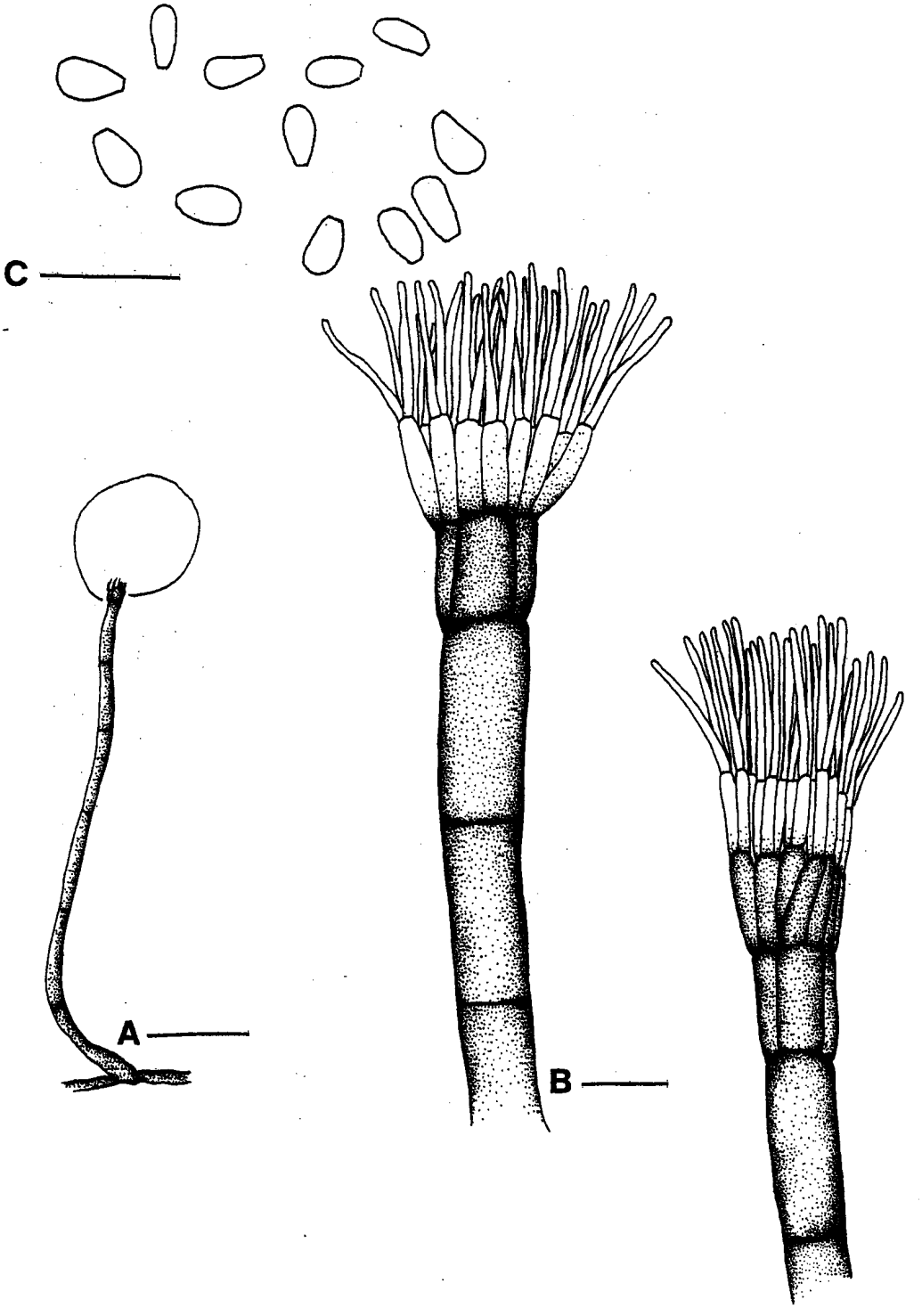

Fig. 57. Conidiophores and conidia of *L. douglasii* (CMW 2078). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

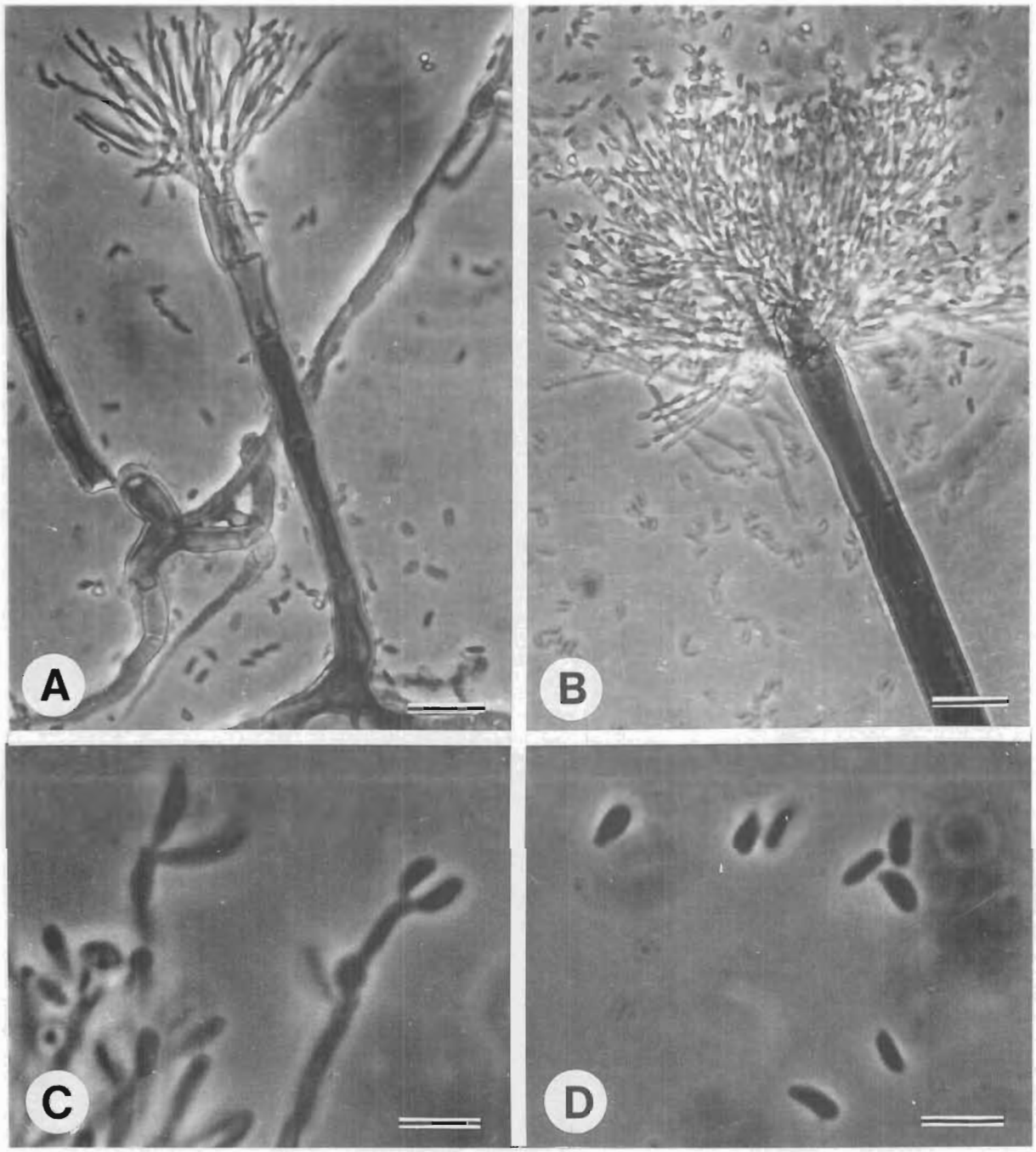

Fig. 58. Light micrographs of the conidiophores and conidia of *L. douglasii* (CMW 2078). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous apparatus (Bar = 20 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

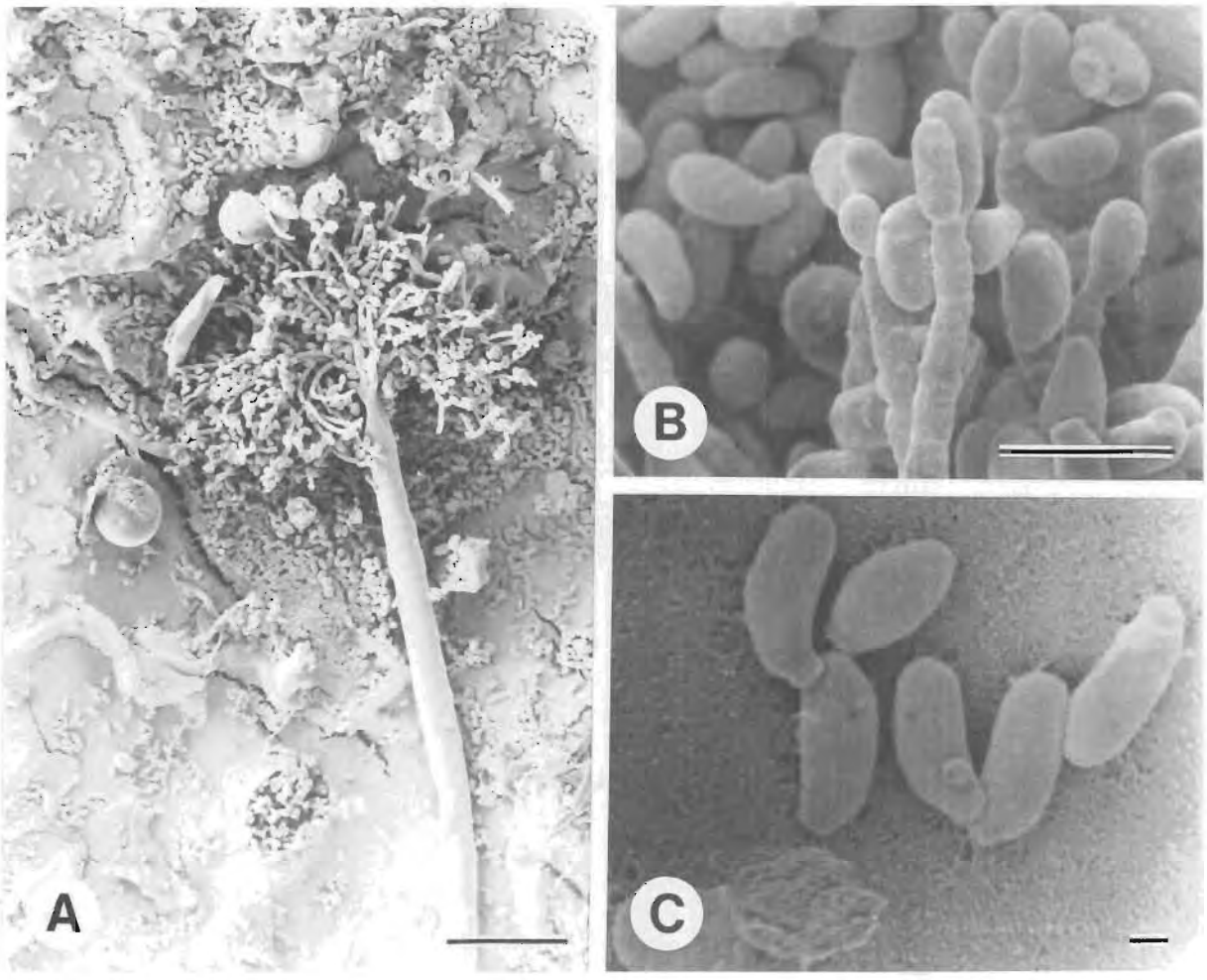

Fig. 59. Scanning electron micrographs of the conidiophores and conidia of *L. douglasii* (CMW 2078). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 1 µm).

15. *Ophiostoma dryocoetidis* (W.B. Kendr. & Molnar) De Hoog & R.J. Scheff., *Mycologia* **76**, 297. 1984. **(Figs. 60 - 61).**

≡ *Ceratocystis dryocoetidis* W.B. Kendr. & Molnar, *Canadian Journal of Botany* **43**, 39. 1965.

Anamorph: *Leptographium dryocoetidis* (W.B. Kendr. & Molnar) M.J. Wingf., *Transactions of the British Mycological Society* **85**, 92. 1985.

Etymology: dry-o-coé-ti-dis: connected with *Dryocoetus*. This specific epithet refers to the association of this fungus with the bark beetle *Dryocoetus confusus*.

Perithecial bases black, globose and smooth walled, unornamented, 170 - 260 in diam. *Perithecial neck* dark brown to black, cylindrical with a slight apical taper, paler near the apex, smooth, 150 - 560 µm long, 40 - 60 µm above globose base, 35 - 45 µm wide at the apex, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* allantoid, aseptate, hyaline, invested in a sheath, 5.2 - 7 x 2.2 - 3.2 µm (Kendrick & Molnar, 1965).

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (37.5-) 123.5 - 134.5 (-250) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), smooth, cylindrical, simple, 0-8 septate, (27.5-) 88 - 74 (-205) µm long, 2.5 - 8.0 µm wide below primary branches, apical cell not swollen, (2.5-) 4.0 - 7.5 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (34-) 44 - 75 (-99) µm long, excluding the conidial mass, with 2 to 4 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical or barrel shape, 0-1 septate (6.0-) 10.5 - 15 (-20) µm long and 3.0 - 6.0 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21"k), aseptate, (4.0-) 10 -11.5 (-13.5) µm long, 2.5 - 4.0 µm wide, tertiary branches light olivaceous (21"k), aseptate, (5.5-) 9.0 - 11 (-15) µm long, 2.5 - 4.0 µm wide, quaternary branches aseptate, 7.0 - 13 (-14) µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (10-) 15.5 - 19 (-25) µm long and 1.0 - 2.5 µm wide. *Conidia* hyaline, aseptate, oblong with truncate ends and rounded apices, 9.0 - 18 x

2.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, turning amber (21'b) when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 27 mm in diam. in 9 days. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies brownish olive (21"m). *Colony margin* smooth. *Hyphae* submerged on agar with little aerial mycelium, hyaline to light olivaceous (21"k), smooth, straight, frequently constricted at the septa, 2.5 - 7.5 µm diam.

Specimens examined: Holotype: British Columbia, Bolean Lake, *Abies lasiocarpa*, Oct. 1963, collected: A.C. Molnar and W.B. Kendrick, DAOM 97704. **Cultures**: British Columbia, Bolean Lake, *Abies lasiocarpa*, Oct. 1963, collected: A.C. Molnar and W.B. Kendrick, CMW 442 (same as CBS 376.66 and DAOM 97704).

Known distribution: Canada.

Hosts/substrate: *Abies lasiocarpa* (Kendrick and Molnar, 1965; Molnar, 1965).

Associated insects: *Dryocoetus confusus* (Kendrick and Molnar, 1965; Molnar, 1965).

Notes: This is one of four *Leptographium* spp. with conidia that are three times as long as they are wide. The others are *O. penicillatum, O. americanum* and *L. eucalyptophilum.* Although this fungus closely resembles *O. penicillatum,* by having similar conidia, Kendrick and Molnar (1965) distinguished these two species based on their perithecial characters. *Ophiostoma dryocoetidis* can be distinguished from *O. americanum* based on the more curved conidia of the former, in contrast to the needle shaped conidia of the latter species (Jacobs *et al.,* 1998). *Leptographium eucalyptophilum* is distinguished from *O. dryocoetidis* based on the more needle shaped conidia and longer conidiophores of the former, compared to the curved conidia and shorter conidiophores of the latter species (Jacobs *et al.,* 1999). This

fungus was the most common species isolated from stained wood of alpine fir, and it was also shown to have the ability to kill this host in inoculation trials (Molnar, 1965).

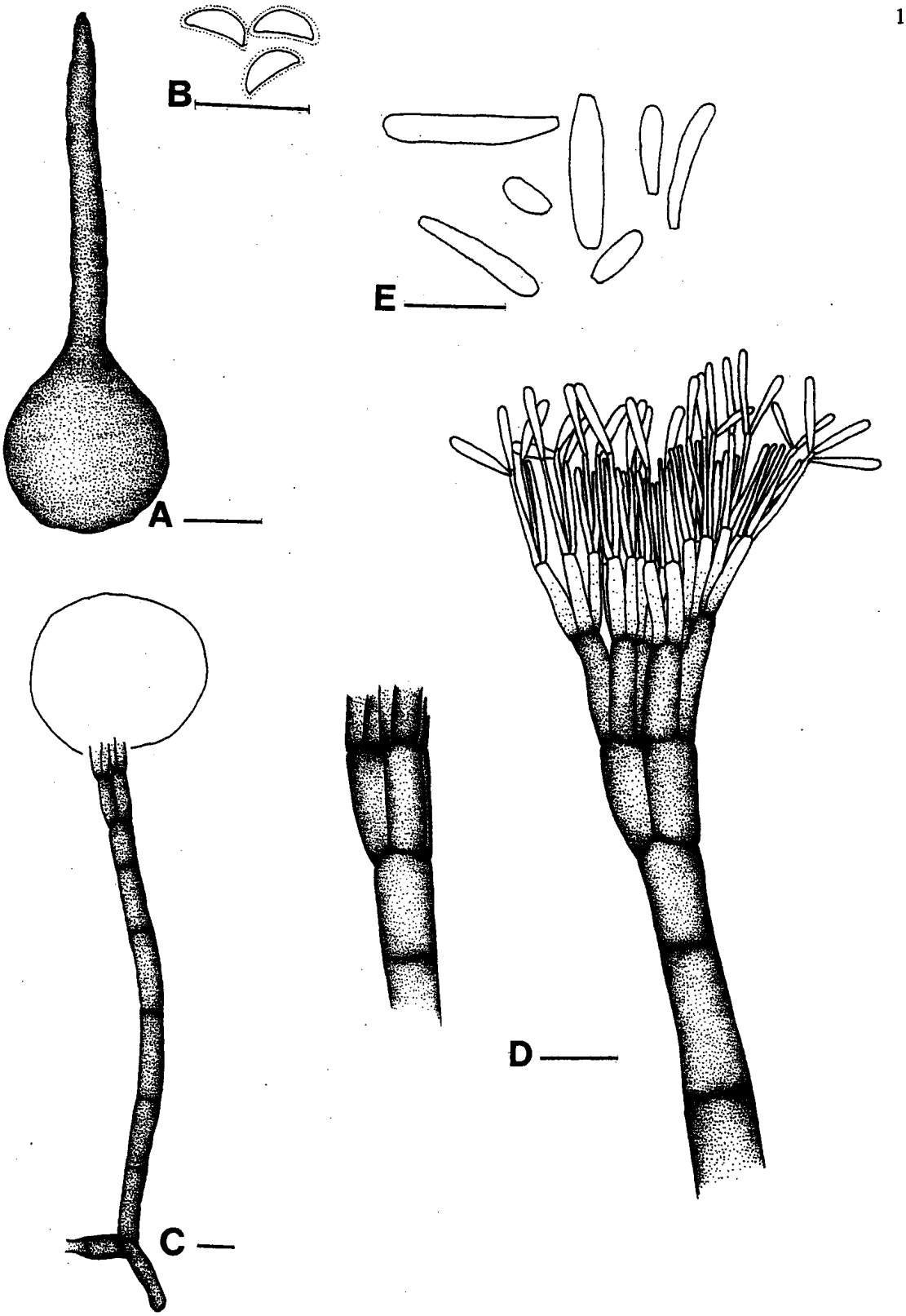

Fig. 60. Teleomorph and anamorph structures of *O. dryocoetidis* (CMW 442). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

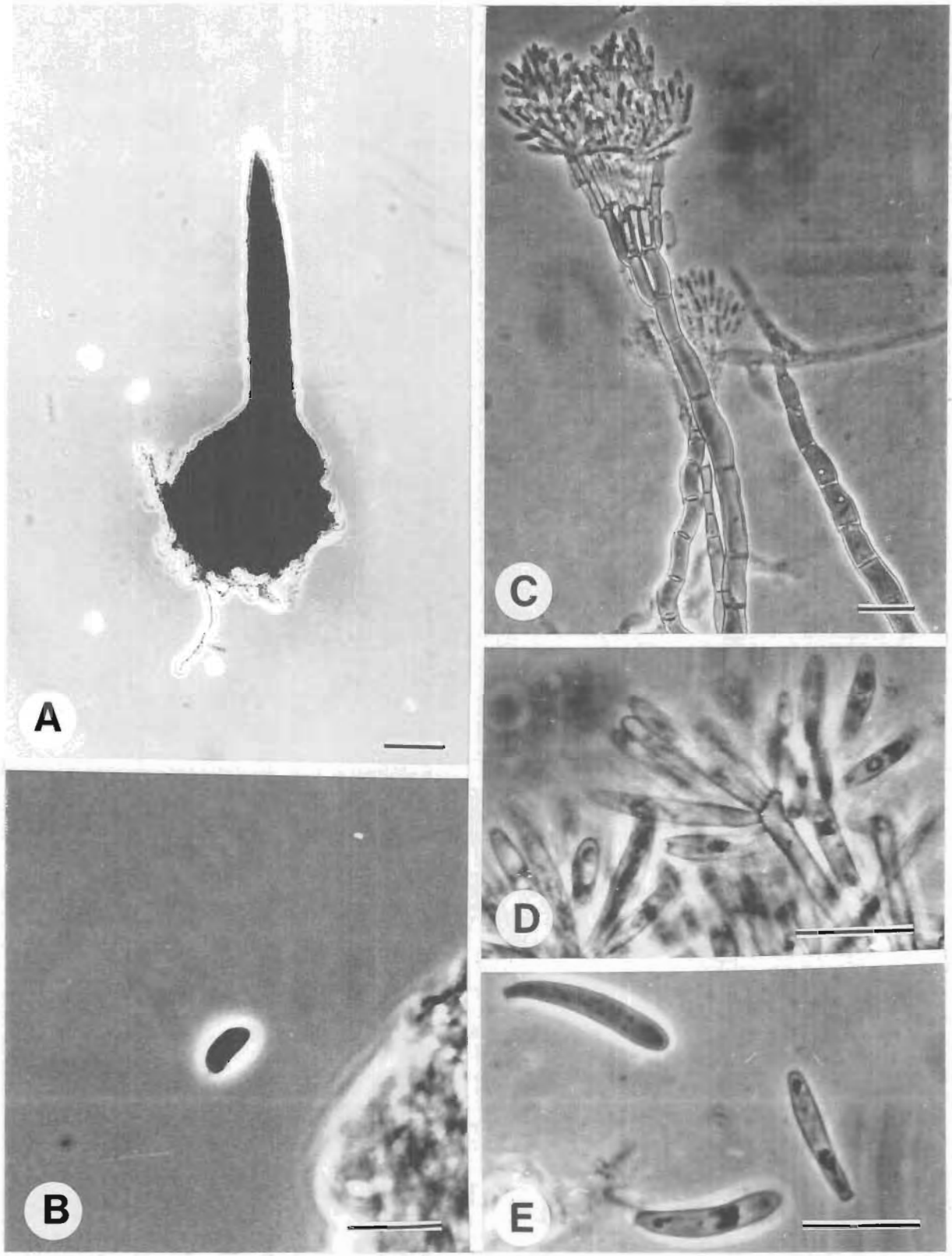

Fig. 61. Light micrographs of the teleomorph and anamorph structures of *O. dryocoetidis* (CMW 442). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

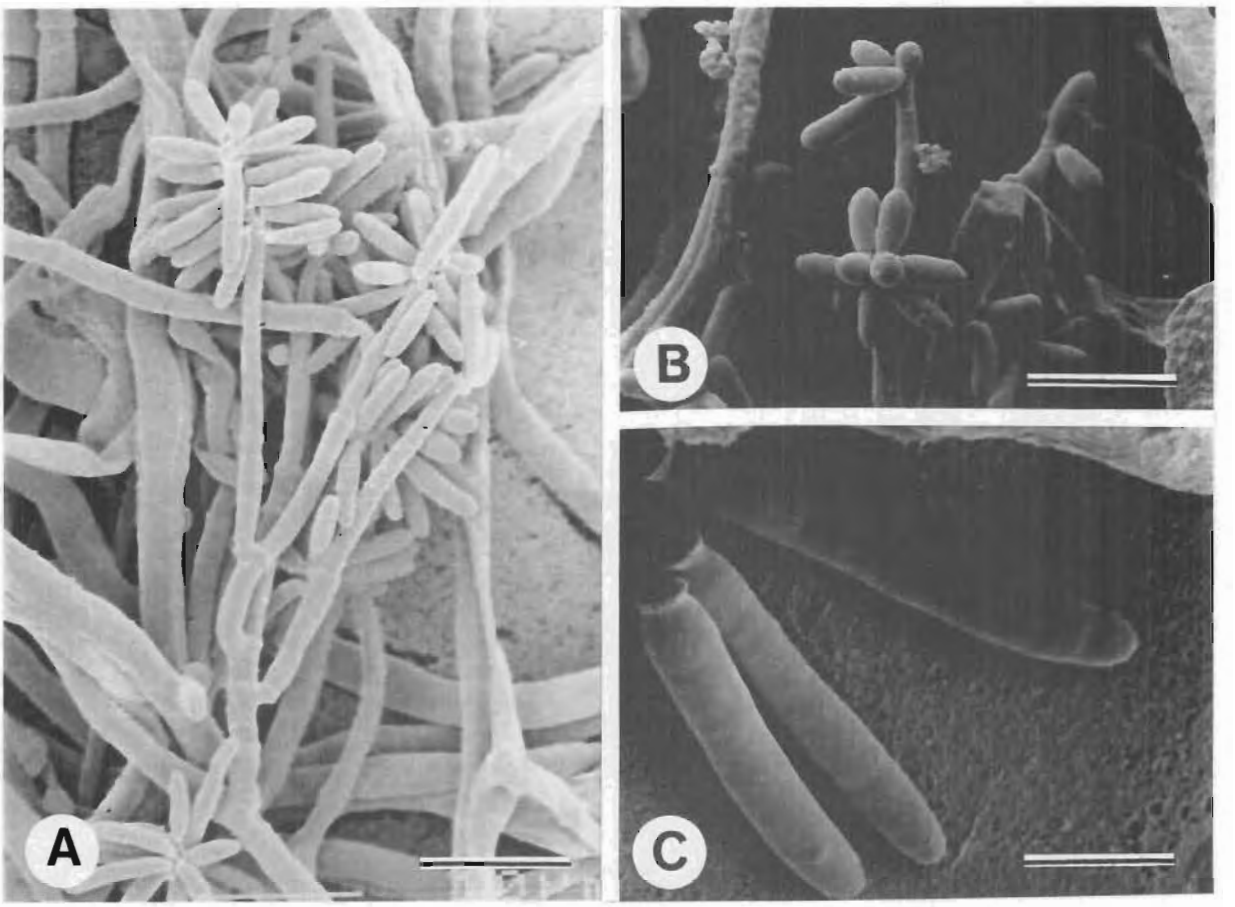

Fig. 62. Scanning electron micrographs of the conidiophores and conidia of *O. dryocoetidis* (CMW 442). **A**. Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 5 µm).

16. *Leptographium elegans* M.J. Wingf., Crous & Tzean *Mycological Research* **98**, 783. 1994. **(Figs. 63-65).**

Teleomorph: Not known.

Etymology: é-le-gans: elegant. From the Latin adjective elegans: choice, fine, neat. The specific epithet refers to the small, fine, conidiophores of this fungus.

Conidiophores occurring mostly singly, arising directly from the mycelium, erect, macronematous, mononematous, (102.5-) 237 - 241 (-432.5) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), smooth, cylindrical, simple, 2-8 septate, (62 5-) 195 - 188 (-377.5) µm long, 2.5 - 5.0 µm wide below primary branches, apical cell not swollen, 4.0 - 6.0 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (30-) 41 - 48.5 (-82.5) µm long, excluding the conidial mass, with 2 to occasionally 3 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical, aseptate, (10-) 11.5 - 22 (-25) µm long and 2.0 - 4.0 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous, aseptate, 7.0 - 13.5 (-15) µm long, 2.0 - 5.5 µm wide, tertiary branches hyaline, aseptate, (6.0-) 9.0 -11.5 (-13) µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (13.5-) 18 - 20 (-26) µm long and 1.0 - 4.0 µm wide. *Conidia* hyaline, aseptate, oblong, 3.0 - 5.0 x 1.0 - 2.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 9 mm in diam. in 8 days. No growth below 10°C with very little growth at 35°C. Able to withstand high concentrations of cycloheximide with a 18% reduction in growth on 0.5 g/l cycloheximide after 12 days at 20°C in the dark. Colonies olivaceous (21"m). *Colony margin* smooth. *Hyphae* submerged on agar with little aerial mycelium, hyaline to light olivaceous (21"k), smooth, slightly serpentine, strands of hyphae aggregated, not constricted at the septa, (2.5-) 3.5 - 5.0 (-7.5) µm diam.

Specimens examined: Holotype: N.E. of Taiwan, Lotung, Ilan country, *Chamaecyparis* sp., 1992, collected: M.J. Wingfield, PREM 51442. **Cultures**: N.E. of Taiwan, Lotung, Ilan country, *Chamaecyparis* sp., 1992, collected: M.J. Wingfield, CMW 2245, CMW 2248 (PREM 56345), CMW 2249 (PREM 56344).

Known distribution: Taiwan.

Hosts/substrate: *Chamaecyparis formosensis* (Wingfield *et al.*, 1994).

Associated insects: Not known.

Notes: *Leptographium elegans* is distinguished from other species in this genus based on the presence of a prominent *Sporothrix* synanamorph in culture. *Sporothrix* synanamorphs have also been observed in other species of *Ophiostoma,* especially those with *Pesotum* anamorphs, but not those with *Leptographium* anamorphs. *Leptographium elegans* is morphologically similar to *L. sibiricum.* These species can, however, be distinguished based on the absence of a *Sporothrix* anamorph in the latter species. Nothing is known about the pathogenicity of this fungus.

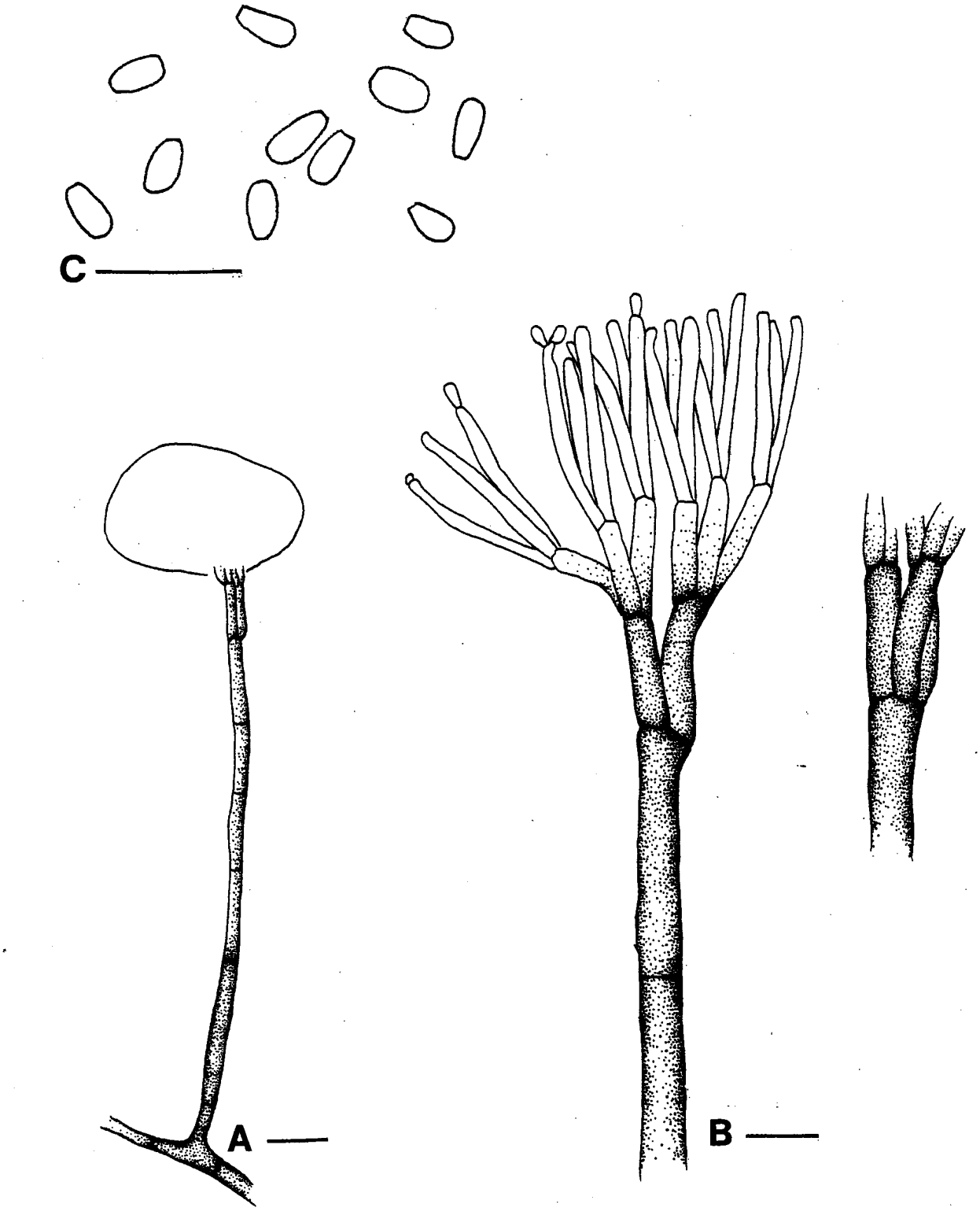

Fig. 63. Conidiophores and conidia of *L. elegans* (CMW 2245). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

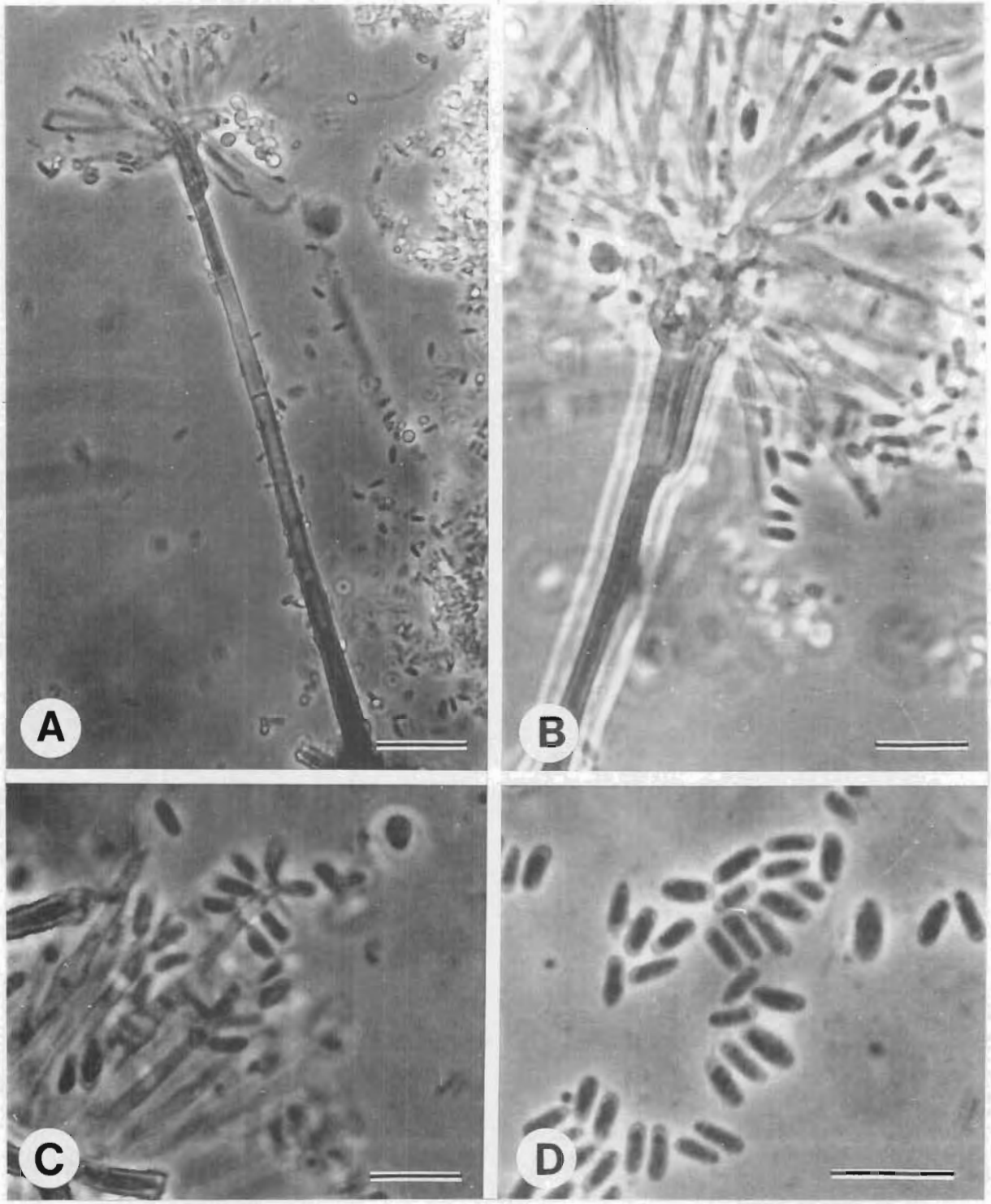

Fig. 64. Light micrographs of the conidiophores and conidia of *L. elegans* (CMW 2245). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

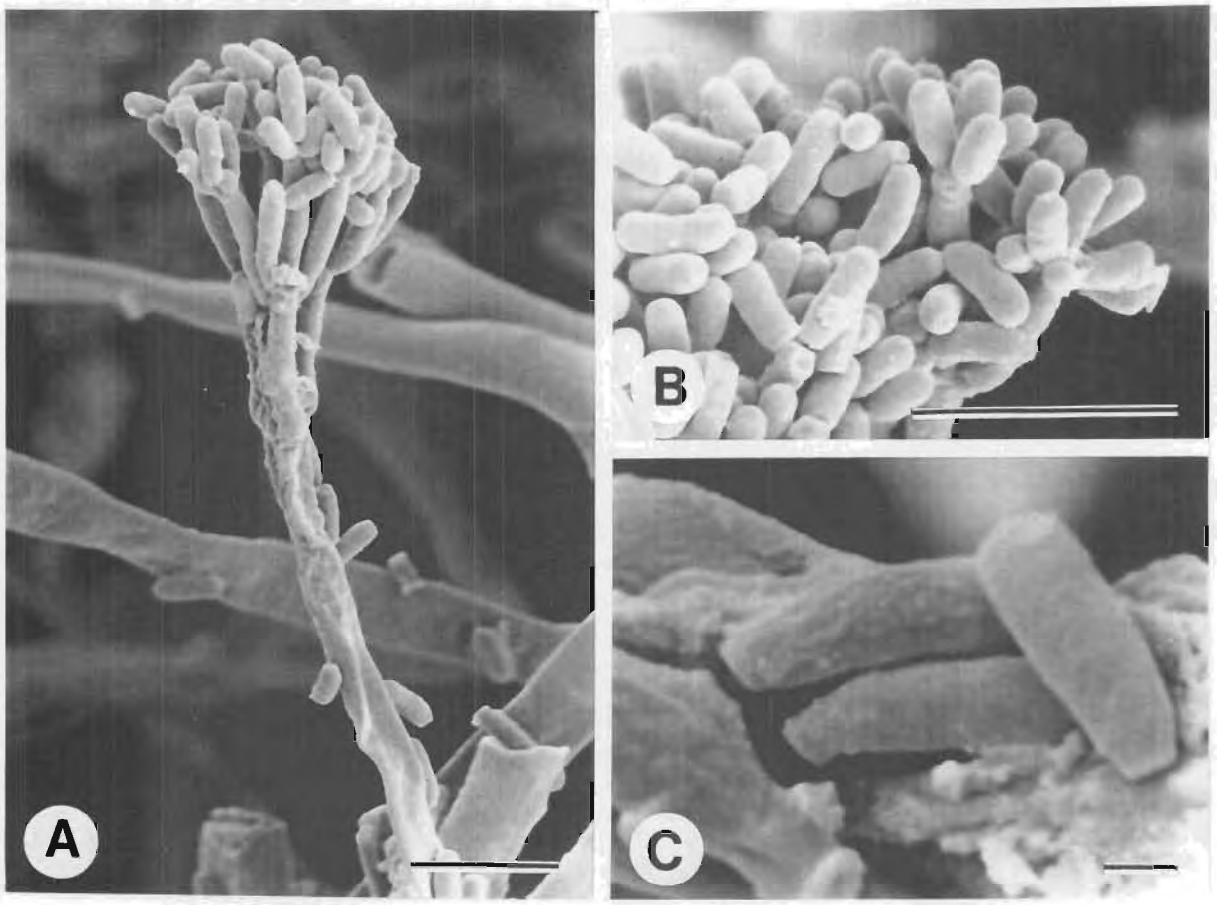

Fig. 65. Scanning electron micrographs of the conidiophores and conidia of *L. elegans* (CMW 2245). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 1 µm).

17. *Leptographium eucalyptophilum* K. Jacobs, M.J. Wingf. & J. Roux, *South African Journal of Botany*, 1999. **(Figs. 66-68)**.

Teleomorph state: Not known.

Etymology: eu-ca-lyp-to-phí-lum: loving the Eucalypt. From Eucalyptus and the Greek adjective φιλος: loving. This specific epithet refers to *Eucalyptus* which is the only host of this fungus.

Conidiophores occurring singly or in groups of up to three, arising directly from the mycelium, erect, macronematous, mononematous, (180-) 203 - 443.5 (-500) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous, smooth, cylindrical, simple, 4 - 9 septate, (140-) 152 - 392 (-440) µm long, 4.0 - 5.0 µm wide below primary branches, apical cell not swollen, 5.0 - 8.5 (-10) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* 30 - 80 µm long, excluding the conidial mass, with 2 - 3 series of cylindrical branches, 2-3 primary branches, light olivaceous to hyaline, smooth, cylindrical, aseptate, 12 - 22.5 (-26) µm long and 3.0 - 5.0 (-6.0) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, aseptate, (7.0-) 9.0 - 10.5 (-13) µm long, 1.0 - 4.0 µm wide, tertiary branches hyaline, aseptate, 5.0 - 10 µm long, 2.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 7.0 - 13 µm long and 1.0 - 2.0 µm wide. *Conidia*, hyaline, aseptate, oblong to obovoid, 6.0 - 9.0 x 3.0 - 5.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Colonies with optimal growth at 30 °C on 2 % MEA, reaching 27 mm in diameter in 6 days. No growth below 10 °C or above 35 °C. Able to withstand high concentrations of cycloheximide with a 15 % reduction in growth on 0.1 g/l cycloheximide after 6 days at 30 °C in the dark. Colonies dark green olivaceous (23'''). *Colony margin* smooth. *Hyphae* submerged or on top of agar with abundant aerial mycelium, light olivaceous to hyaline, smooth, not constricted at the septa, 2.0 - 5.0 µm diameter.

Holotype: PREM 56312, isolated from the xylem of diseased *Eucalyptus urophylla* X

E. pellita hybrid, collected: J. Roux, Kissoko plantation, Point Noire area, Republic of Congo, June 1998. **Paratypes**: PREM 56313, PREM 56314, PREM 56315, PREM 56316, PREM 56317, PREM 56318, PREM 56319 PREM 56320, isolated from the xylem of diseased *Eucalyptus urophylla* X *E. pellita* hybrid, collected: J. Roux, Kissoko plantation, Point Noire area, Republic of Congo, June 1998. **Cultures:** C1(ex holotype), C2, C3, C4, C5, C6, C7, isolated from the xylem of diseased *Eucalyptus urophylla* X *E. pellita* hybrid, collected: J. Roux, Kissoko plantation, Point Noire area, Republic of Congo, June 1998.

Known distribution: Central Africa, Republic of Congo.

Host/substrate: *Eucalyptus urophylla* X *E. pellita* hybrid (Jacobs *et al.*, 1999).

Associated insects: Not known.

Notes: *Leptographium eucalyptophilum* closely resembles the other *Leptographium* spp. with long conidia namely *L. americanum, L. penicillatum* and *L. dryocoetidis* (Grosmann, 1932; Kendrick & Molnar, 1965; Jacobs *et al.*, 1997). It can, however, easily be distinguished from *O. penicillatum* and *O. dryocoetidis* based on the long allantoid and oblong conidia of these species, respectively. These are twice as broad as those in *L. eucalyptophilum* (Grosmann, 1932; Kendrick & Molnar, 1965). Rhamoconidia are occasionally observed in *L. eucalyptophilum,* and these have never been reported from the other species. In addition, *O. penicillatum* and *O. dryocoetidis* are characterized by teleomorph structures. No teleomorph has been found in association with *L. eucalyptophilum. Leptographium eucalyptophilum* can be distinguished from *O. americanum* based on the considerably longer conidia of the latter species.

Leptographium eucalyptophilum is found on *Eucalyptus* and has thus far not been associated with any insect activity. Pathogenicity trials showed that *L. eucalyptophilum* most likely does not play a primary role in disease development on *Eucalyptus* trees (Jacobs *et al.*, 1999).

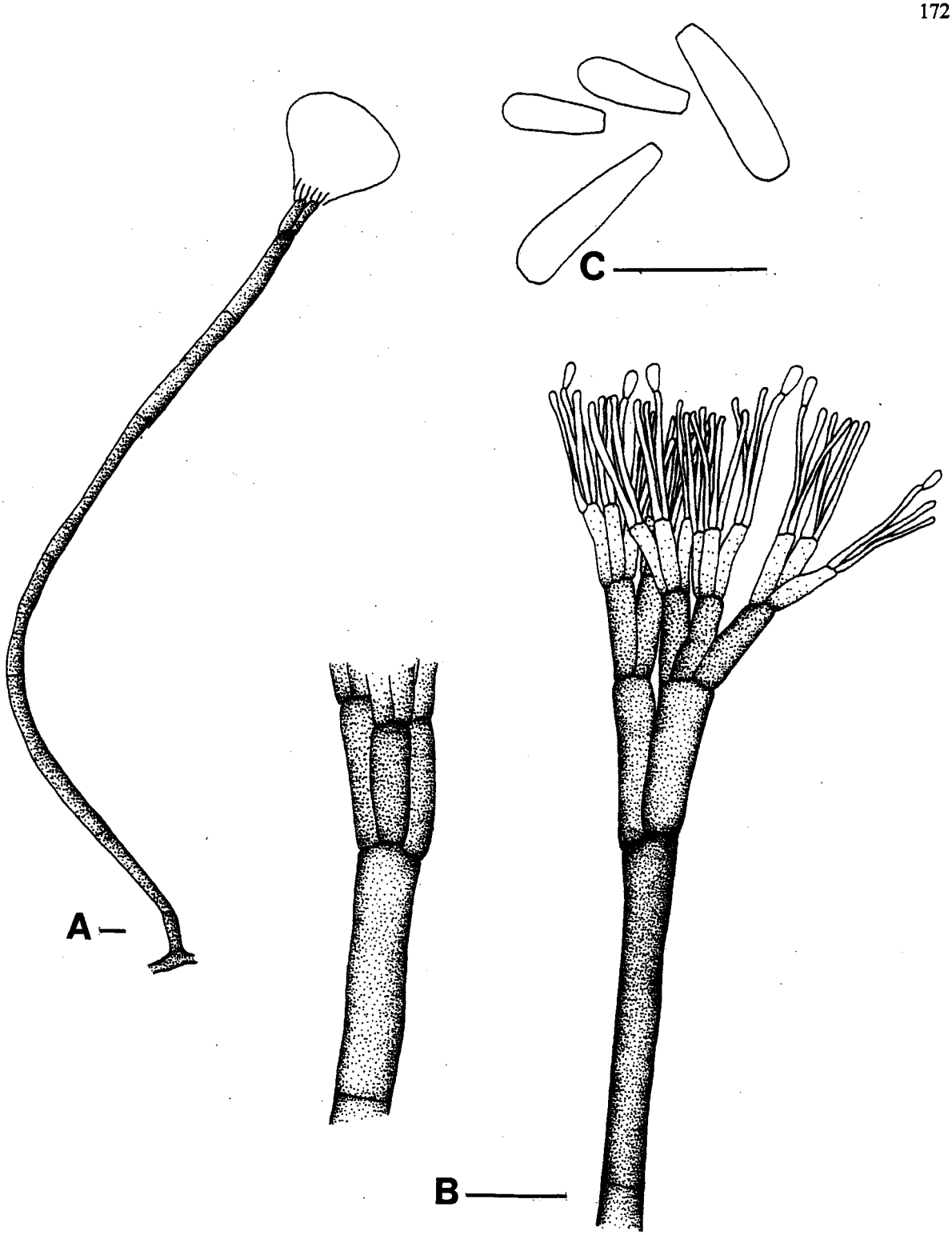

Fig. 66. Conidiophores and conidia of *L. eucalyptophilum* (PREM 56312). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

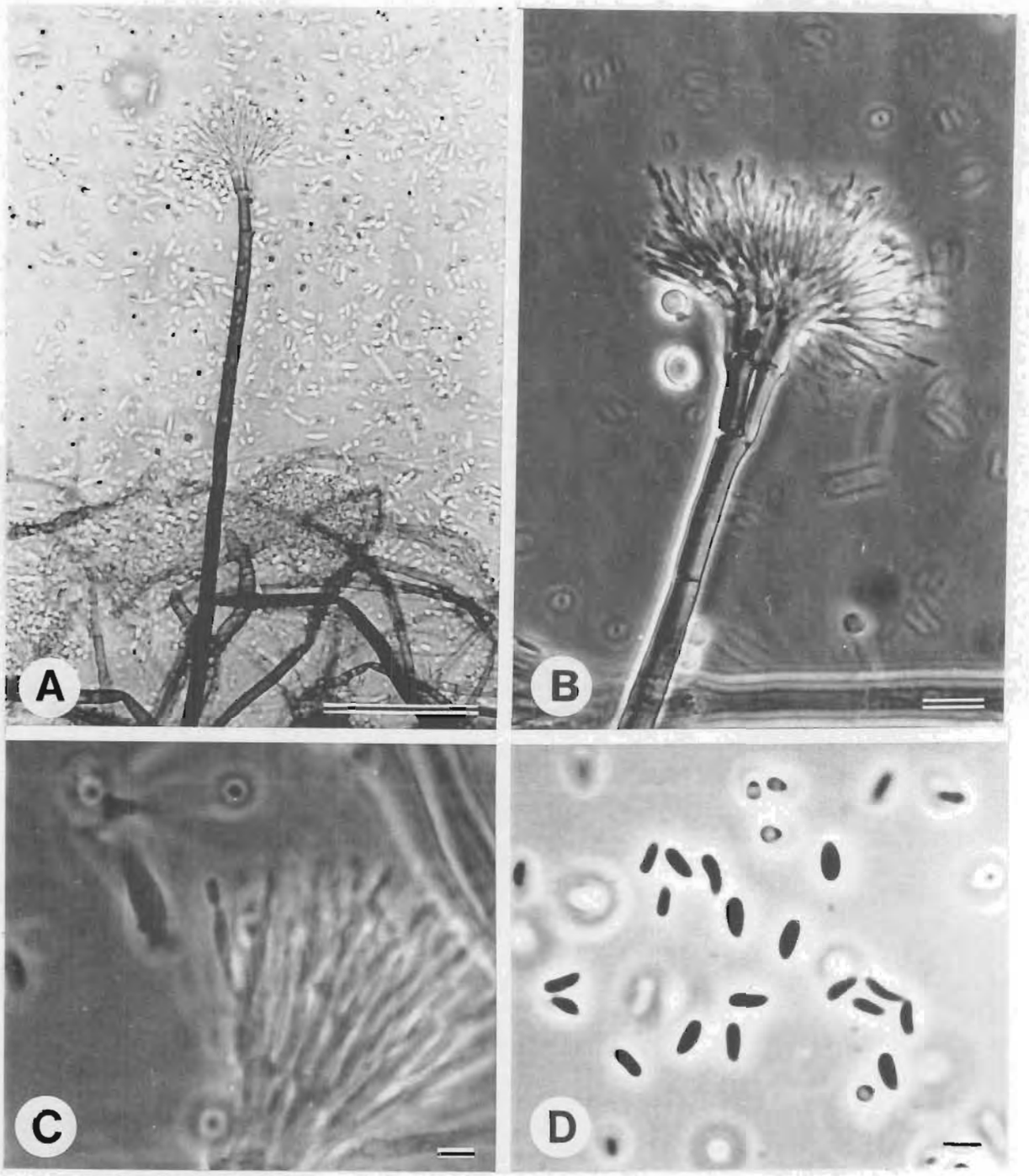

Fig. 67. Light micrographs of the conidiophores and conidia of *L. eucalyptophilum* (PREM 56312). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

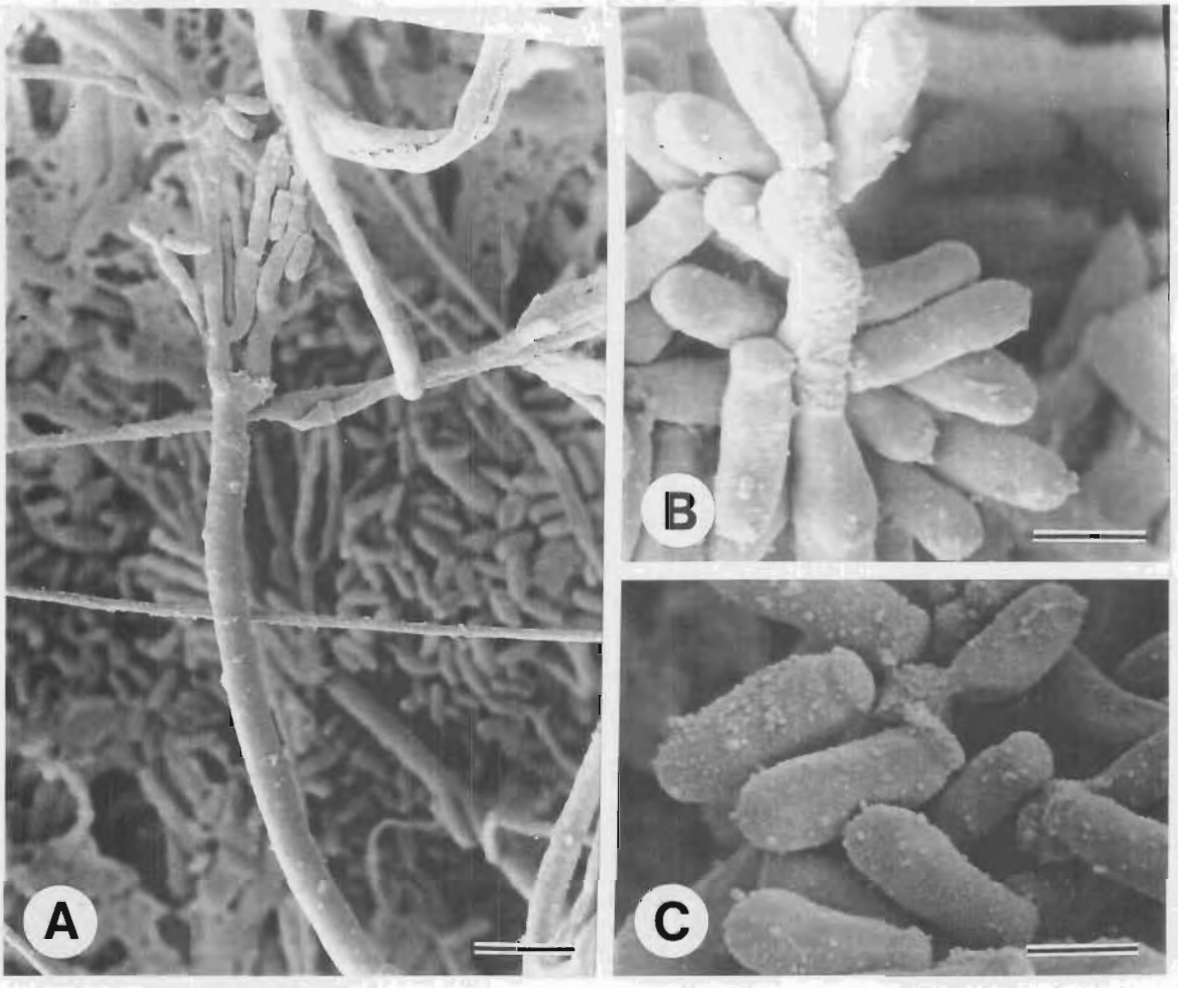

Fig. 68. Scanning electron micrographs of the conidiophores and conidia of *L. eucalyptophilum* (PREM 56312). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 5 µm).

18. *Leptographium euphyes* K. Jacobs & M.J. Wingf., *Mycological Research,* 1999. **(Figs. 69 - 71).**

Teleomorph state: Not known

Etymology: eu-phý-es: shapely. From the Greek adjective εὐφυης: well-grown, shapely. This specific epithet refers to the small, but shapely conidiophores of this fungus.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (204-) 265 - 335 (-415) µm in length, rhizoid-like structures present. *Stipes* olivaceous, smooth, cylindrical, simple, 3 - 9 septate, (142.5-) 194 - 255 (-353.5) µm long, 6.0 - 9.0 µm wide below primary branches, apical cell not swollen, 6.0 - 12.5 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (31-) 68 - 77 (-93) µm long, excluding the conidial mass, with 3 - 4 series of cylindrical branches, 2 - 3 primary branches, light olivaceous, smooth, cylindrical, aseptate, (11-) 16 - 20.5 (-47) µm long and 5.0 - 7.0 (-8.0) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous to hyaline, aseptate, 8.0 - 16.5 (-18) µm long, (3.0-) 4.0 - 5.0 (-6.0) µm wide, tertiary branches hyaline, aseptate, 8.0 - 13 µm long, 2.0 - 3.0 µm wide, quaternary branches aseptate, hyaline, 7.0 - 12 µm long, 2.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (10-) 12.5 -17 (-20) µm long and 1.0 - 2.0 µm wide. *Conidia* aseptate, obovoid with truncate ends, occasionally oblong, 4.0 -5.0 (-6.0) x 2.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Colonies with optimal growth at 25°C on 2% MEA, reaching 19 mm in diameter in 6 days. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide no reduction in growth on 0.1 g/l cycloheximide after days at 25°C in the dark. Colonies olivaceous (19"f). *Colony margin* smooth. *Hyphae* submerged or on top of agar with no aerial mycelium, light olivaceous to hyaline, smooth, occasionally constricted at the septa, (2.0-) 2.5 - 4.0 (-5.0) µm diameter.

Specimens examined: Holotype: New Zealand, *Pinus strobus,* collected: M. Dick,

PREM 45703. **Paratypes**: New Zealand, *P. radiata,* collected: M. Dick, PREM 45701; *Pinus strobus,* New Zealand, collected: M. Dick; PREM 56363. **Cultures:** New Zealand, *Pinus strobus,* collected: M. Dick, CMW 259; *Pinus strobus,* New Zealand, collected: M. Dick; CMW 301.

Known distribution: New Zealand.

Host/substrate: *Pinus strobus* (Jacobs *et al.*, 1999).

Associated insects: not known.

Notes: Isolates of *Leptographium euphyes* have been mistakenly identified as *L. procerum* (Wingfield & Marasas, 1983). However, this species is unlike isolates of *L. procerum* and can be distinguished based on its short robust conidiophores (Jacobs *et al.*, 1999). *Leptographium euphyes* is morphologically similar to *L. grandifoliae.* These species could, however, be distinguished based on the presence of a teleomorph in the latter species (Davidson, 1976) and its absence in the former species. In the absence of a teleomorph, *L. euphyes* can be distinguished from *O. grandifoliae* based on more complex conidiogenous apparatuses as well as larger conidia (4 - 6 µm) compared to *O. grandifoliae* (2.5 - 4 µm).

Leptographium euphyes is commonly isolated together with *L. procerum* in New Zealand. The fungus originates from a collection of isolates that were linked to a report of a root disease of *Pinus strobus* in New Zealand (Shaw & Dick, 1980). Later, Wingfield and Marasas (1983) studied this collection of isolates and noted that it represented isolates having two distinct morphological forms. These included one group that was typical of *L. procerum* and another which were considered to be different. This latter group represents *Leptographium euphyes.* Nothing is known about the pathogenicity of *L. euphyes* although we expect that they are mildly pathogenic or saprophytic associates of the insects with which they are associated.

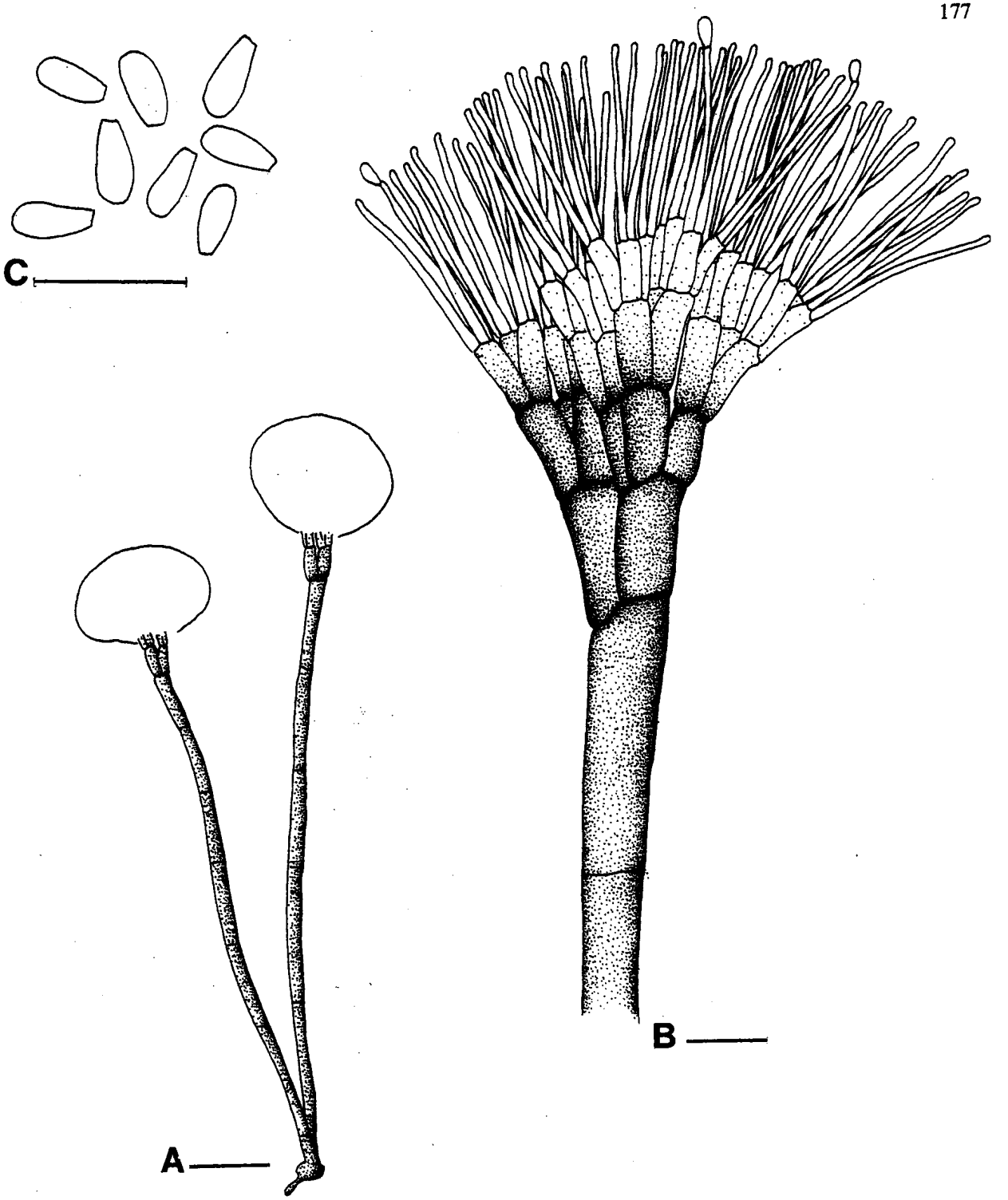

Fig. 69. Conidiophores and conidia of *L. euphyes* (PREM 45703). **A.** Habit sketch (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

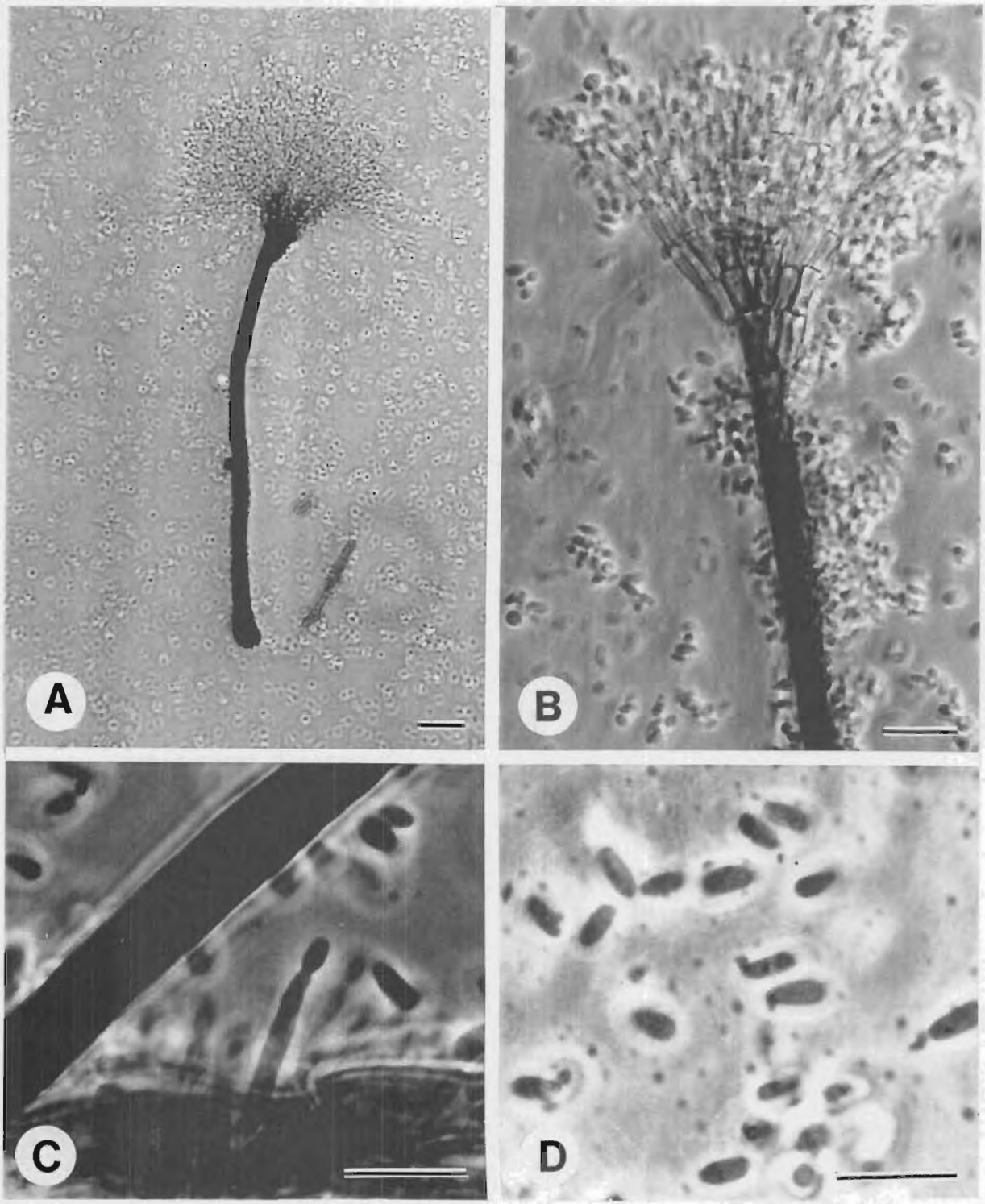

Fig. 70. Light micrographs of the conidiophores and conidia of *L. euphyes* (PREM 45703). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous apparatus (Bar = 20 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

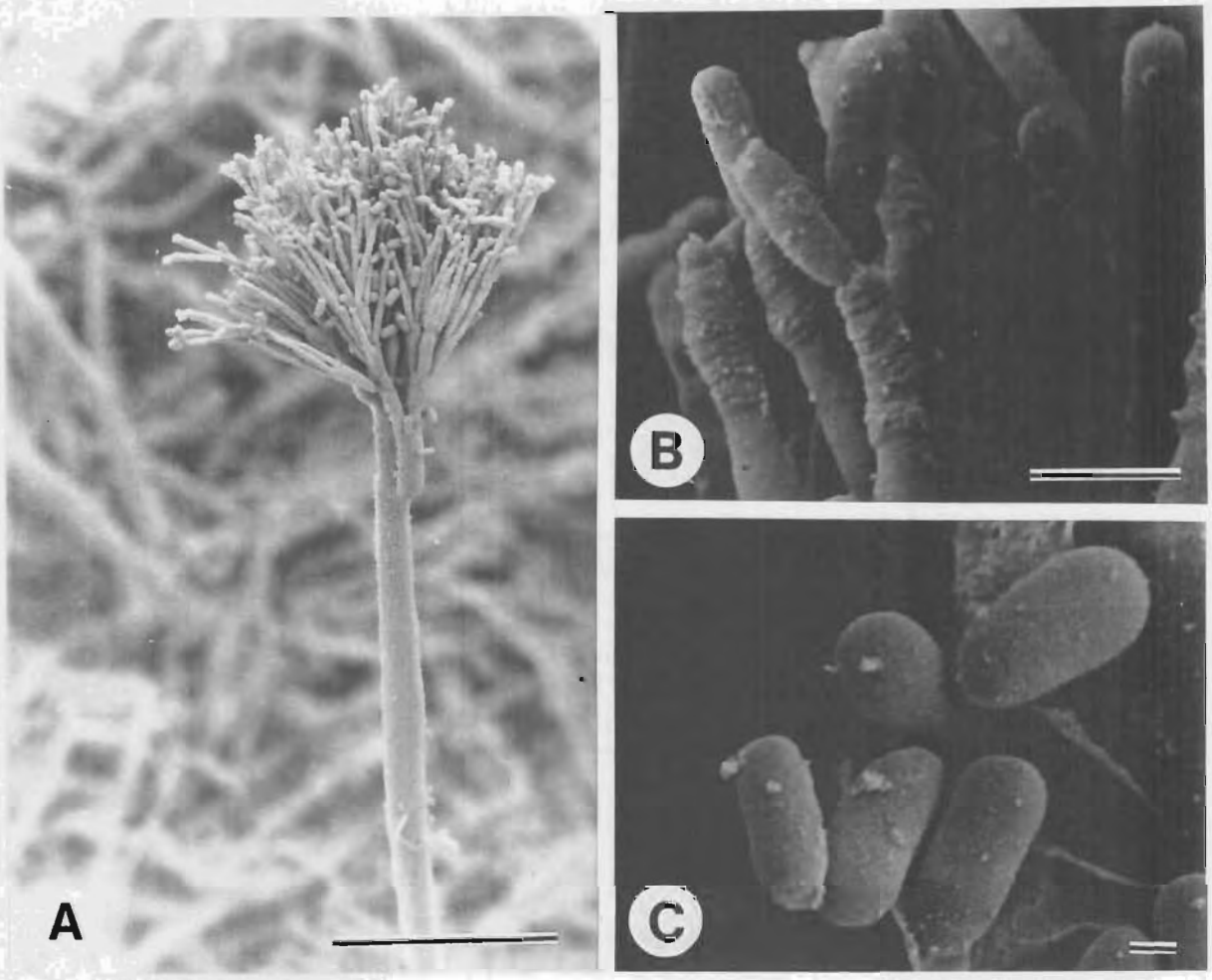

Fig. 71. Scanning electron micrographs of the conidiophores and conidia of *L. euphyes* (PREM 45703). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

19. *Ophiostoma francke-grosmanniae* (R.W. Davidson) De Hoog & R.J. Scheff., *Mycologia* **76**, 297. 1984. **(Figs. 72-74).**

≡ *Ceratocystis francke-grosmanniae* R.W. Davidson, *Mycologia* **63**, 6. 1971.

Anamorph: *Leptographium francke-grosmanniae* Jacobs & Wingfield sp. nov.

Etymology: fran-cke-gros-mán-ni-ae: genitive of Francke-Grosmann. This specific epithet honors Helene Francke-Grosmann, who made major contributions in the study of blue-stain fungi and in particular species in the genera *Ophiostoma* and *Leptographium.*

Perithecial bases black to dark brussels brown (15m), globose and smooth walled, unornamented, 115 - 160 µm in diam. *Perithecial neck* dark brussels brown to black, cylindrical with a slight apical taper, smooth, 200 - 300 µm long, 30 - 35 µm above globose base, 20 - 25 µm wide at the apex, *ostiolar hyphae* convergent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* reniform, almost cucullate, aseptate, hyaline, invested in a sheath, 3.0 - 4.0 x 1.0 - 2.5 µm, without sheath (Davidson, 1971).

Conidiophores occurring singly or in groups up to 4, arising directly from the mycelium, erect, macronematous, mononematous, (59-) 105.5 - 134 (-170.5) µm in length, rhizoid-like structures present. *Stipes* light olivaceous (21"k), smooth, cylindrical, simple, 4-7 septate, (43.5-) 91.5 - 93.5 (-150) µm long, 3.0 - 6.0 µm wide below primary branches, apical cell not swollen, (6.0-) 8.5 - 10.5 (-11) µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* (15.5-) 26.5 - 29 (-39) µm long, excluding the conidial mass, with 1 to 2 series of cylindrical branches, 2-3 primary branches, hyaline to light olivaceous (21"k), smooth, cylindrical, aseptate (8.0-) 9.5 - 11.5 (-14) µm long and 3.0 - 6.0 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, aseptate, (6.0-) 9.0 - 10.5 (-12.5) µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (6.0-) 7.5 - 10 (-11) µm long and 2.0 - 3.0 µm wide. Tightly packed annelations gives a phialidic appearance to the conidiogenous cells under the light microscope (Mouton, Wingfield and Van Wyk,

1992). *Conidia* hyaline, aseptate, broadly ellipsoid with truncate ends and rounded apices, (2.5-) 3.0 - 4.5 (-5.0) x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline. Conidial mass hyaline when wet, turning cream colored (19'f) when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 9 mm in diam. in 8 days. No growth below 10°C with little growth at 35°C. Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.5 g/l cycloheximide after 12 days at 20°C in the dark. Colonies cartridge buff (19"f) at first becoming olivaceous (21"m) with age. *Colony margin* smooth. *Hyphae* submerged on agar with no aerial mycelium, hyaline, smooth, straight, not constricted at the septa, (1.5-) 3.0 - 4.5 (-6.0) µm diam.

Specimens examined: Holotype: West Germany, *Quercus* sp., May 1967, collected: R.W. Davidson, BPI 595654 (received from NFC). **Cultures**: Germany, Reinbeck, *Quercus* infested with *Hylecoetus dermestoides,* May 1967, collected: Helene Francke-Grosmann, CMW 445, CMW 2857.

Known distribution: Germany.

Hosts/substrate: *Quercus* sp. (Davidson, 1971).

Associated insects: *Hylecoetus dermestoides* (Davidson, 1971).

Notes: *Ophiostoma francke-grosmanniae* was considered to be similar to *O. leptographioides* but could be distinguished based on perithecial dimensions (Davidson, 1971). The conidiogenous cells of this species superficially resemble phialides and it was at one time thought to reside in *Phialocephala* (Upadhyay, 1981). Mouton *et al.,* (1992) determined that the conidiogenous cells are annelidic and that this species can tolerate high concentrations of cycloheximide, in contrast to species of *Phialocephala* (Harrington, 1988; Marais, 1996). Nothing is known about the pathogenicity of this species.

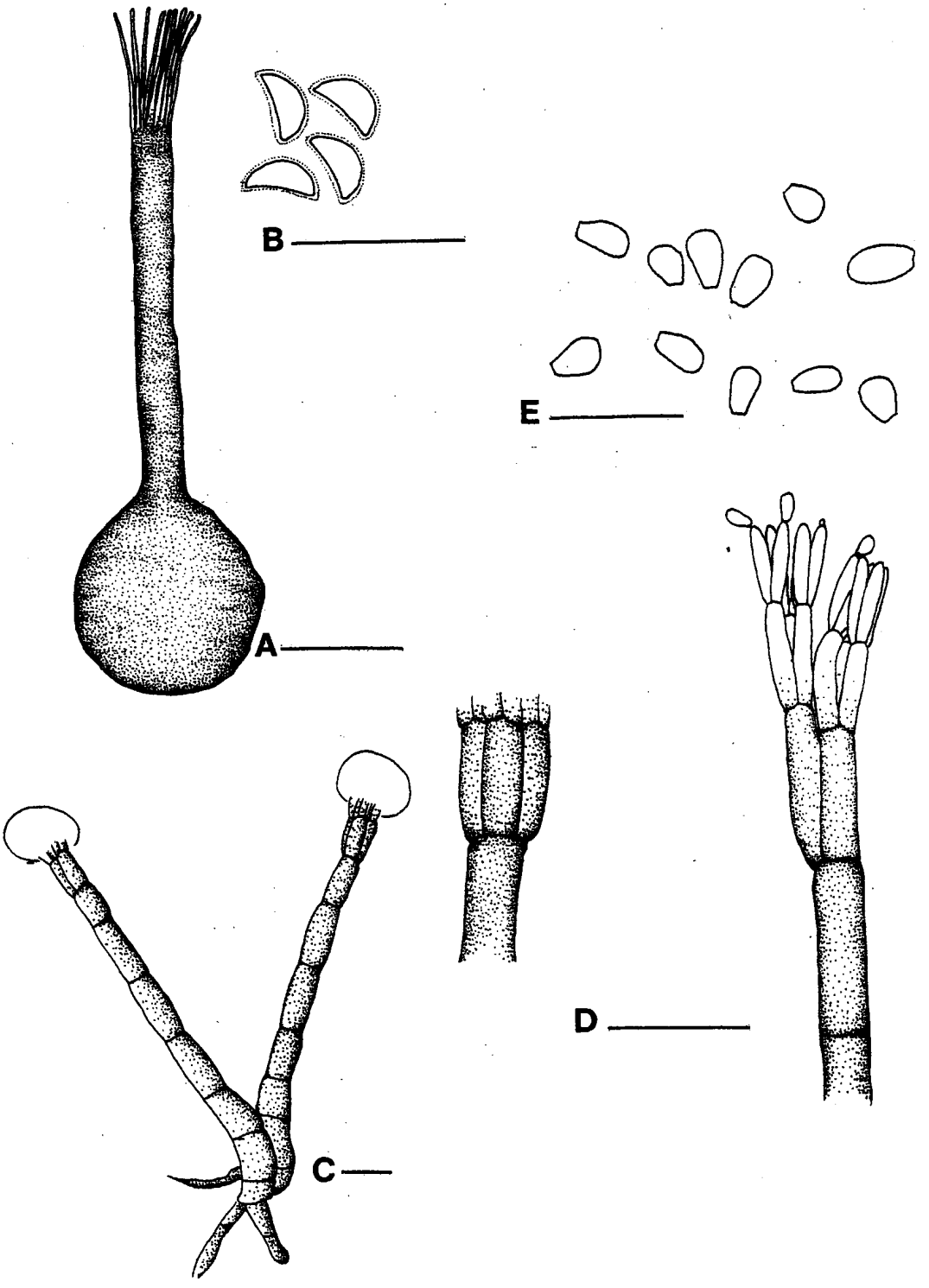

Fig. 72. Teleomorph and anamorph structures of *O. francke-grosmanniae* (CMW 445). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

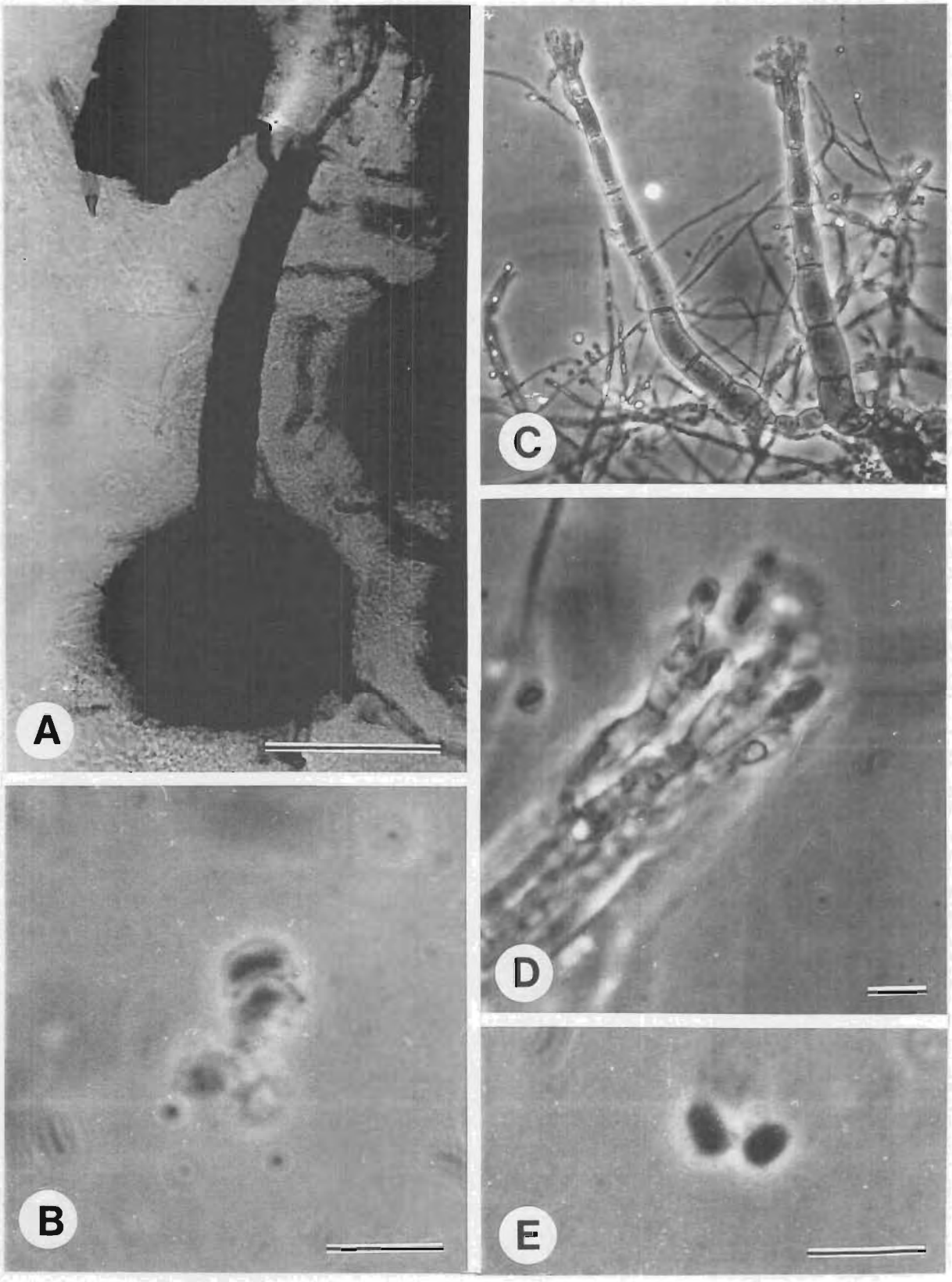

Fig. 73. Light micrographs of the teleomorph and anamorph structures of *O. franke-grosmanniae* (CMW 445). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

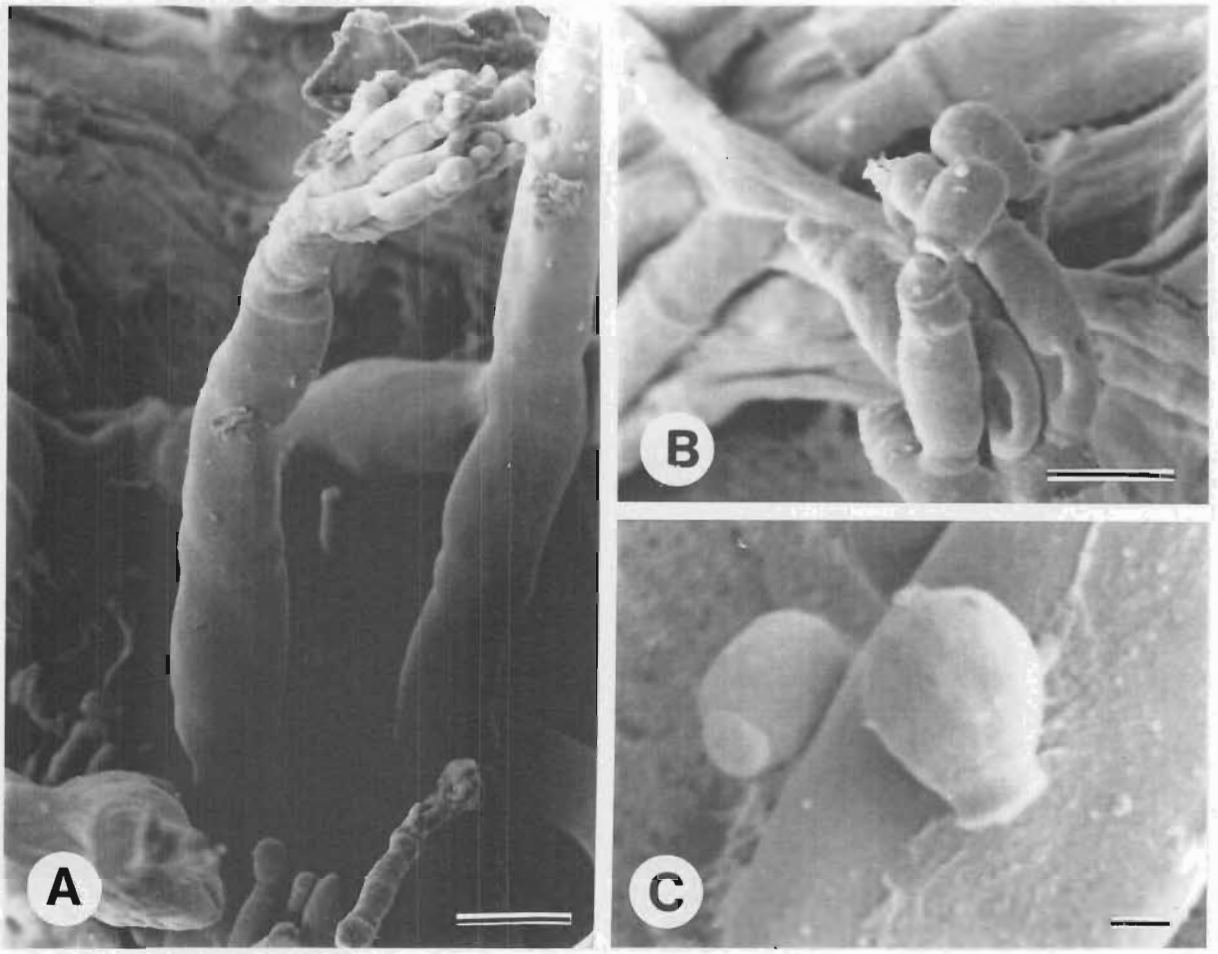

Fig. 74. Scanning electron micrographs of the conidiophores and conidia of *O. francke-grosmanniae* (CMW 445). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

20. *Ophiostoma grandifoliae* (R.W. Davidson) T.C. Harr., *Mycotaxon* **28**, 41. 1987. **(Figs. 75-77).**

≡ *Ceratocystis grandifoliae* R.W. Davidson, *Memoirs of the New York Botanical Garden* **28**, 45. 1976.

Anamorph: *Leptographium grandifoliae* (R.W. Davidson) M.J. Wingf., *Transactions of the British Mycological Society* **85**, 92. 1985.

Etymology: gran-di-fo-li-ae: of the large leaves. From the Latin adjective grandis: large and Latin noun folium: a leaf. This specific epithet refers to *Fagus grandifoliae* which is the host of this fungus.

Perithecial bases black, globose and smooth walled, unornamented, 170 - 200 µm in diam. *Perithecial neck* black, cylindrical with a slight apical taper, smooth, 500 - 1700 µm long, 40 - 55 µm above globose base, 20 - 30 µm wide at the apex, *ostiolar hyphae* convergent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* allantoid, aseptate, hyaline, invested in a sheath, 3.2 - 4.5 x 1.6 - 2.5 µm (Davidson, 1976).

Conidiophores occurring in groups of up to 8, mostly on aerial mycelium, erect, macronematous, mononematous, 80 - 374 (-397.5) µm in length, rhizoid-like structures present. *Stipes* light olivaceous (21"k), smooth, cylindrical, simple, 2-14 septate, 62.5 - 327 (-347.5) µm long, (2.5-) 3.0 - 4.5 (-5.0) µm wide below primary branches, apical cell not swollen, 4.0 - 7.5 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (20-) 21.5 - 46 (-62.5) µm long, excluding the conidial mass, with 1 to occasionally 3 series of cylindrical branches, 2 - 3 primary branches, light olivaceous (21"k), smooth, cylindrical, aseptate, (6.0-) 8.5 - 10 (-13) µm long and 2.0 - 3.5 (-5.0) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21"k) to hyaline, aseptate, (5.5-) 7.0 - 8.5 (-12) µm long, 2.0 - 3.0 µm wide, tertiary branches hyaline, aseptate, 5.5 - 9.0 µm long, 1.0 - 2.5 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (10-) 12 - 15.5 (-18.5) µm long and 1.0 - 2.0 µm wide. *Conidia* hyaline, aseptate, obovoid with truncate ends and rounded apices, 2.5 - 4.0 x 1.0 - 2.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous

apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, turning amber (21'b) when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 9 mm in diam. in 8 days. No growth below 10°C with some growth at 35°C. Able to withstand high concentrations of cycloheximide with a 22% reduction in growth on 0.5 g/l cycloheximide after 12 days at 20°C in the dark. Colonies gray olivaceous (21""). *Colony margin* smooth. *Hyphae* above on agar with abundant aerial mycelium, hyaline, smooth, straight, not constricted at the septa, 1.5 - 3.0 µm diam.

Specimens examined: Holotype: U.S.A., eastern Iowa, *Fagus grandifoliae*, 1976, collected: R.W. Davidson, BPI 596248 (received from NFC). **Cultures:** U.S.A., eastern Iowa, *Fagus grandifoliae*, 1976, collected: R.W. Davidson, CMW 703 (same as ATCC 28746).

Known distribution: U.S.A.

Hosts/substrate: *Fagus grandifoliae* (Davidson, 1976).

Associated insects: Not known.

Notes: *Ophiostoma grandifoliae* is similar to *L. sibiricum* and *O. leptographioides*. *Ophiostoma grandifoliae* can be distinguished from *L. sibiricum* based on the presence of rhizoids at the base of conidiophores, and the absence of these structures in *L. sibiricum*. In addition, *O. grandifoliae* is associated with a teleomorph, whereas no teleomorph has been reported for *L. sibiricum*.

Ophiostoma grandifoliae is one of a small number of *Leptographium* spp. not isolated from a coniferous host. This is also true for *O. leptographioides*. *Ophiostoma leptographioides* is associated with an *Ophiostoma* teleomorph, which makes it similar to *O. grandifoliae*. *Ophiostoma grandifoliae* and *O. leptographioides* can be distinguished from each other based on the longer conidia of the latter species. Furthermore, *Ophiostoma grandifoliae* is characterized by perithecia with long necks and small allantoid ascospores. This is in contrast to the short-necked

perithecia and pillow-shaped ascospores of *O. leptographioides*. *Ophiostoma grandifoliae* has been isolated from blue-stain in sapwood of *Fagus grandifolia* (Davidson, 1976). This is the only report of this fungus to date (Harrington, 1988).

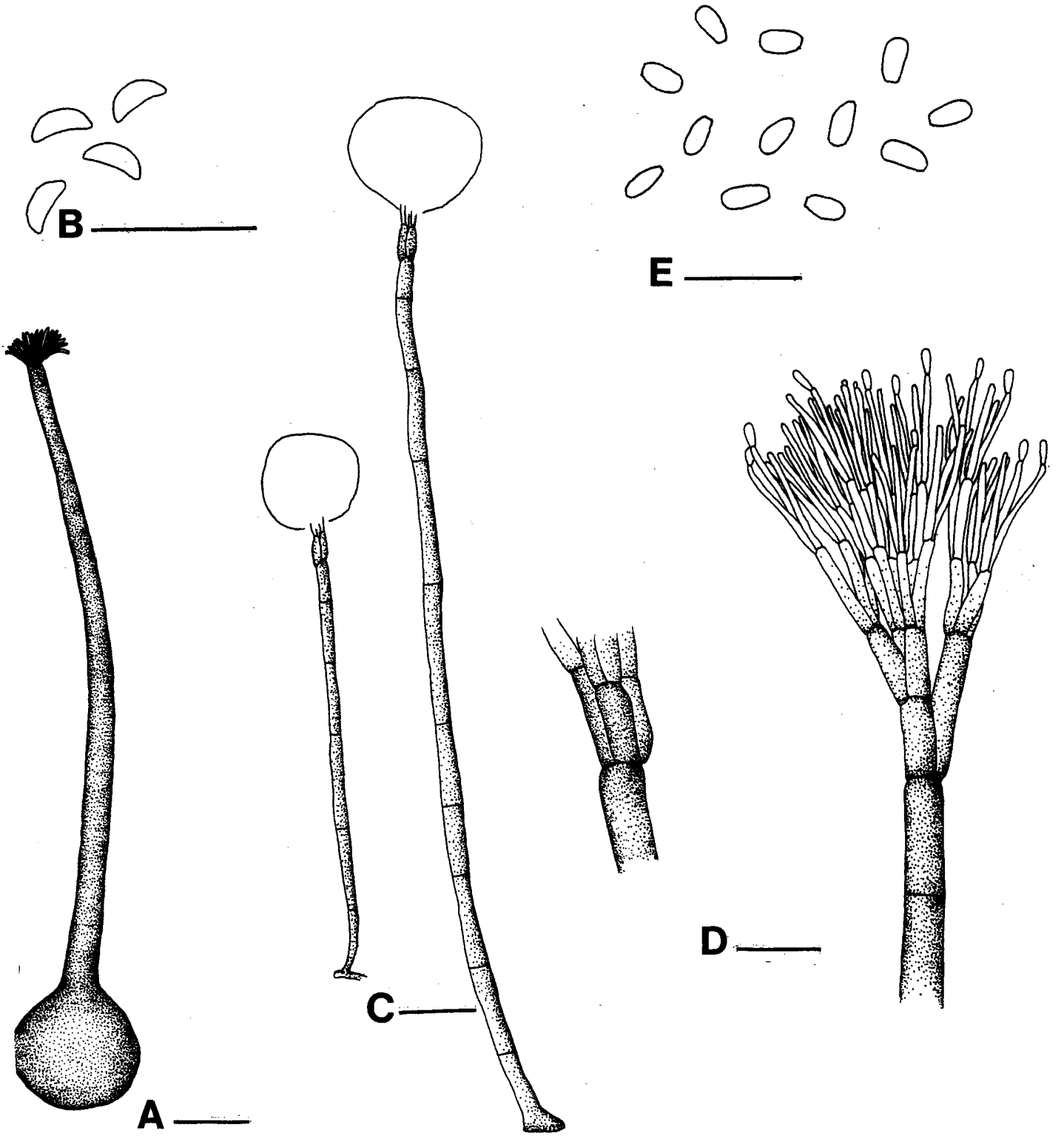

Fig. 75. Teleomorph and anamorph structures of *O. grandifoliae* (CMW 703). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

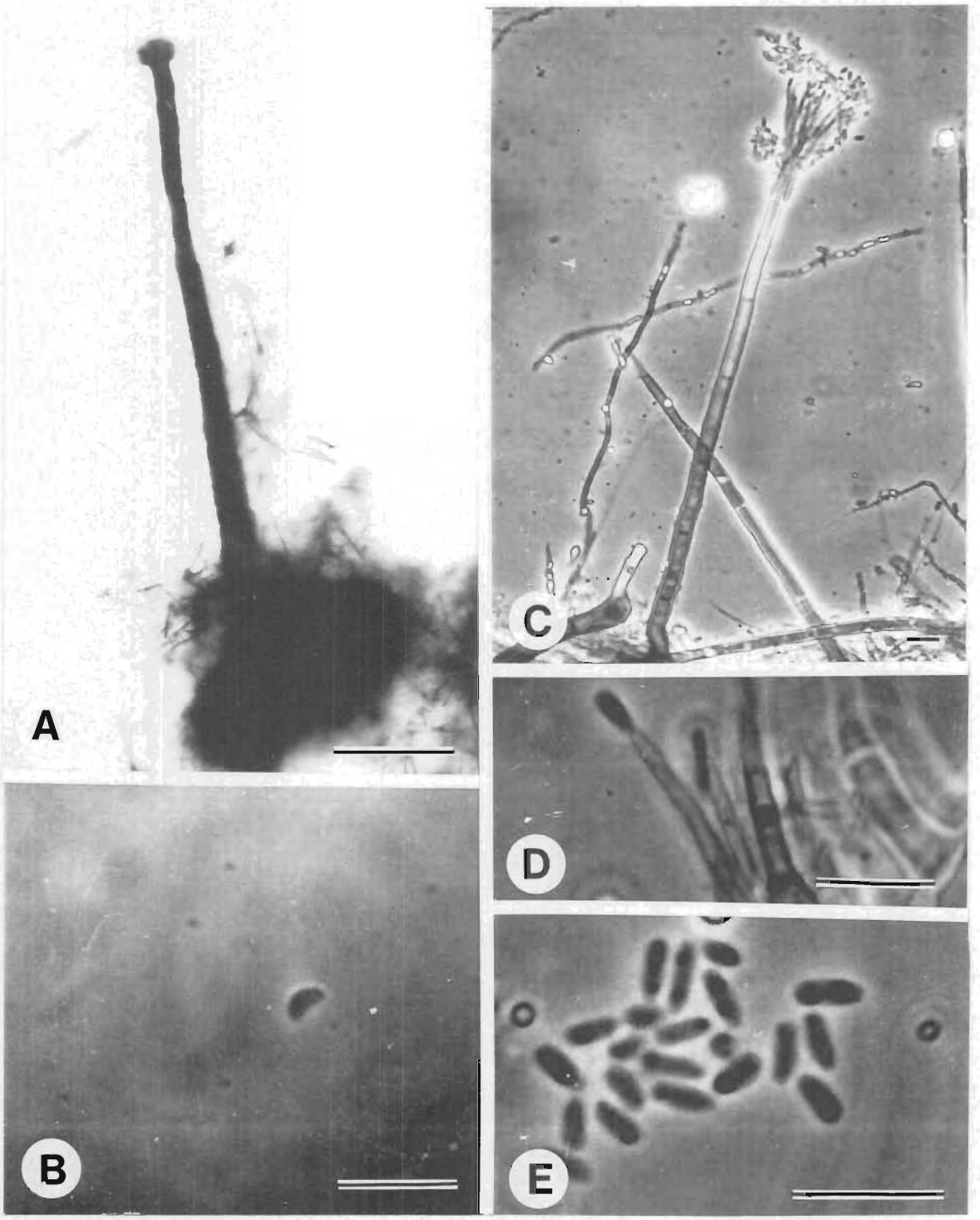

Fig. 76. Light micrographs of the teleomorph and anamorph structures of *O. grandifoliae* (CMW 703). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

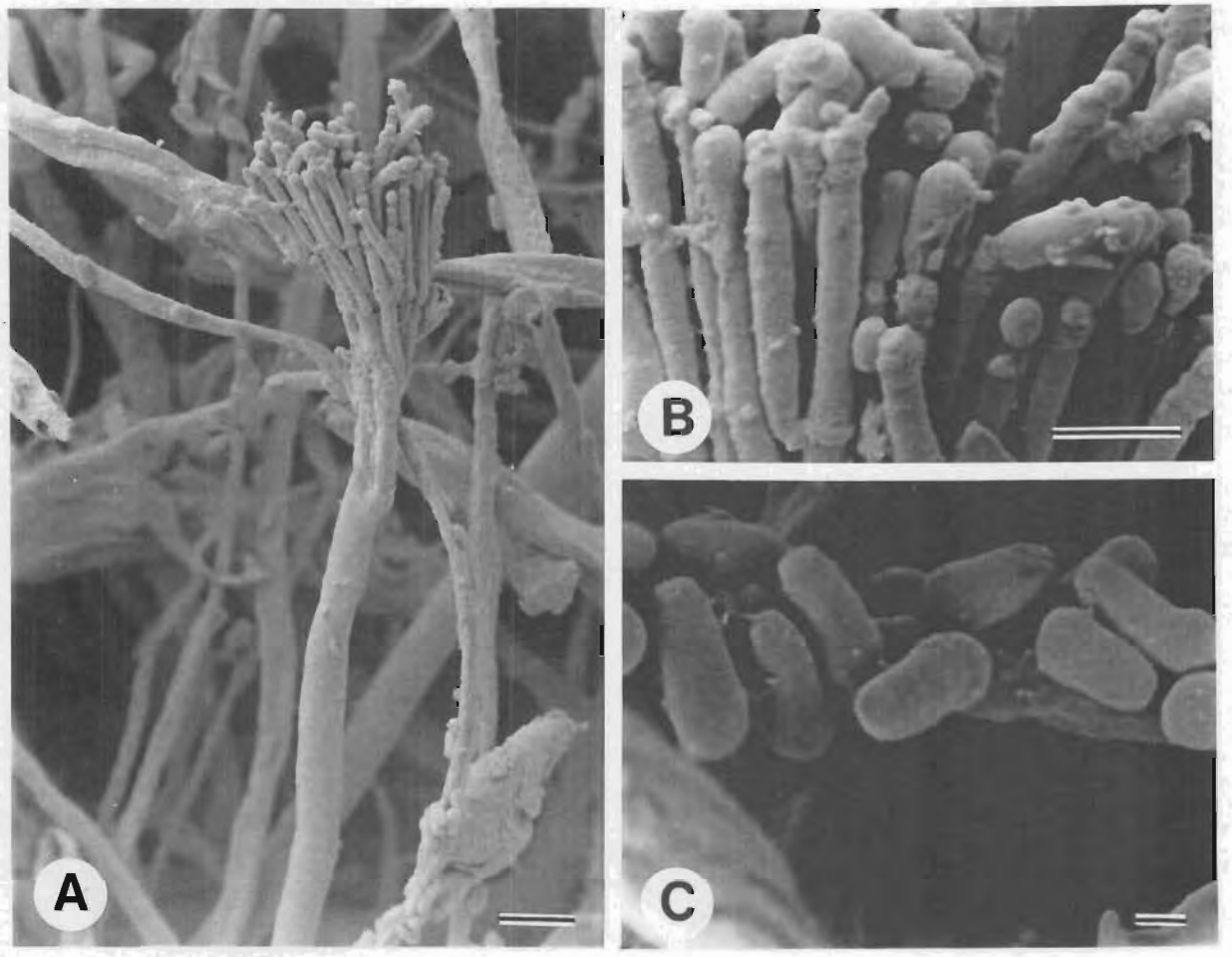

Fig. 77. Scanning electron micrographs of the conidiophores and conidia of *O. grandifoliae* (CMW 703). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

21. *Leptographium guttulatum* M.J. Wingf. & K. Jacobs, *Mycological Research*. 1999. **(Figs. 78-80).**

Teleomorph: Not known.

Etymology: gut-tu-lá-tum: provided with droplets. From the Latin noun guttula: a little drop. This specific epithet refers to the guttules or droplets that are characteristic of the conidia of this species.

Conidiophores occurring singly, arising directly from the mycelium or aerial mycelium, erect, macronematous, mononematous, (200-) 365 - 465 (-810) µm in length, rhizoid-like structures absent. *Stipes* olivaceous (21"k), smooth, cylindrical, simple, 2 - 7 septate, (120-) 258 - 343 (-670) µm long, (5.0-) 6.5 - 10 (-12) µm wide below primary branches, apical cell not swollen, 5.0 - 12 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (60-) 107 - 121 (-200) µm long, excluding the conidial mass, with 2 to 4 series of cylindrical branches, 2-4 primary branches, arrangement of the primary branches - type B, light olivaceous (21"k) to olivaceous (21"m), smooth, cylindrical, aseptate, (18-) 25.5 - 33 (-40) µm long and 5.0 - 10 µm wide, secondary branches light olivaceous (21"k), aseptate, (15-) 19 - 30 (-35) µm long, (3.0-) 4.0 - 7.5 (-8.0) µm wide, tertiary branches hyaline, aseptate, (10-) 16 - 26.5 (-33) µm long, 3.0 - 5.0 µm wide, quaternary branches hyaline, aseptate, (9.0-) 10 - 19.5 (-25) µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (10-) 11.5 - 21.5 (-27) µm long and 2.0 - 3.0 µm wide. *Conidia* hyaline, aseptate, oblong to slightly obovoid, prominent guttulate, (4.0-) 5.0 - 9.0 (-10) x 2.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apices of conidiogenous apparatus, hyaline at first, becoming cream coloured (19'f) with age. Conidial masses cream coloured when wet, remaining the same colour when dry.

Colonies with optimal growth at 25°C on 2 % MEA, reaching 36 mm in diameter in 9 days. Little growth at 5°C and no growth above 30°C. Able to withstand high concentrations of cycloheximide with a 5% increase in growth on 0.5 g/l cycloheximide after 9 days at 25°C in the dark. Colonies dark olive (21"m). *Colony*

margin smooth. *Hyphae* submerged on agar with little aerial mycelium, olivaceous (21"k), smooth, straight, not constricted at the septa, 5.0 - 13 µm diameter.

Specimens examined: Holotype: France, Orleans, isolated from *Tomicus* sp. from *Pinus sylvestris,* 1984, M. Morelet, PREM 56307. **Paratypes:** England, Fresley Cannon Wood, Hampshire, isolated from *Tomicus piniperda* from *Pinus*, 1988, J.N. Gibbs, PREM 56310; Austria, Niederösterreich, Flatz, isolated from *Hylurgops palliatus* from Norway spruce, 28 May 1997, T. Kirisits, PREM 56311; Austria, Glein, Styria, isolated from *Hylurgops glabratus* from Norway spruce, 3 August 1993, T. Kirisits, PREM 56309; Austria, Niederösterreich, Flatz, isolated from *Hylurgops palliatus* from Norway spruce, 28 May 1998, T. Kirisits, PREM 56308. **Cultures:** France, Orleans, isolated from *Tomicus* sp. from *Pinus sylvestris,* 1984, M. Morelet, CMW 742; England, Fresley Cannon Wood, Hampshire, isolated from *Tomicus piniperda* from *Pinus*, 1988, J.N. Gibbs, CMW 1310; Austria, Niederösterreich, Flatz, isolated from *Hylurgops palliatus* from Norway spruce, 28 May 1997, T. Kirisits, CMW 4921; Austria, Glein, Styria, isolated from *Hylurgops glabratus* from Norway spruce, August 1993, T. Kirisits, CMW 4922; Austria, Niederösterreich, Flatz, isolated from *Hylurgops palliatus* from Norway spruce, 28 May 1998, T. Kirisits, CMW 4923.

Known distribution: Europe (England, France, Austria, Sweden).

Hosts/substrate: *Picea abies, Pinus sylvestris* (Wingfield & Gibbs, 1991; Jacobs *et al.,* 1999)

Associated insects: *Hylurgops glabratus* (Jacobs *et al.,* 1999), *Hylurgops palliatus* (Mathiesen, 1950; Harrington, 1988; Wingfield & Gibbs, 1991; Jacobs *et al.,* 1999), *Hylastes ater* (Wingfield & Gibbs, 1991), *Hylastes opacus* (Wingfield & Gibbs, 1991), *Ips typographus* (Mathiesen, 1950), *Tetropium* sp. (Mathiesen, 1950), *Tomicus piniperda* (Jacobs *et al.,* 1999).

Notes: Mathiesen (1950) described this species as a variety of *Ophiostoma penicillatum.* Harrington (1988) noted that this variety as well as the "pini" variety

could easily and consistently be distinguished morphologically from *O. penicillatum.* He also indicated that these fungi might represent distinct taxa. Comparison of *L. guttulatum* with *L. penicillatum,* revealed that these species could easily be distinguished from each other. Whereas *L. penicillatum* is characterized by large allantoid conidia, the conidia of *L. guttulatum* are also large, but more globose than those of *L. penicillatum.* The conidiogenous apparatuses of *L. guttulatum* have a similar brush-shaped appearance to those found in *L. piceaperdum.* These species can, however, be distinguished based on the larger, guttulate conidia of *L. guttulatum.* These two species were clearly separated when comparing ribosomal DNA sequences (Coetsee, 1999).

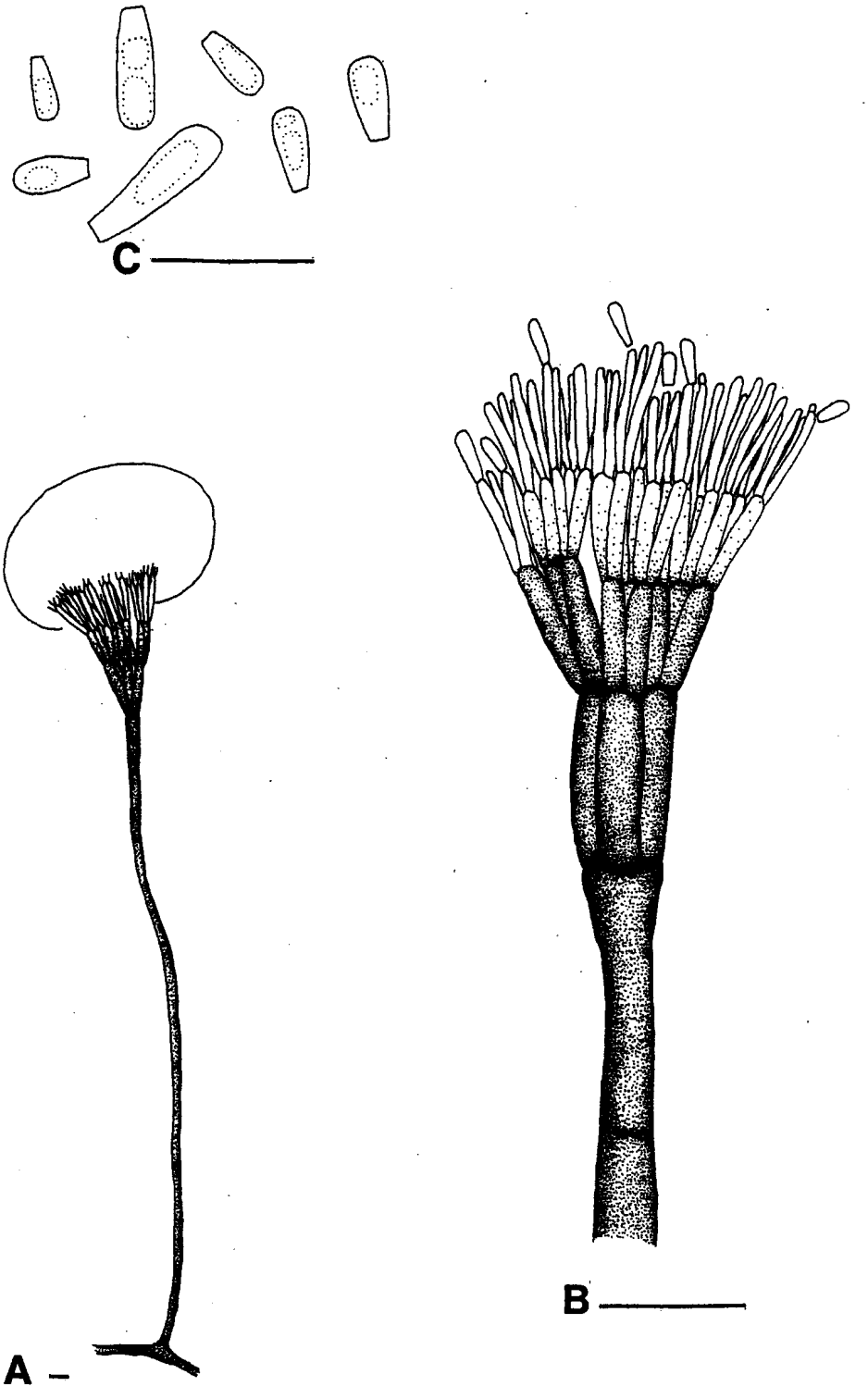

Fig. 78. Conidiophores and conidia of *L. guttulatum* (PREM 56307). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

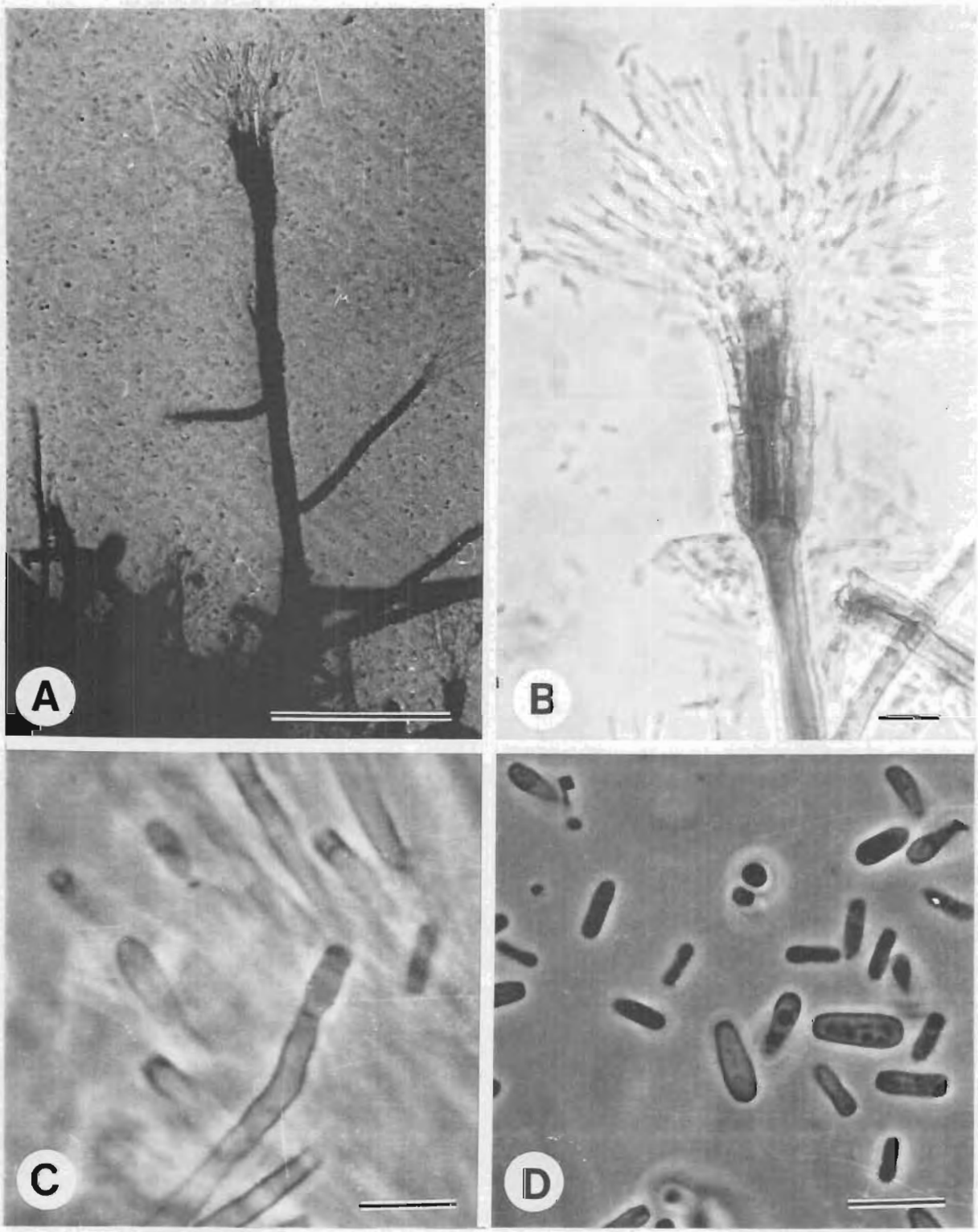

Fig. 79. Light micrographs of the conidiophores and conidia of *L. guttulatum* (PREM 56307). **A.** Conidiophore (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

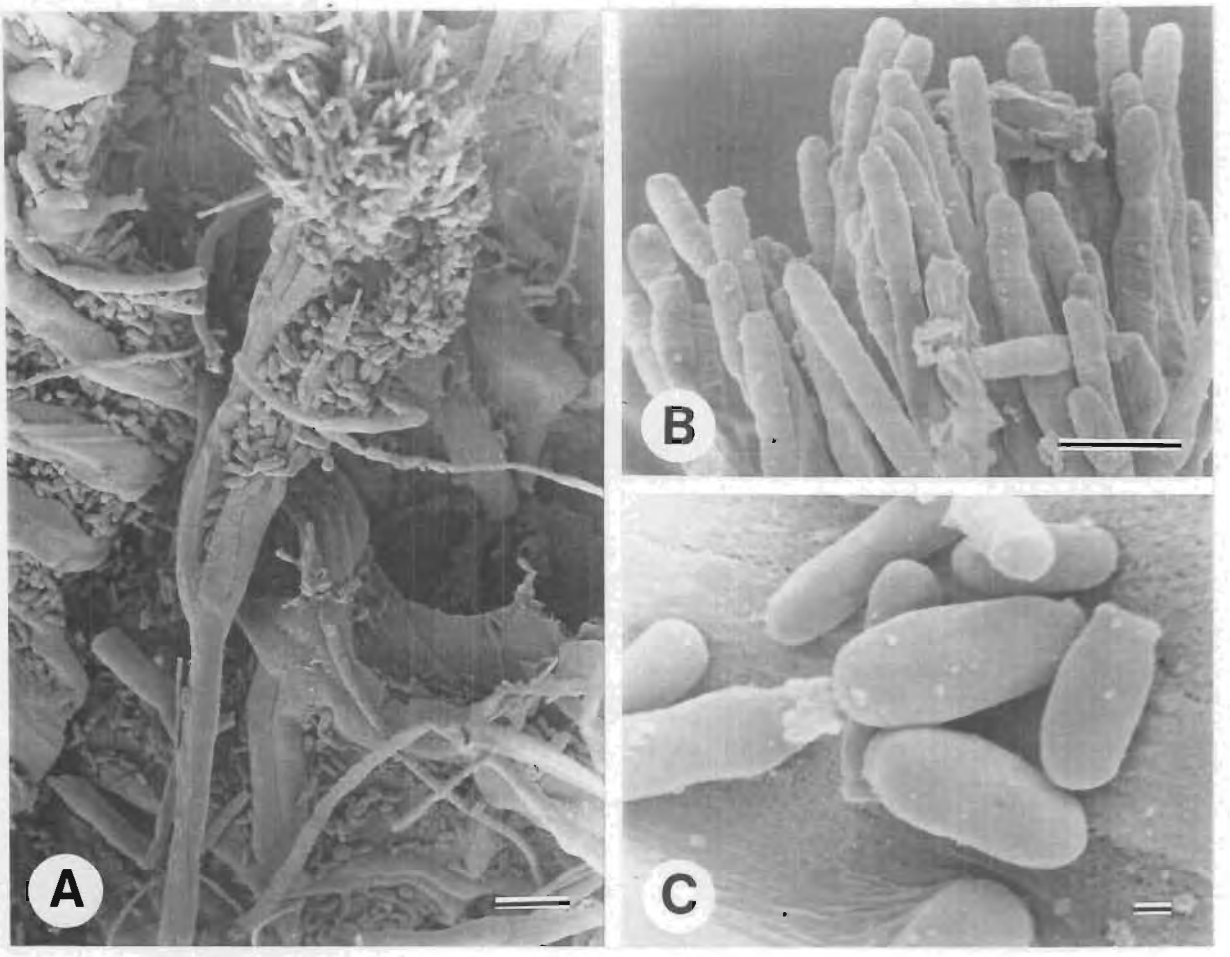

Fig. 80. Scanning electron micrographs of the conidiophores and conidia of *L. guttulatum* (PREM 56307). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 1 µm).

22. *Leptographium hughesii* K. Jacobs, M.J. Wingf. & T.C. Harr., *Canadian Journal of Botany* **76,** 1662. 1999. **(Figs. 81-83).**

Teleomorph: Not known.

Etymology: húghe-si-i: genitive of Hughes. This specific epithet honors S.J. Hughes who established the genus *Verticicladiella* in 1953.

Conidiophores occurring singly or in groups of up to eight, arising directly from the agar or aerial mycelium, erect, macronematous, mononematous, (240-) 559.5 - 913 (-1200) µm in length, rhizoid-like structures present at the base. *Stipes* olive-buff (21'"b), smooth, cylindrical, simple, 4 - 18 septate, (210-) 484.5 - 711 (-1130) µm long (3.5-) 4.0 - 7.5 µm wide below primary branches, apical cell not swollen, 5.0 - 12.0 µm wide at base, basal cell slightly swollen. *Conidiogenous apparatus* (30.0-) 67.5 - 89 (-175) µm long, excluding the conidial mass, with 2 to 3 (occasionally 4) series of cylindrical branches, 2 to 3 primary branches, olive-buff (21'"b), arrangement of primary branches - type B, smooth, cylindrical, aseptate, (7.5-) 16 - 25 (-35.5) µm long and (2.0-) 3.5 - 5.5 (-6.0) µm wide, secondary branches hyaline to olive-buff (21"b), aseptate, (6.0-) 10 - 14 (-16) µm long, 2.0 - 4.0) µm wide, tertiary branches hyaline, aseptate, 4.0 - 13.5 µm long, 1.0 - 3.0 µm wide, quaternary branches aseptate, 6.0 - 8.5 µm long, 1.0 - 2.0 µm wide. *Conidiogenous cells* discrete, 2 to 4 per branch, tapering slightly from the base to the apex, (8.0-) 9.0 - 15.5 (-18.5) µm long and 1.0 - 2.0 µm wide. *Conidia* hyaline, aseptate, ellipsoid to obovoid, occasionally slightly curved, 1.0 - 2.5 x 3.0 - 5.0 µm . Conidia accumulating in white, slimy droplets at the apex of conidiogenous apparatus.

Colonies with optimal growth at 25°C on 2% MEA, reaching 8 mm in diam. after 8 days, with little growth at 5°C and no growth at 35°C. Colonies olivaceous (21"m), with laciniate margins. Able to withstand high concentrations of cycloheximide with a 60% increase in linear growth on 0.1 mg/ml cycloheximide, with a 63% reduction in growth on o.5 g/l cycloheximide after 8 days at 20°C in the dark. Colony covered in a dense mat of aerial mycelium, hyphae mostly submerged, hyaline, smooth, straight, not constricted at the septa, 1.0 - 6.0 µm diam.

Specimens examined: Holotype: England, on a ship from Borneo, Princess Risborough, *Parashorea plicata*, December 1957, Savary, DAOM 62102. **Cultures**: Southern part of Vietnam, Phu Quoc island, isolated from the wounds of live *Aquilana* sp., June, 1996, B. Blanchette, CMW 4052.

Known distribution: Borneo, Vietnam.

Hosts/substrate: *Parashorea plicata* (Kendrick, 1962; Jacobs *et al.*, 1999).

Associated insects: Not known (Kendrick, 1962; Jacobs *et al.*, 1999).

Notes: This fungus was initially identified as a specimen of *L. abietinum* (Kendrick, 1962) due to the fact that its conidia are slightly curved, similar those of *L. abietinum*. Closer examination of the herbarium type as well as new isolates revealed that this specimen could readily be distinguished from other isolates identified as *L. abietinum*. The colonies of *L. hughesii* are characterized by a thick mat of aerial mycelium that covers the whole colony. This character has not been observed in isolates of *L. abietinum*. *Leptographium hughesii* originates from a non-coniferous host in Asia, which is in contrast to *L. abietinum* that occurs exclusively in North America, and is almost always associated with conifers, especially spruce (Kendrick, 1962; Jacobs *et al.*, 1999). *Leptographium abietinum* is also associated with insect infesting spruce, whereas no insect associations has been reported for *L. hughesii*.

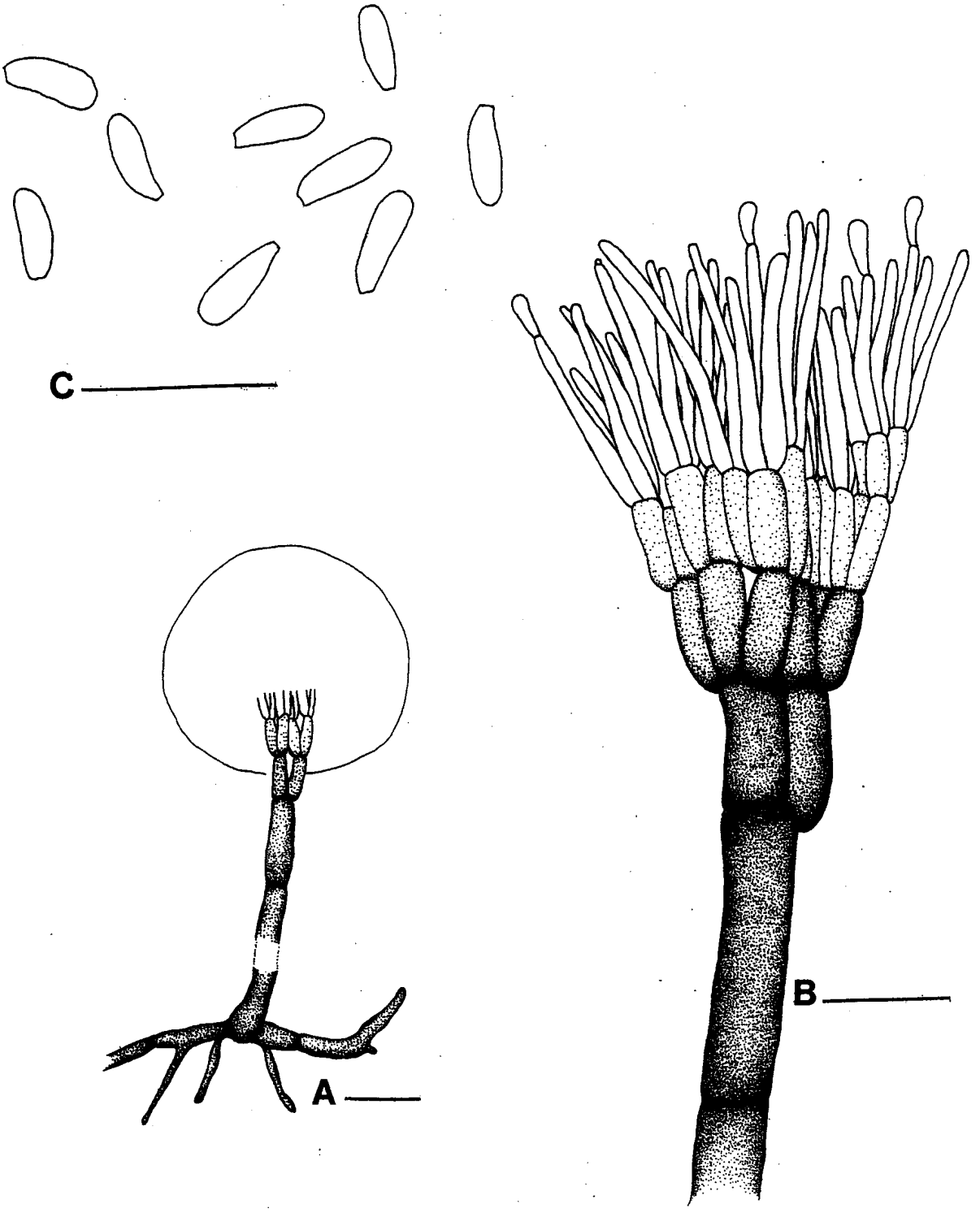

Fig. 81. Conidiophores and conidia of *L. hughesii* (CMW 4052). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

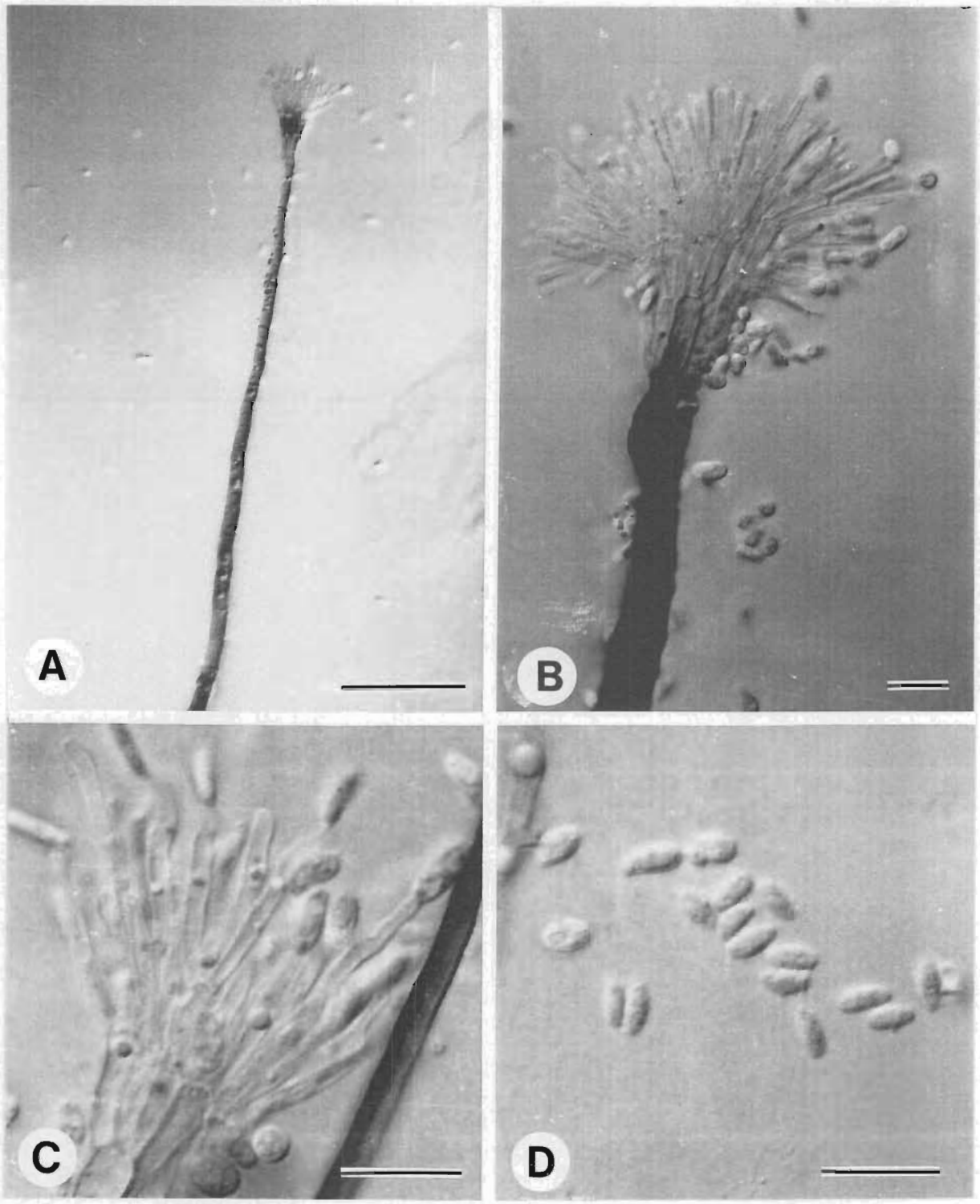

Fig. 82. Light micrographs of the conidiophores and conidia of *L. hughesii* (CMW 4052). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

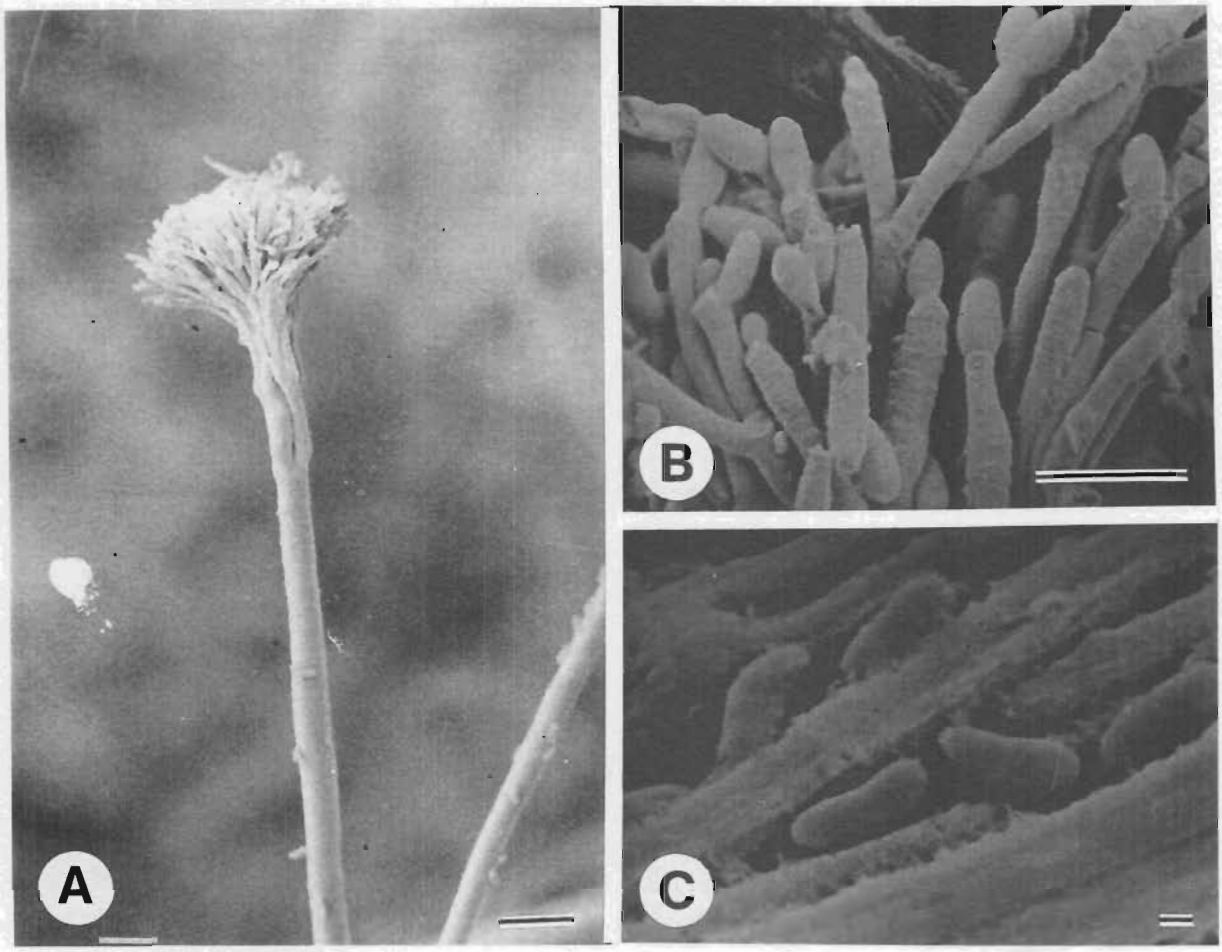

Fig. 83. Scanning electron micrographs of the conidiophores and conidia of *L. hughesii* (CMW 4052). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 1 µm).

23. *Ophiostoma huntii* (Robinson) De Hoog & R.J. Scheff., *Mycologia* **76**, 197. 1984. **(Figs. 84-86).**

≡ *Ceratocystis huntii* Robinson, *Canadian Journal of Botany* **42**, 528. 1964.

Anamorph: *Leptographium huntii* (Robinson) M.J. Wingf., *Transactions of the British Mycological Society* **85**, 92. 1985.

Etymology: hún-ti-i: genitive of Hunt. This specific epithet honors J. Hunt who made a considerable contribution towards the taxonomy of the ophiostomatoid fungi.

Perithecial bases black, globose and smooth walled, ornamented with fragile hyphal hairs, 280 - 448 µm in diam. *Perithecial neck* dark brown to black, cylindrical with a slight apical taper, smooth, 140 - 720 µm long, 40 - 70 µm above globose base, 21 - 42 µm wide at the apex, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* cucullate, aseptate, hyaline, invested in a sheath, 3.0 - 4.0 x 1.5 - 2.0 µm (Robinson-Jeffrey & Grinchenko, 1964).

Conidiophores occurring singly or in groups, arising directly from the mycelium but mostly on aerial mycelium, erect, macronematous, mononematous, (135-) 216.5 - 541.5 (-775) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), smooth or occasionally constricted at septa, cylindrical, simple, 5-18 septate, (100-) 145.5 - 484.5 (-720) µm long, (5.0-) 6.0 - 10 µm wide below primary branches, apical cell not swollen, 7.5 - 15 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (30-) 47.5 - 61 (-95) µm long, excluding the conidial mass, with 1 to 3 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical to barrel shaped, 0-1 septate (8.0-) 8.5 - 21 (-28) µm long and (3.0-) 4.5 - 5.5 (8.0) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21"k), aseptate, (8.0-) 11 - 15 (-20) µm long, (3.0-) 3.5 - 5.0 (-7.0) µm wide, tertiary branches hyaline, aseptate, (7.0-) 10.5 - 12 (-17) µm long, 2.0 - 6.0 µm wide, quaternary branches aseptate, (6.0-) 9.0 - 11.5 (-13) µm long, (2.0-) 2.5 - 4.0 (-5.0) µm wide. *Conidiogenous cells* discrete, 2-4 per branch, cylindrical, tapering slightly at the apex, (10-) 17.5 - 20.5 (-33) µm long and 2.0 - 4.0 µm wide. *Conidia* hyaline, aseptate, obovoid with truncate ends and

rounded apices, (3.0-) 4.0 - 6.0 (-8.5) x 1.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 43 mm in diam. in 8 days. No growth below 5°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 22% reduction in growth on 0.5 g/l cycloheximide after 12 days at 20°C in the dark. Colonies greenish olivaceous (23''') to olivaceous (21''m), losing color with continuous subculturing. *Colony margin* effuse. *Hyphae* submerged on agar with abundant aerial mycelium, hyaline to olivaceous (21''m), smooth, serpentine, occasionally constricted at the septa, (2.5-) 3.5 - 10.5 (-15) µm diam.

Specimens examined: Holotype: British Columbia, Toby Creek, near Invermere, *Pinus contorta* var. *latifolia* in bark beetle galleries, 6 Aug. 1962, collected: R. C. Jeffrey, DAOM 90235. **Cultures:** New Zealand, Mulberry Road, pine infested with *Hyalstes ater,* collected: M. Mackenzie, CMW 185 (same as IMI 5551, CMW 2820), CMW 1003; Scots pine infested with *Tomicus piniperda,* collected: J. Gibbs, CMW 1790 (same as CMW 2851); U.S.A., collected T.C. Harrington, C113 (same as CMW 2824); U.S.A., *Pinus resinosa,* collected: C.J. Randall, C583, (same as CMW 2768).

Known distribution: Canada, Australia, New Zealand.

Hosts/substrate: *Pinus contorta* var. *latifoliae* (Robinson-Jeffrey & Grinchenko, 1964; Solheim, 1995c), *Pinus ponderosa* (Davidson & Robinson-Jeffrey, 1965), *Pinus monticola* (Davidson & Robinson-Jeffrey, 1965), *Pinus banksiana* (Olchowecki & Reid, 1974), *Pinus strobus* (Davidson & Robinson-Jeffrey, 1965), *Picea mariana* (Olchowecki & Reid, 1974).

Associated insects: *Dendroctonus ponderosae* (Robinson-Jeffrey & Grinchenko, 1964; Harrington, 1988; Perry, 1991; Solheim, 1995c), *Hylastes ater* (Jacobs *et al.,* 1998), *Hylastes macer* (Harrington, 1988), *Ips pini* (Davidson & Robinson-Jeffrey, 1965; Harrington, 1988), *Tomicus piniperda* (Gibbs & Inman, 1991).

Notes: Griffin (1968) proposed that *O. huntii* might be a possible synonym of *O. penicillatum*. However, as in the case of *O. piceaperdum*, the ascospores of *O. huntii* are hat-shaped in contrast to the curved ascospores of *O. penicillatum*. Robinson-Jeffrey and Grinchenko (1964) distinguished *O. piceaperdum* and *O. huntii* based on small differences in the teleomorph states, although the anamorph states are morphologically similar. Olchowecki and Reid (1974) also noted the similarity of this fungus to *O. piceaperdum,* but could distinguish them based on the smaller ascospores in *O. huntii.* *Ophiostoma huntii* can be distinguished from *O. piceaperdum* based on the presence of serpentine hyphae in the latter species and the absence of these in *O. huntii.* *Ophiostoma piceaperdum* also has conidiogenous apparatuses that are more brush-like than those of *O. huntii.* *Ophiostoma huntii* does not readily produce conidiophores in culture and when these are present, they are mostly on the aerial mycelium. Nothing is known about the pathogenicity of this species.

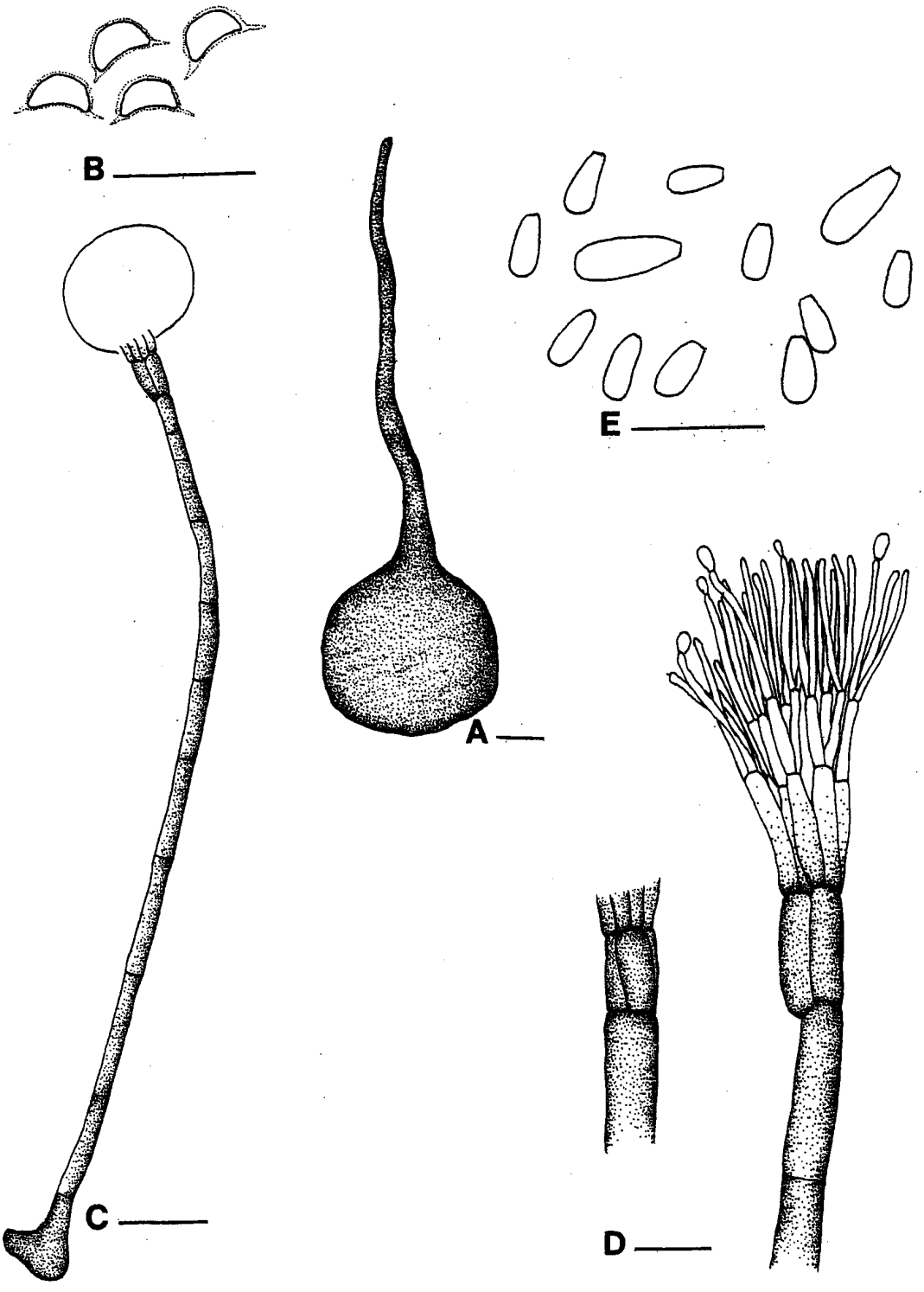

Fig. 84. Teleomorph and anamorph structures of *O. huntii* (CMW 2824). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 100 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

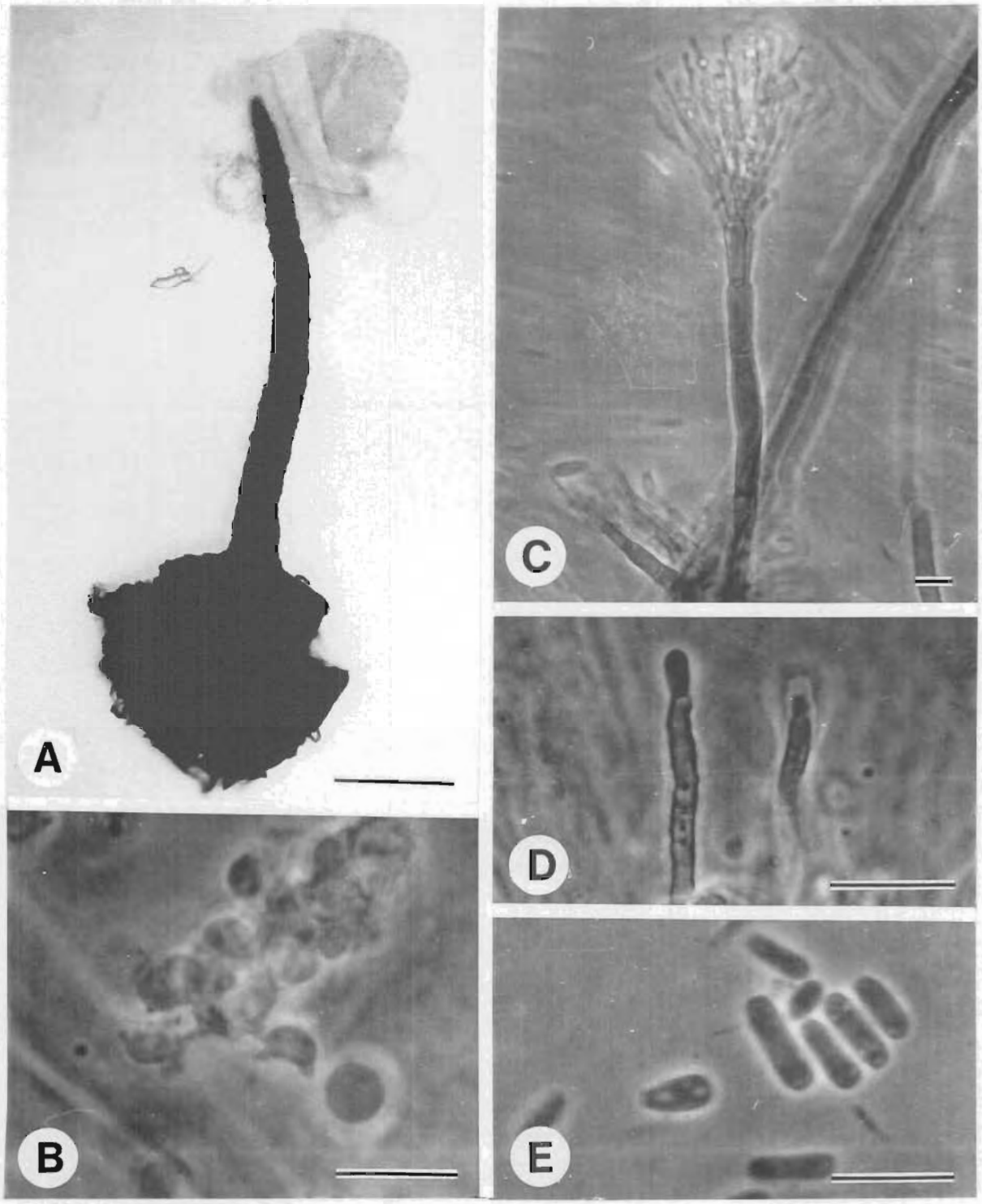

Fig. 85. Light micrographs of the teleomorph and anamorph structures of *O. huntii* (CMW 2824). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

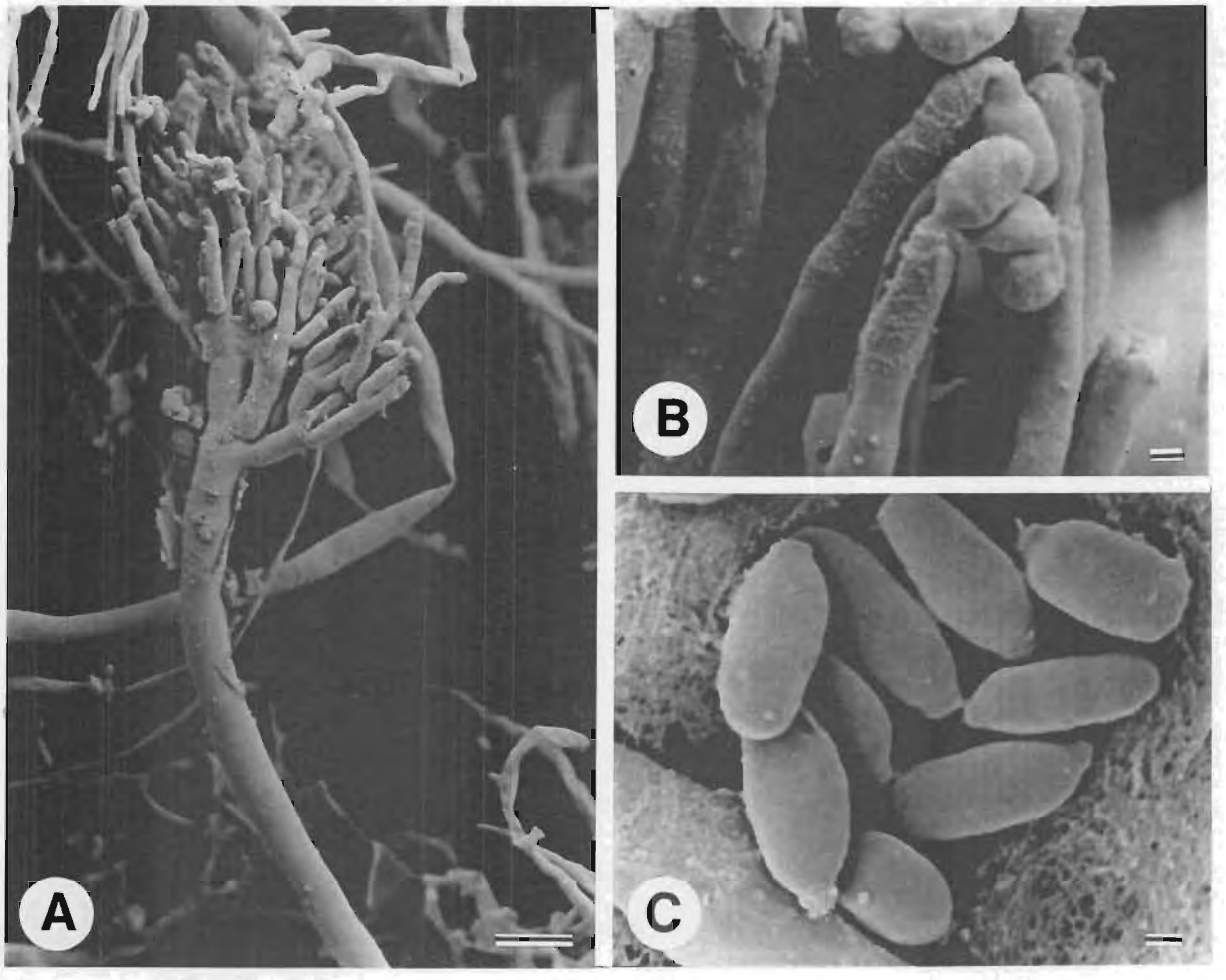

Fig. 86. Scanning electron micrographs of the conidiophores and conidia of *O. huntii* (CMW 2824). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 1 µm). **C.** Conidia (Bar = 1 µm).

24. *Ophiostoma laricis* K. van der Westh., Yamaoka & M.J. Wingf., *Mycological Research* **99**, 1336. 1995. **(Figs. 87-89).**

Anamorph: *Leptographium laricis* K. van der Westh., Yamaoka & M.J. Wingf., *Mycological Research* **99**, 1336. 1995.

Etymology: lá-ri-cis: of the larch tree. From the Latin noun larix: a larch tree. This specific epithet refers to *Larix* which is the host of this fungus.

Perithecial bases black, globose and smooth walled, unornamented, 210 - 310 µm in diam. *Perithecial neck* dark brown to black, cylindrical with a slight apical taper, smooth, 400 - 1320 µm long, 50 - 70 µm above globose base, 20 - 50 µm wide at the apex, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* curved, aseptate, hyaline, invested in a sheath, 6.0 - 11 x 2.0 - 4.0 µm (Van der Westhuizen *et al.,* 1995).

Conidiophores occurring singly or in groups of up to four, arising directly from the mycelium, erect, macronematous, mononematous, (130-) 207.5 - 214.5 (-260) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), smooth, cylindrical, simple, 1-7 septate, (55-) 98 - 126.5 (-200) µm long, 5.0 - 10 µm wide below primary branches, apical cell not swollen, 5.0 - 10 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (60-) 95 - 102 (-135) µm long, excluding the conidial mass, with 3 to 5 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical, 0-1 septate (12.5-) 21.5 - 24 (-28) µm long and (3.0-) 4.0 - 6.5 (-8.0) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21"k), 0-1 septate, (12.5-) 16.5 - 19 (-26) µm long, 3.0 - 6.0 µm wide, tertiary branches hyaline to light olivaceous, 0-1 septate, (9.0-) 10.5 - 19 (-22) µm long, 3.0 - 5.0 (-6.0) µm wide, quaternary branches aseptate, (8.0-) 11.5 - 14 (-17) µm long, 1.5 - 4.5 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (10-) 13.5 - 16.5 (-21) µm long and 1.0 - 2.0 µm wide. *Conidia* hyaline, aseptate, obovoid with truncate ends and rounded apices, (3.0-) 5.0 - 6.0 (8.0) x 1.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming

cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 25 °C on 2% MEA, reaching 40 mm in diam. in 8 days. No growth below 5°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 13% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies hyaline to light olivaceous (21"k). *Colony margin* smooth. *Hyphae* submerged on agar with little aerial mycelium, light olivaceous (21"k), smooth, straight, not constricted at the septa, (3.0-) 4.5 - 6.0 (8.0) µm diam.

Specimens examined: Holotype: Japan, Mt. Fuji, *Larix kaempferi*, August 1990, collected: Y. Yamaoka and M.J. Wingfield, PREM 51810. **Paratypes**: Japan, Mt. Fuji, *Larix kaempferi*, August 1990, collected: Y. Yamaoka and M.J. Wingfield, PREM 51811, PREM 51812, PREM 51813. **Cultures**: Japan, Mt. Fuji, *Larix kaempferi*, August 1990, collected: Y. Yamaoka and M.J. Wingfield, CMW 1980 (same as IMI 363664), CMW 1913 (same as IMI 363665), CMW 1957 (same as IMI 363666), CMW 2014 (same as IMI 363667).

Known distribution: Japan.

Hosts/substrate: *Larix kaempferi* (Van der Westhuizen *et al.*, 1995).

Associated insects: *Ips cembrae* (Van der Westhuizen *et al.*, 1995).

Notes: This species is similar to *O. penicillatum* and *O. piceaperdum*. It can be distinguished from *O. penicillatum* based on differences in conidial shape, the former species having obovoid conidia while the latter species is characterized by large allantoid conidia. *O. piceaperdum* is distinguished from *O. laricis* based on the presence of hat-shaped ascospores in *O. piceaperdum* and curved ascospores in the case of *O. laricis* (Van der Westhuizen *et al.*, 1995).

Very little is known about the pathogenicity of this species or its ecological role. Inoculation studies with this species on Japanese larch (*L. kaempferi*), resulted in large lesions. However, these were considerably smaller than those produced by the

pathogenic *Ceratocystis laricicola* (Yamaoka *et al.*, 1998) and *O. laricis*, therefore, does not appear to be an important pathogen.

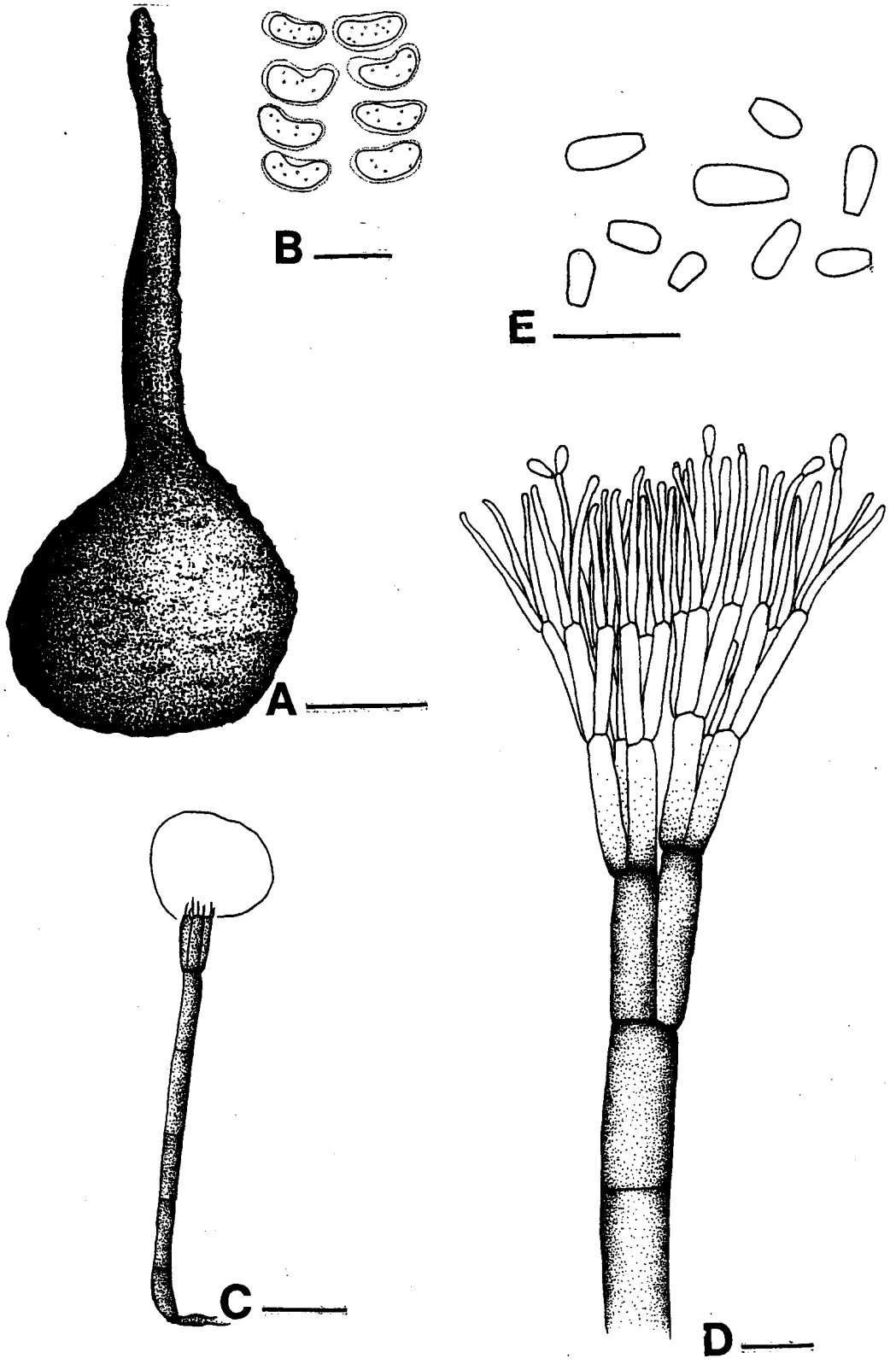

Fig. 87. Teleomorph and anamorph structures of *O. laricis* (PREM 51810). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

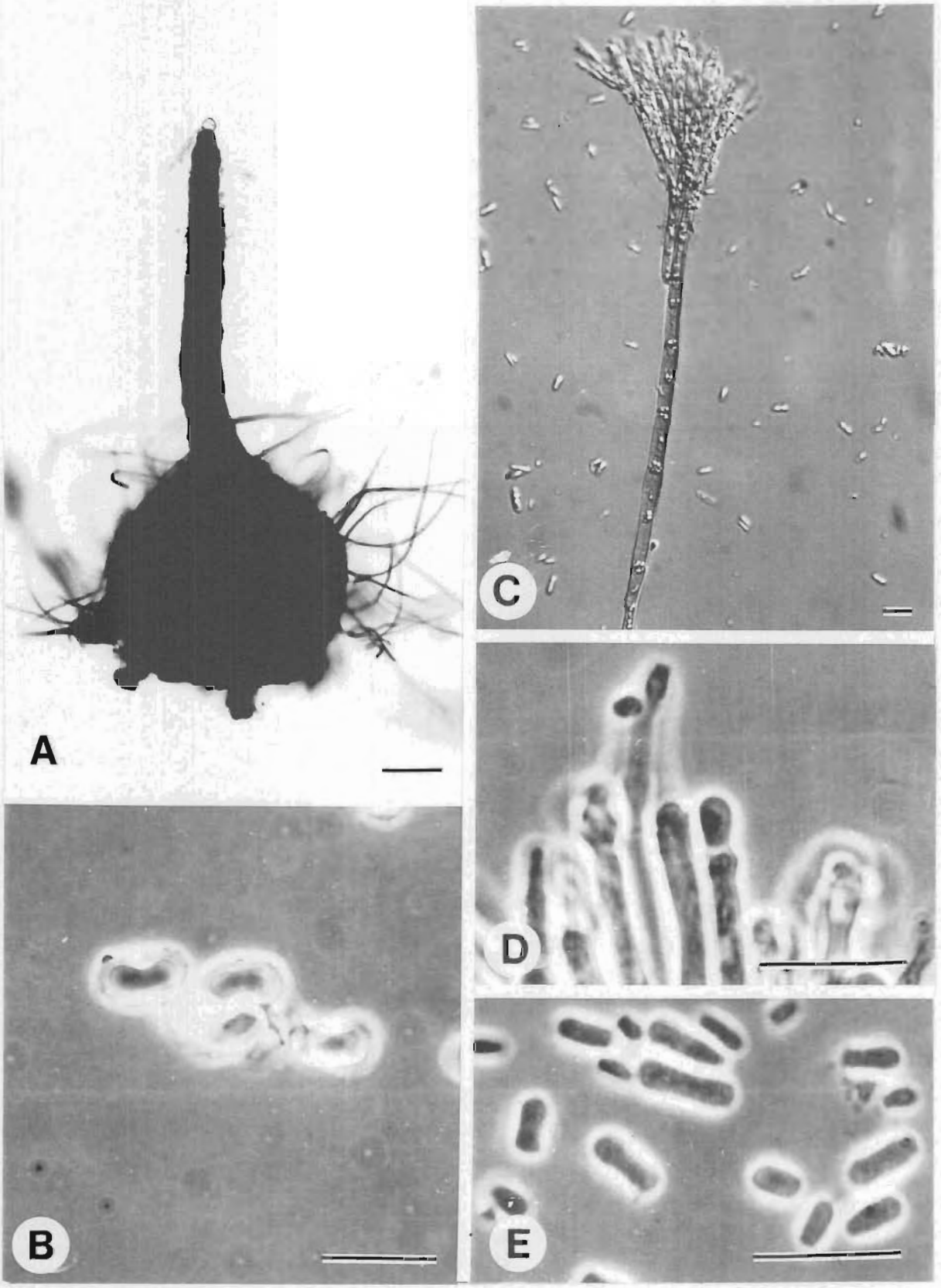

Fig. 88. Light micrographs of the teleomorph and anamorph structures of *O. laricis* (PREM 51810). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

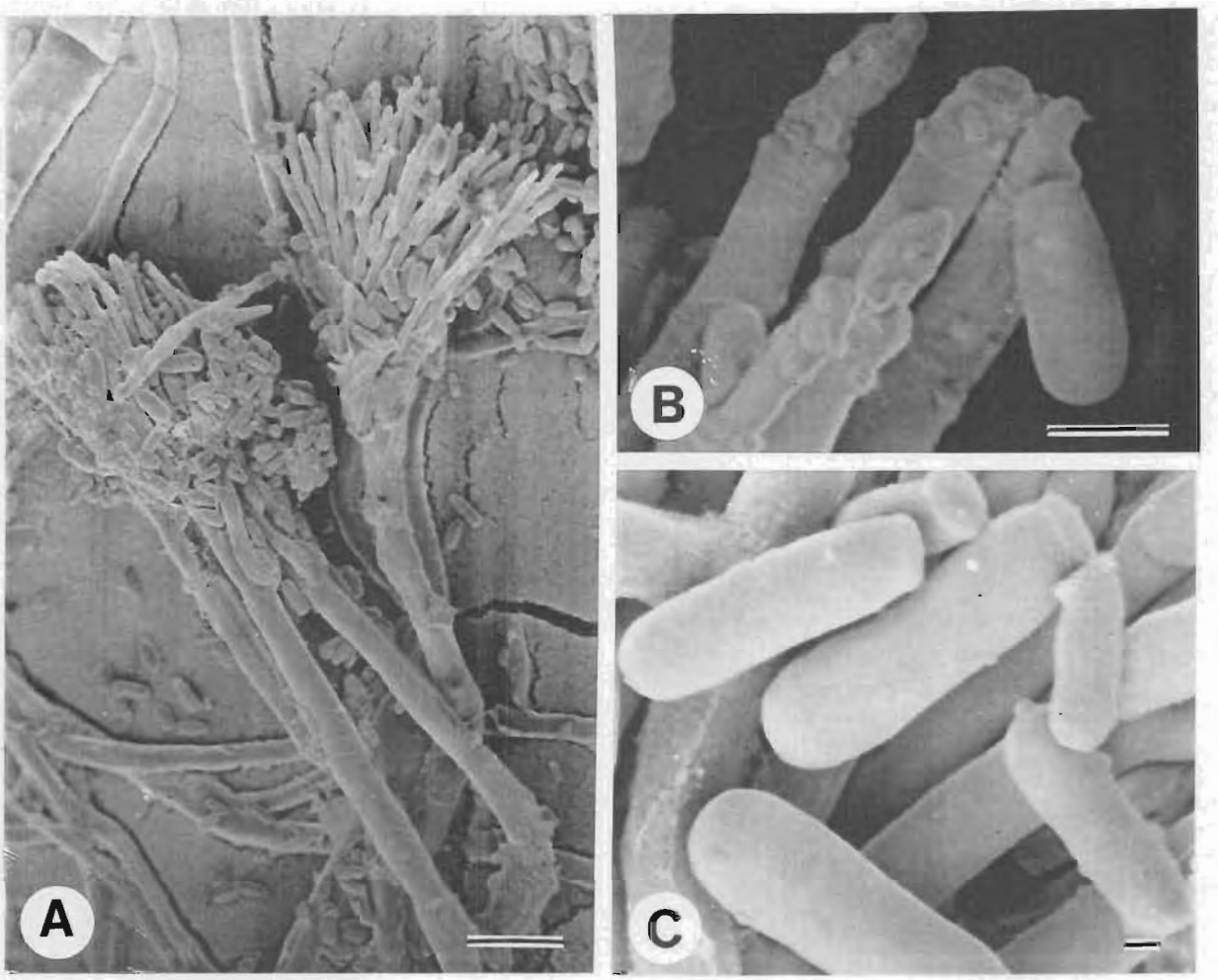

Fig. 89. Scanning electron micrographs of the conidiophores and conidia of *O. laricis* (PREM 51810). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

25. *Ophiostoma leptographioides* (R.W. Davidson) Arx, *Antonie von Leeuwenhoek* **18**, 211. 1952. **(Figs. 90-92).**

≡ *Ceratostomella leptographioides* R.W. Davidson, *Mycologia* **34**, 657. 1942.

≡ *Ceratocystis leptographioides* (R.W. Davidson) Hunt, *Lloydia* **19**, 28. 1956.

Anamorph: *Leptographium leptographioides* K. Jacobs & M.J. Wingf. sp. nov.

Etymology: lep-to-gra-phi-ói-des: resembling *Leptographium*. From the Greek adjective λεπτος fine, thin slender and the Greek noun γραφισν: a brush. This specific epithet refers to the brush-like structures of this fungus.

Perithecial bases black, globose and smooth walled, unornamented, 100 - 150 µm in diam. *Perithecial neck* dark brown to black, cylindrical with a slight apical taper, smooth, 150 - 180 µm long, 35 - 40 µm above globose base, 15 - 25 µm wide at the apex, *ostiolar hyphae* divergent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* reniform, aseptate, hyaline, invested in a sheath giving it a pillow-shaped and occasionally a hat-shaped appearance, 6.0 - 7.5 x 3.0 - 4.0 µm (Davidson, 1942).

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (77.5-) 130.5 - 141 (-237.5) µm in length, rhizoid-like structures present. *Stipes* olivaceous (21"m), smooth, cylindrical, simple, 2-10 septate, (50-) 106 - 109.5 (-192.5) µm long, 4.0 - 7.5 µm wide below primary branches, apical cell not swollen, (5.0-) 7.0 - 10.5 (-12.5) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (17.5-) 27.5 - 31 (-42.5) µm long, excluding the conidial mass, with 1 to 2 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical, aseptate, (8.0-) 11 - 13.5 (-16) µm long and 2.0 - 5.5 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline to light olivaceous, aseptate, (6.0-) 9.0 - 10.5 (-12.5) µm long, 2.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 8.5 - 14 (-15.5) µm long and 1.0 - 2.0 µm wide. *Conidia*

hyaline, aseptate, obovoid to ellipsoid with truncate ends and rounded apices, 4.0 - 9.5 (-12) x 1.0 - 4.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 30 °C on 2% MEA, reaching 38 mm in diam. in 8 days. No growth below 5°C with significant growth at 35°C. Able to withstand high concentrations of cycloheximide with a 12% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies olivaceous (21"m). *Colony margin* smooth. *Hyphae* submerged on agar with little aerial mycelium, hyaline, smooth, straight, not constricted at the septa, (2.5-) 4.5 - 6.0 (-10) µm diam.

Specimens examined: Holotype: U.S.A., 3 March 1943, collected: R.W. Davidson, BPI 595702 (59117) (received from NFC), grown on malt agar. **Cultures**: California, Richland, *Quercus albus,* collected: B. Moss, C528 (same as CMW 2803); U.S.A, collected: R.W. Davidson, CMW 481 (PREM 56387).

Known distribution: U.S.A.

Hosts/substrate: *Quercus* sp. (Davidson, 1942).

Associated insects: Not known.

Notes: Davidson (1942) described the teleomorph of this species in the genus *Ceratostomella.* However, only brief mention was made of the presence of a *Leptographium* - type anamorph and the anamorph of this species has not been formally named in *Leptographium.* The anamorph of *O. leptographioides* is similar to those of *O. francke-grosmanniae* and *O. grandifoliae.* It can be distinguished from *O. franke-grosmanniae* based on the distinct annelidic conidiogenous cells of the former species. These are in contrast to the conidiogenous cells of *L. francke-grosmanniae* that have closely packed annellations, giving them a phialidic appearance when viewed with the light microscope. The teleomorph states of *O. leptographioides* and *O. francke-grosmanniae* can also easily be distinguished based

on the short-necked perithecia and pillow shaped ascospores of the former species. This is in contrast to the long necked perithecia and curved ascospores of *O. francke-grosmanniae.*

Ophiostoma leptographioides can be distinguished from *O. grandifoliae* based on the presence of longer oblong conidia in the former species, in contrast to the smaller obovoid conidia of the latter species. These species can further be distinguished based on their teleomorph characters. *Ophiostoma leptographioides* has pillow-shaped ascospores, in contrast to the cucullate ascospores of *O. grandifoliae.* Nothing is known about the pathogenicity or ecology of this species.

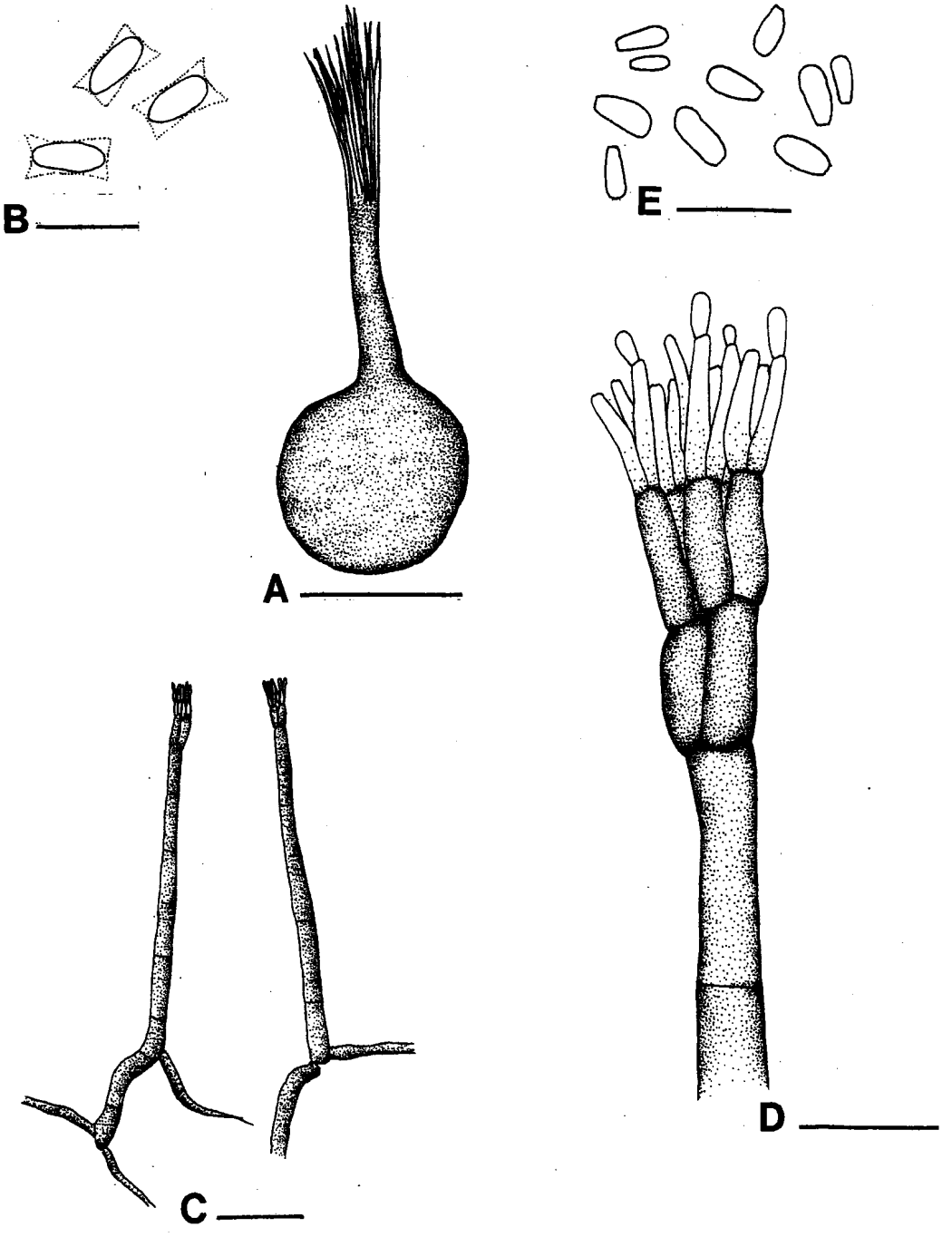

Fig. 90. Teleomorph and anamorph structures of *O. leptographioides* (CMW 2803). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 50 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

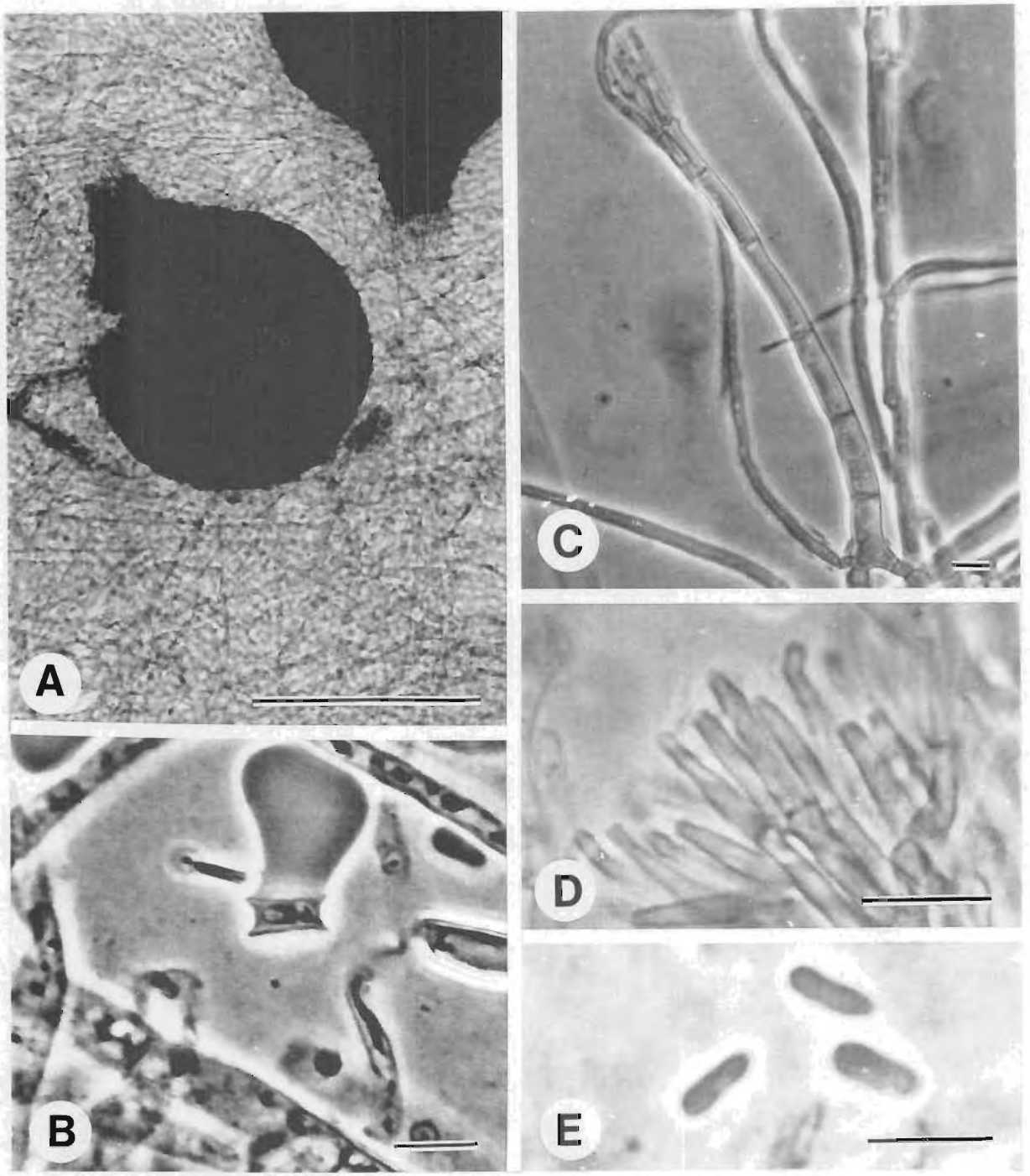

Fig. 91. Light micrographs of the teleomorph and anamorph structures of *O. leptographioides* (CMW 2803). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

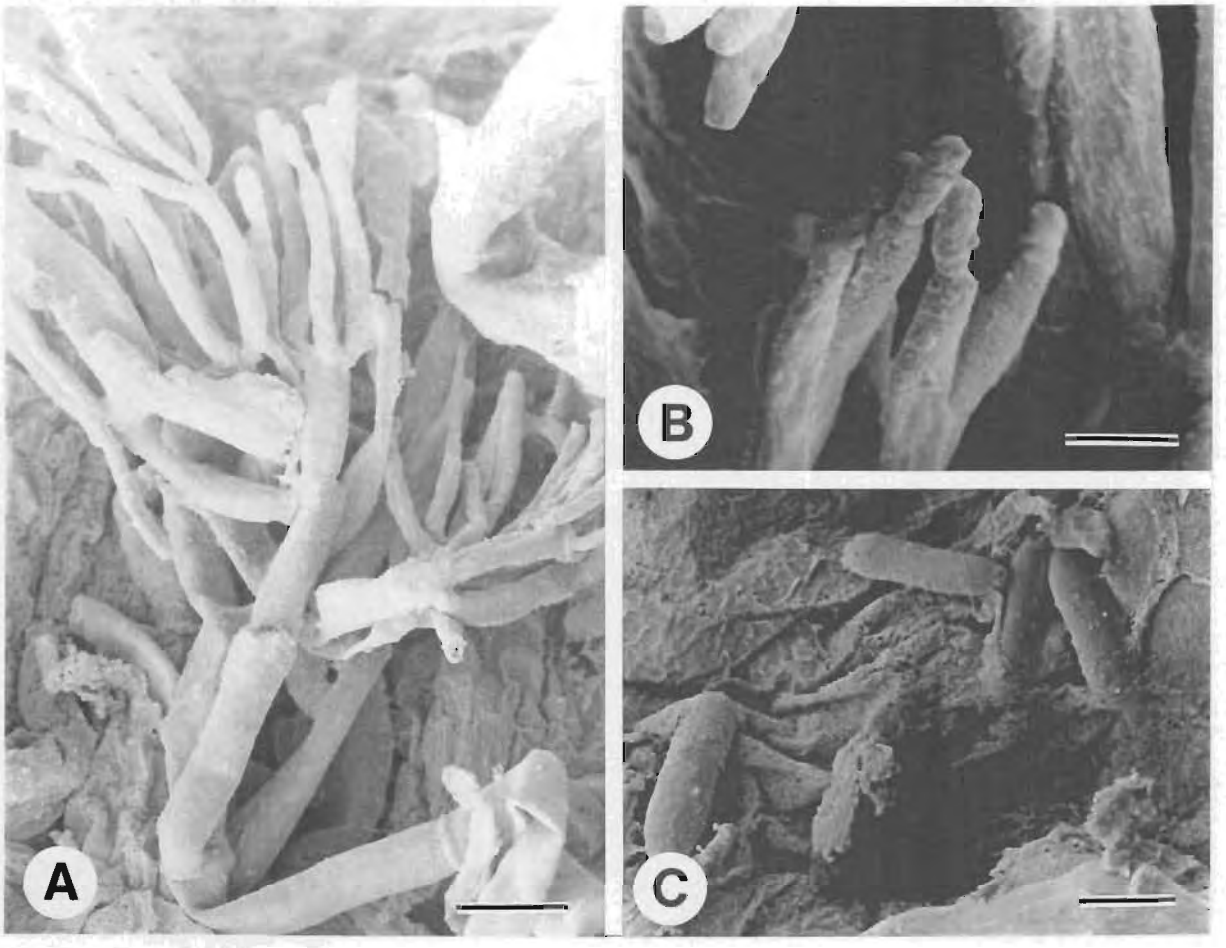

Fig. 92. Scanning electron micrographs of the conidiophores and conidia of *O. leptographioides* (CMW 2803). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 5 µm).

26. *Leptographium lundbergii* Lagerb. & Melin *Sv. Skogsvardsf. Tidskr.* **25**, 249. 1927. **(Figs. 93-95).**

= *Scopularia venusta* Preuss, 1851.

= *Verticicladiella truncata* M.J. Wingf. & Marasas, *Transactions of the British Mycological Society* **80**, 232. 1983.

= *Leptographium truncatum* (M.J. Wingf. & Marasas) M.J. Wingf., *Transactions of the British Mycological Society* **85**, 92. 1985.

Teleomorph: Not known.

Etymology: lund-bér-gi-i: genitive of Lundberg. This specific epithet honors G. Lundberg who played an important role in the study of blue-stain of pine and spruce in Europe.

Conidiophores occurring singly or in groups of up to six, arising directly from the mycelium, erect, macronematous, mononematous, (90-) 246 - 409 (-685) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), smooth, cylindrical, simple, 1 - 16 septate, (35-) 214.5 - 306.5 (-635) µm long, 2.5 - 5.0 µm wide below primary branches, apical cell not swollen, 2.5 - 5.5 (-6.0) µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* (35-) 42.5 - 85 (-150) µm long, excluding the conidial mass, with 2 to 3 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical, 0-2 septate, 11.0 - 41.5 (-57) µm long and 4.5 - 9.0 (-11) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline to light olivaceous (21"k), 0-1 septate, 8.0 - 30.5 (-39) µm long, 3.0 - 7.5 (-9.0) µm wide, tertiary branches hyaline to light olivaceous (21"k), aseptate, (8.0-) 14.5 - 17 (-22) µm long, (3.0-) 4.0 - 6.0 (-8.0) µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (11-) 16.5 - 25.5 -(37) µm long and 1.5 - 3.0 µm wide. *Conidia* light gray olivaceous (19""), aseptate, broadly ellipsoid with truncate ends and rounded apices, 3.0 - 5.0 x 2.0 - 4.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 25 °C on 2% MEA, reaching 39 mm in diam. in 7 days. No growth below 5°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 21% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15''''k). *Colony margin* smooth. *Hyphae* submerged on agar with very little aerial mycelium on the edges of the colony, greenish olivaceous (23''') to olivaceous (21''m), smooth, straight, occasionally constricted at the septa, (3.0-) 4.5 - 8.5 (-14) µm diam.

Specimens examined: Neotype: *Pinus* sp., 1929, collected T. Lagerberg & E. Melin, PREM 50548. **Paratypes:** South Africa, Sabie, Wilgeboom seed orchard, *Pinus taeda* roots, collected: M.J. Wingfield, Dec. 1978, PREM 45698; South Africa, Sabie, Wilgeboom seed orchard, from *Hylastes* sp. collected M.J. Wingfield, Feb 1980, collected: M.J. Wingfield, PREM 45696; South Africa, East Transvaal, Maraiti Plantation, *Pinus taeda* roots, Feb, 1979, collected: M.J. Wingfield, PREM 45697; New Zealand, Gwavas State Forest, *Pinus strobus* roots, May 1979, collected M. Dick, PREM 45699; New Zealand, Horner State Forest, *Pinus radiata* roots, May, 1979, collected: M. Dick. PREM 45700. **Cultures**: New Zealand, *Pinus strobus,* 1986, collected: M. Dick, CMW 30; New Zealand, *Pinus strobus,* 1986, collected: M. Dick, CMW 21; *Pinus* sp., 1929, collected T. Lagerberg & E. Melin, CMW 217 (CBS352.29); United Kingdom, isolated from *Hylastes opacus,* 1986; collected: J.N. Gibbs, CMW 836 (PREM 56388).

Known distribution: Europe, South Africa, New Zealand.

Hosts/substrate: *Pinus* spp., *Picea* spp. (Lagerberg *et al.,* 1927), *Pinus densiflora* (Kaneko & Harrington, 1990, *Pinus thunbergii* (Kaneko & Harrington, 1990), *Pinus strobus* (Wingfield & Marasas, 1983), *Pinus ponderosa* (Rumbold, 1931), *Pinus sylvestris* (Wingfield & Gibbs, 1991), *Pinus radiata* (Wingfield & Marasas, 1983), *Pinus taeda* (Wingfield & Marasas, 1983; Wingfield *et al.,* 1988), *Picea abies, Larix leptolepis* (Bakshi, 1950).

Associated insects: *Trypodendron lineatum* (Bakshi, 1950; Harrington, 1988),

Hylastes opacus (Wingfield & Gibbs, 1991), *H. ater* (Harrington, 1988), *H. angustatus* (Wingfield & Marasas, 1983; Harrington, 1988, Wingfield *et al.*, 1988), *H. opacus* (Wingfield & Gibbs, 1991), *Hylurgops palliatus, H. ligniperda* (Harrington, 1988), *H. palliatus* (Wingfield & Gibbs, 1991), *Blastophagus minor* (Mathiesen-Käärik, 1953), *Blastophagus piniperda* (Mathiesen-Käärik, 1953), *Dendroctonus ponderosae* (Rumbold, 1931), *Ips acuminatus* (Mathiesen-Käärik, 1953; Harrington, 1988), *Myelophilus minor* (Harrington, 1988), *M. piniperda* (Harrington, 1988), *Orthotomicus proximus* (Mathiesen-Käärik, 1953; Harrington, 1988), *Pissodes pini* (Mathiesen-Käärik, 1953), *Pityogenes quadridens* (Mathiesen-Käärik, 1953).

Notes: This species was described by Lagerberg *et al.* (1927) and represents the type of the genus *Leptographium. Scopularia venusta* described by Preuss (1851), was synonomised with *L. lundbergii* because the description of the former species was found to be unduly vague to verify certain characters (Lagerberg *et al.*, 1927). *Leptographium truncatum* was described by Wingfield and Marasas (1983) and is characterized by broadly truncate conidia. *Leptographium truncatum* was reduced to synonymy with *L. lundbergii* by Strydom *et al.* (1997) as proposed by Wingfield and Gibbs (1991). Due to the lack of a type specimen for *L. lundbergii,* Strydom *et al.* (1997) designated a neotype for it based on the culture CBS 352.29 (PREM 50548) which was collected by E. Melin, one of the original authors of *L. lundbergii.* Illustrations supporting this neotypification were supplied by Wingfield and Gibbs (1991).

Leptographium lundbergii can easily be recognized by its prominently truncate conidia. This is similar to those of *L. pyrinum* and *L. yunnanensis.* These species can, however, be distinguished from *L. lundbergii* based on the granular sheath material around its hyphae. The conidiophores of *L. lundbergii* differed markedly from those observed in *L. pyrinum* and *L. yunnanensis.* Conidiophores of *L. lundbergii* are more structured compared to the more crooked and loosely arranged branches making up the conidiogenous apparatus in isolates of *L. pyrinum* and *L. yunnanensis.*

Leptographium lundbergii is one of many species in the genus that is responsible for blue-stain of timber. Bakshi (1951) indicated that this species is an important blue-stain agent of conifers in Europe. It penetrates the wood quickly, which becomes blue-stained in a short period of time (Bakshi, 1951). Kaneko and Harrington (1990) found that Japanese isolates of this fungus were weakly pathogenic to severely stressed red and black pines. Likewise, Wingfield and Marasas (1983) conducted inoculations in *Pinus elliottii* in South Africa using an isolate of *L. truncatum* (now *L. lundbergii*) and showed that the fungus is able to produce pronounced lesions.

Leptographium lundbergii is associated with a number of pine root-feeding insects. Some of these such as *H. angustatus* and *H. ater* have been introduced into Southern Hemisphere countries and the fungus has been carried with the insects. Thus, *H. ater* occurs in New Zealand, Australia and Chile and *L. lundbergii* is known from New Zealand (Wingfield & Marasas, 1983). The fungus almost certainly will also be present in Australia and Chile. Likewise, *L. lundbergii* was probably introduced into South Africa, together with *H. angustatus* (Wingfield & Knox-Davies, 1980b).

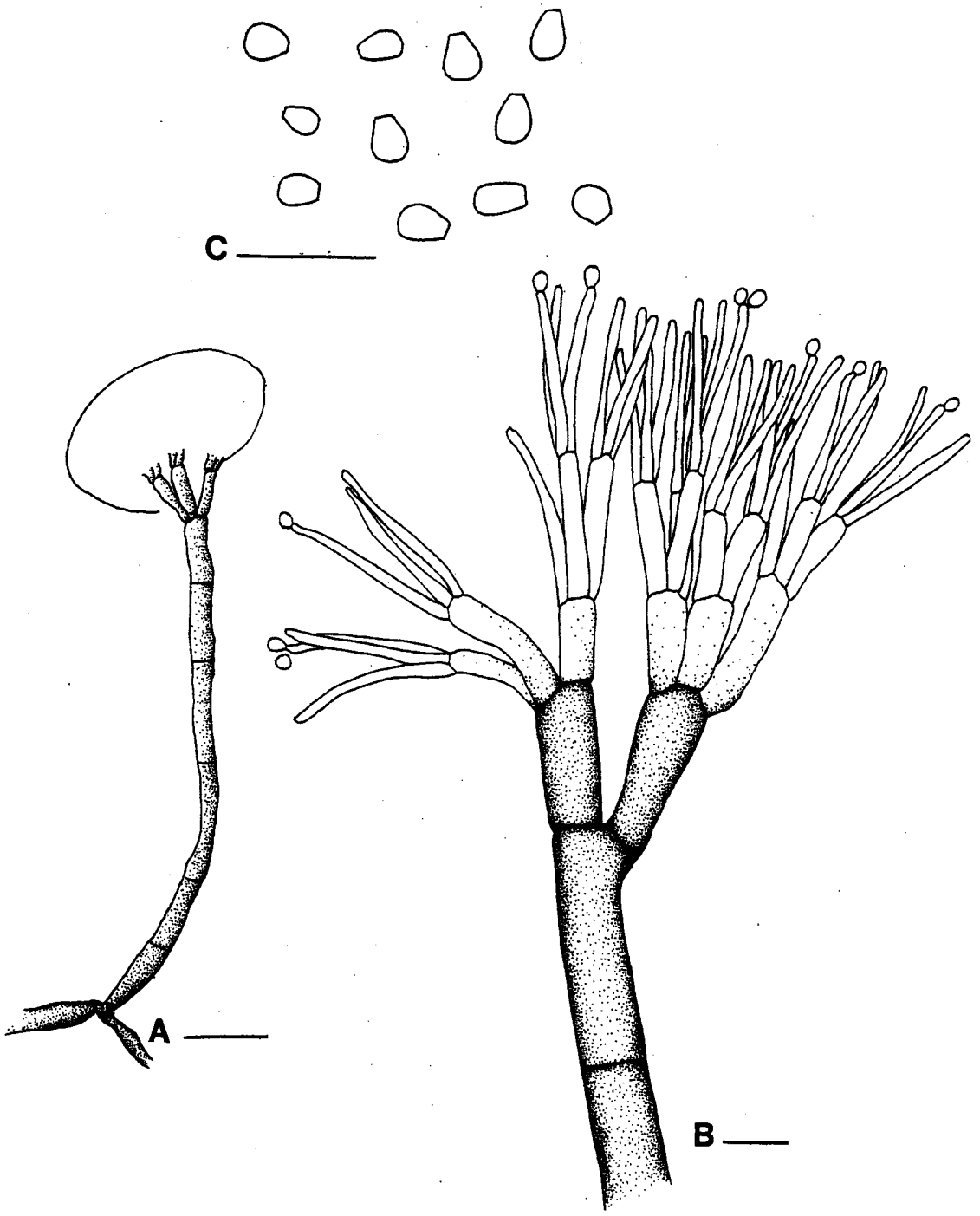

Fig. 93. Conidiophores and conidia of *L. lundbergii* (PREM 45698). **A.** Habit sketch (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

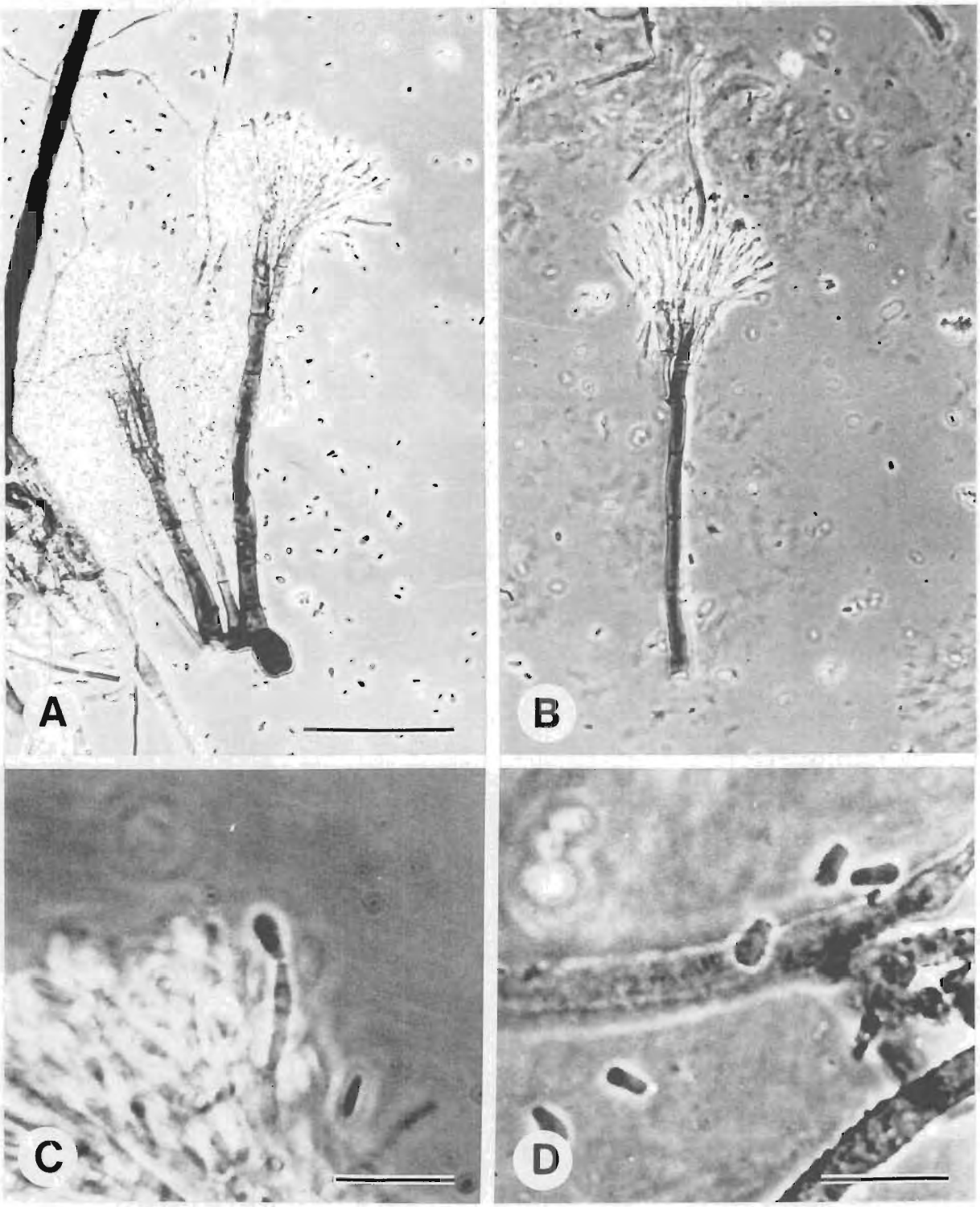

Fig. 94. Light micrographs of the conidiophores and conidia of *L. lundbergii* (PREM 45698). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 50 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

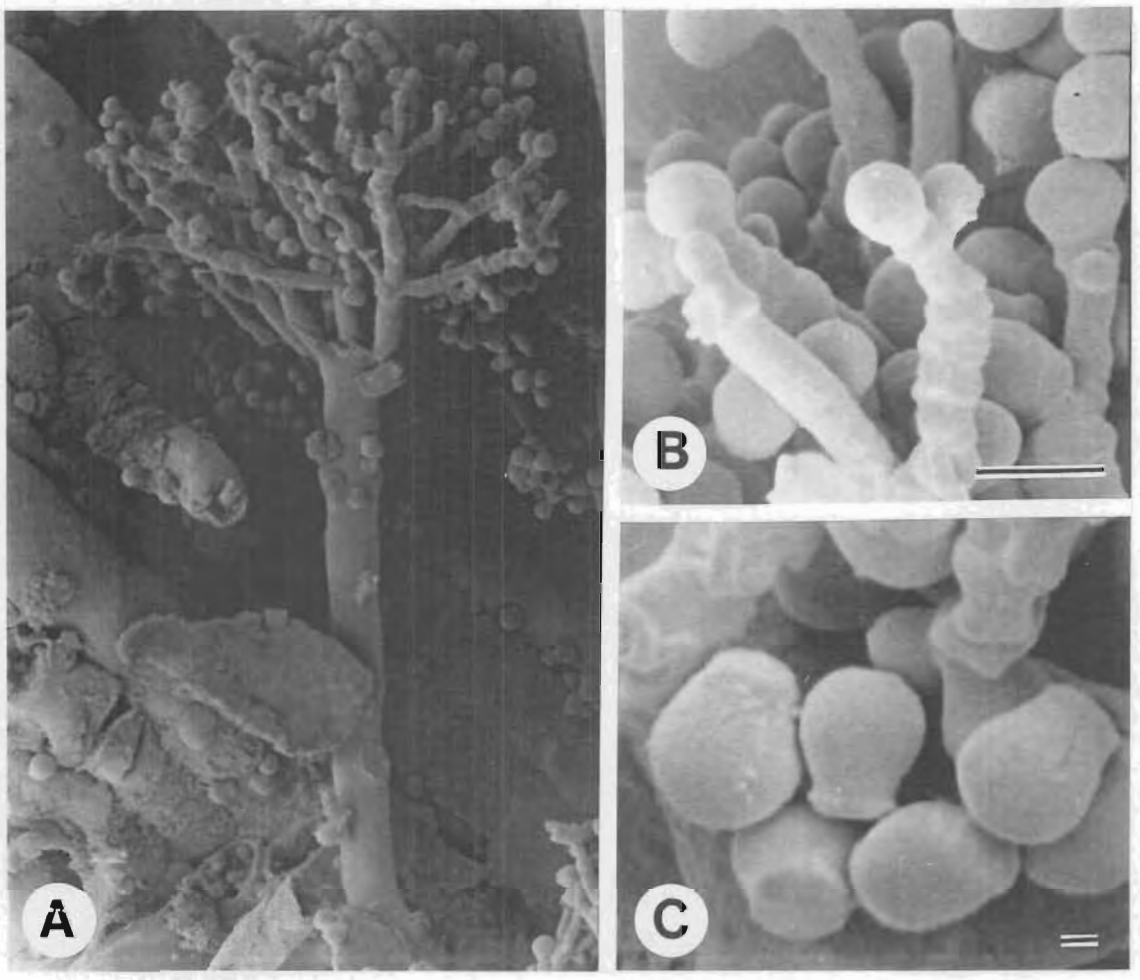

Fig. 95. Scanning electron micrographs of the conidiophores and conidia of *L. lundbergii* (PREM 45698). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

27. *Leptographium neomexicanum* M.J. Wingf., T.C. Harr. & Crous, *Canadian Journal of Botany* **72**, 228, 1994. **(Figs. 96-98).**

Teleomorph: Not known.

Etymology: ne-o-mex-i-cá-num: pertaining to New Mexico. This specific epithet refers to the origin of this fungus in New Mexico, U.S.A.

Conidiophores occurring singly or occasionally in groups of up to three, arising directly from the mycelium, erect, macronematous, mononematous, (84-) 183.5 - 552 (-821.5) µm in length, rhizoid-like structures present. *Stipes* olivaceous (21"m), smooth, cylindrical, simple, 4 - 10 septate, (62-) 163 - 461 (-750) µm long, (4.5-) 8.5 - 13 (-15.5) µm wide below primary branches, apical cell not swollen, (4.5-) 5.5 - 14 (-15.5) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (28-) 30 - 82.5 (-77.5) µm long, excluding the conidial mass, with 2 to 3 series of cylindrical branches, 2-4 primary branches, light olivaceous (21"m), smooth, cylindrical, aseptate (11-) 15 - 17 (-37) µm long and (3.0-) 4.0 - 10.5 (-12.5) µm wide, arrangement of the primary branches on the stipe - type C, secondary branches hyaline to light olivaceous, aseptate, (7.0-) 11 -12 (-20) µm long, (2.0-) 3.0 - 4.5 (-7.5) µm wide, tertiary branches hyaline, aseptate, 7.5 - 18.5 µm long, 1.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, cylindrical, tapering slightly at the apex, (9.0-) 13.5 - 15.5 (-22) µm long and 1.0 - 2.0 µm wide. *Conidia* hyaline, aseptate, obovoid with truncate ends and rounded apices, slightly curved at the apex, (3.0-) 3.5 - 4.5 (-5.5) x 1.0 - 2.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, turning light amber (19'b) when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 33 mm in diam. in 6 days. No growth below 10°C or above 30°C. Able to withstand high concentrations of cycloheximide with a no reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15""'k). *Colony margin* laciniate. *Hyphae* submerged on agar with very little aerial mycelium, light olivaceous (21"k),

smooth, straight, not constricted at the septa, occasionally roughened, 1.5 - 11 µm diam.

Specimens examined: Holotype: U.S.A., New Mexico, *Pinus ponderosa* roots, 1980, collected: T.C. Harrington,. PREM 51443. **Paratype**: U.S.A., New Mexico, *Pinus ponderosa* roots, 1980, collected: W.H. Livingston, PREM 51444, PREM 51445. **Cultures**: U.S.A., Mascalero, New Mexico, *Pinus ponderosa*, 1980, collected: T.C. Harrington, C 32 (same as CMW 2079).

Known distribution: New Mexico.

Hosts/substrate: *Pinus ponderosa* (Wingfield *et al.*, 1994).

Associated insects: Not known.

Notes: This fungus was isolated from the roots of conifers, which is considered to be a distinguishing character, as in the case of *L. albopini* and *L. douglasii*. *Leptographium neomexicanum* is similar to *L. douglasii* but can be distinguished from it based on the shorter conidiophores of the latter species. The hyphae of *L. neomexicanum* are also less serpentine than those of *L douglasii.* This species is further characterized by an abundance of gray aerial mycelium in culture (Wingfield *et al.*, 1994). *Leptographium neomexicanum* has also been shown to have a low level of virulence (Wingfield *et al.*, 1994).

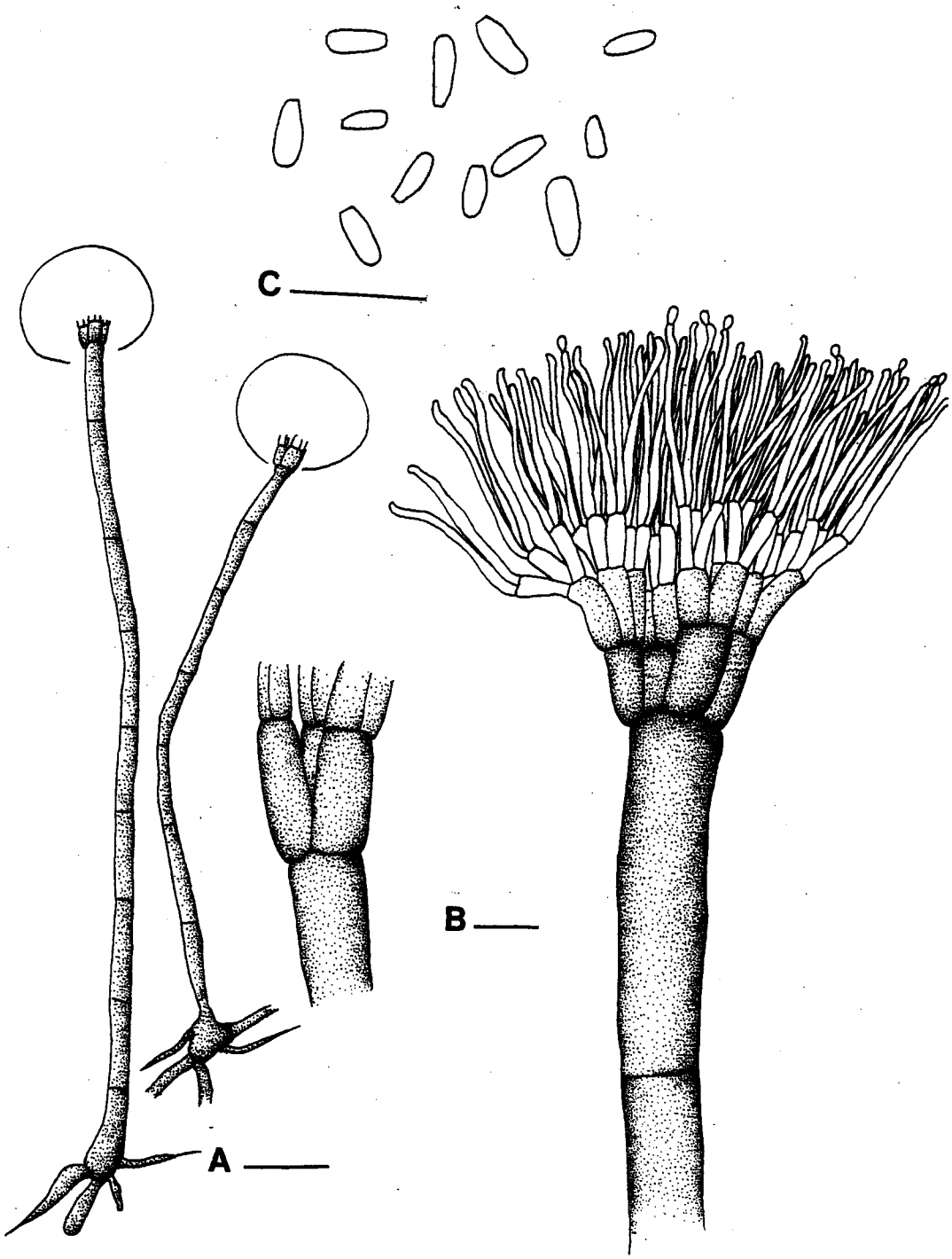

Fig. 96. Conidiophores and conidia of *L. neomexicanum* (CMW 2079). **A.** Habit sketch (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

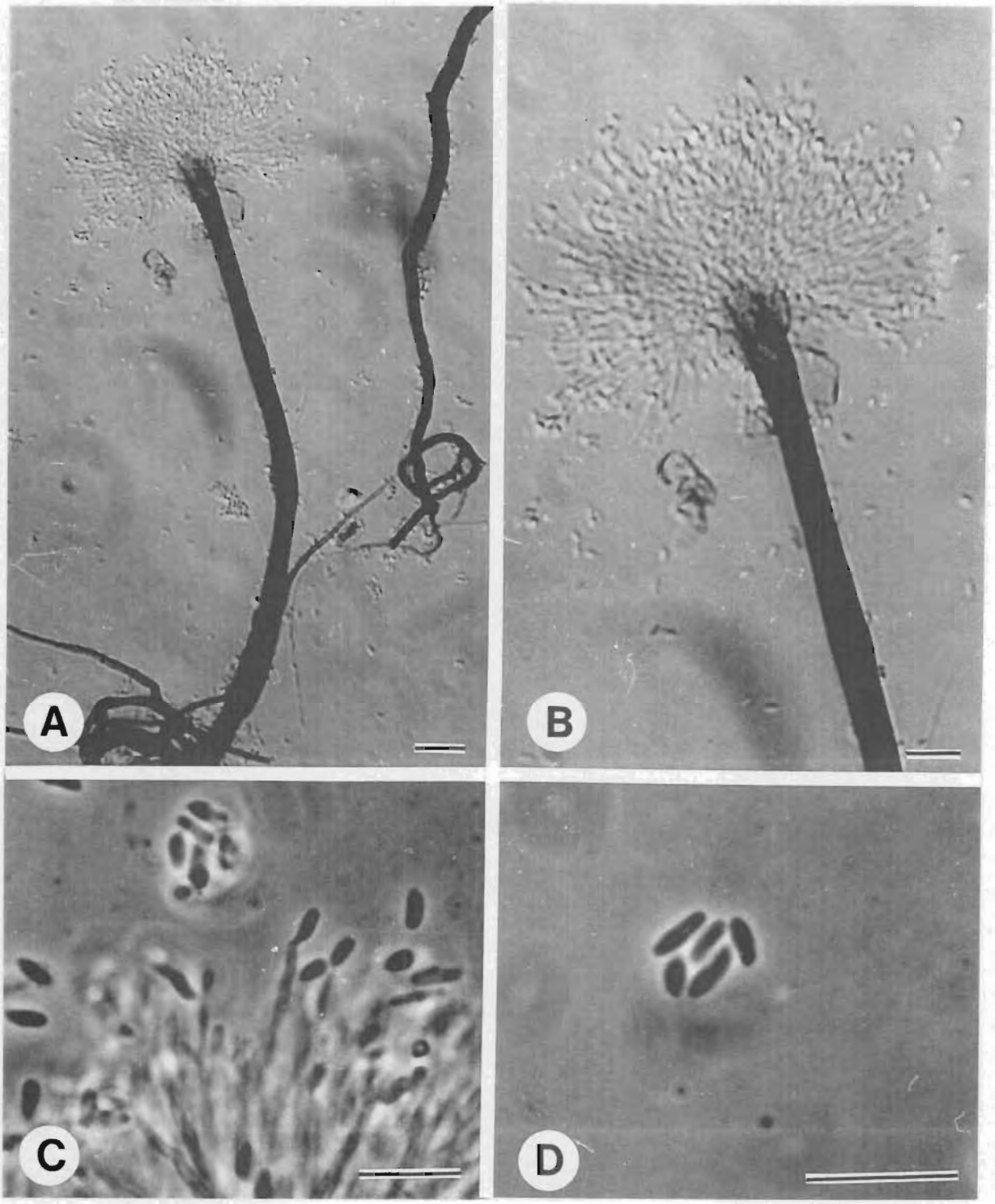

Fig. 97. Light micrographs of the conidiophores and conidia of *L. neomexicanum* (CMW 2079). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

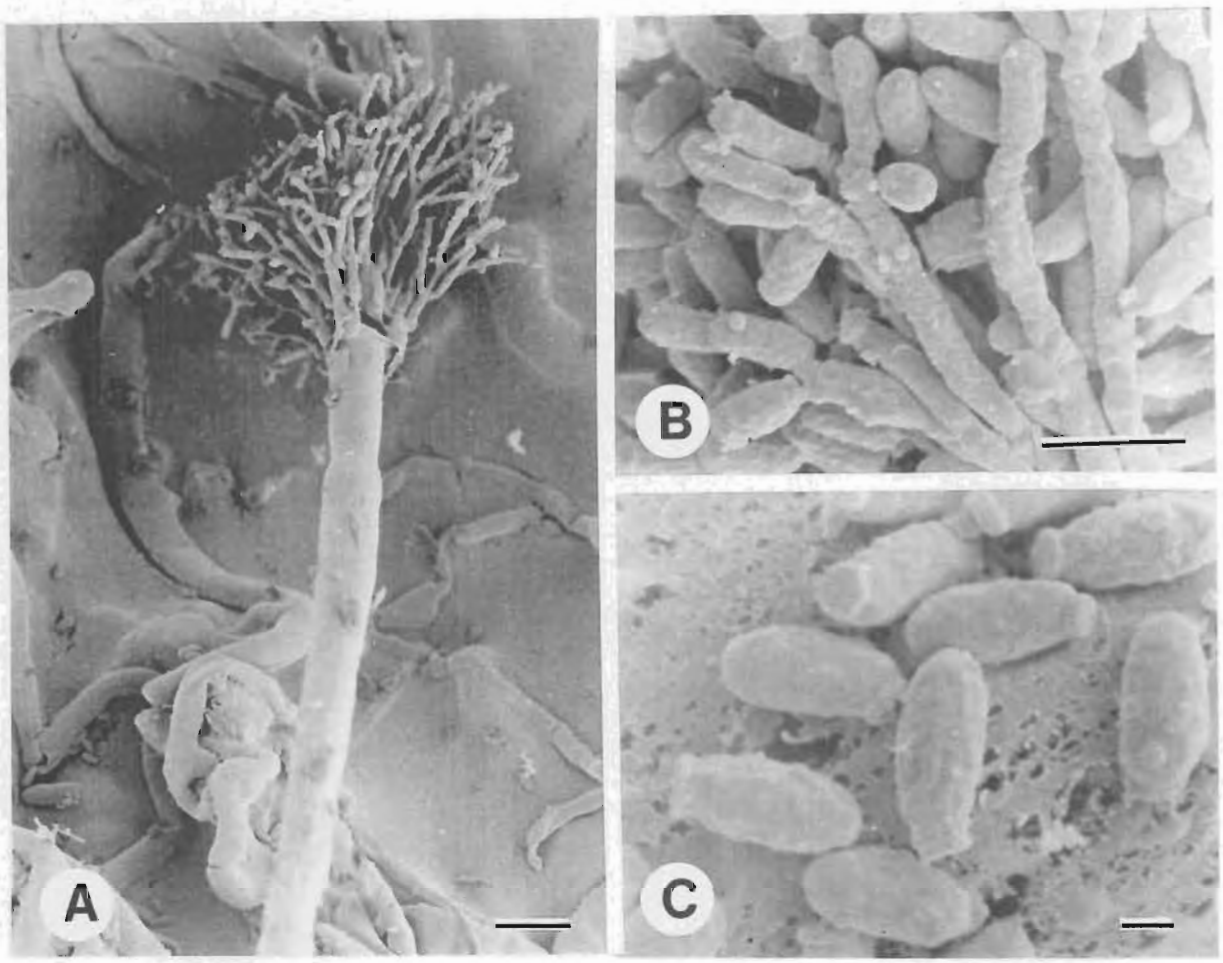

Fig. 98. Scanning electron micrographs of the conidiophores and conidia of *L. neomexicanum* (CMW 2079). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

28. *Ophiostoma penicillatum* (Grosmann) Siemaszko, *Planta Polonica* **7,** 24. 1939. **(Figs. 99 - 101).**

≡ *Grosmannia penicillata* (Grosmann) Goid., *Boll. Staz. Patol. Veg. Roma N.S.* **16**, 39. 1936.

≡ *Ceratostomella penicillata* Grosmann, *Hedwigia* **72**, 190. 1932.

≡ *Ceratocystis penicillata* (Grosmann) C. Moreau, *Rev. Mycol. Suppl. Col.* **17**, 22. 1952.

Anamorph: *Leptographium penicillatum* Grosmann, *Zeitschr. für Parasitenkunde* **3**, 94. 1930.

≡ *Scopularia penicillata* (Grosmann) Goid., *Boll. Staz. Patol. Veg. Roma N.S.* **16**, 39. 1936.

≡ *Verticicladiella penicillata* (Grosmann) W.B. Kendr. *Canadian Journal of Botany* **40**, 776. 1962.

Etymology: pe-ni-cil-lá-tum: possessing penicillia. This specific epithet refers to the penicillate conidiophores of this fungus.

Perithecial bases black, globose and smooth walled, unornamented, 250 - 300 µm in diameter. *Perithecial neck* dark brown to black, cylindrical with a slight apical taper, smooth, 300 - 500 µm long, 50) µm wide, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* allantoid, aseptate, hyaline, invested in a sheath, 6.5 x 2.3 µm (Grosmann, 1932).

Conidiophores occurring singly or in groups of up to eight, arising directly from the mycelium, erect, macronematous, mononematous, (130-) 258.5 - 336.5 (-460) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k) to olivaceous (21"m), smooth, cylindrical, simple, 1 - 10 septate, (75-) 199 -248.5 (-340) µm long, 5.0 - 10 µm wide below primary branches, apical cell not swollen, (5.0-) 6.5 - 10 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (35-) 51.5 - 87 (-110) µm long, excluding the conidial mass, with 2 to 5 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k) to olivaceous (21"m), smooth, cylindrical to barrel shape, 0-1 septate, (12-) 17 - 18.5 (-28) µm long and (5.0-) 5.5 - 8.5 (-11) µm

wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21"k), aseptate, (9.0-) 14 - 15 (-21) µm long, 3.0 - 7.0 µm wide, tertiary branches light olivaceous (21"k), aseptate, (8.0-) 11 - 14 (-18) µm long, 3.0 - 6.0 µm wide, quaternary branches hyaline, aseptate, (6.0-) 7.5 - 13 (-15) µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (10-) 12 -16.5(-25) µm long and 2.0 - 3.0 µm wide. *Conidia* hyaline, aseptate, allantoid with truncate ends and rounded apices, (4.0-) 6.5 - 7.5 (-10.5) x 2.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colour (19'f) with age. Conidial mass cream colour when wet, turning amber (21'b) to dark olive (21'"m) when dry.

Colonies with optimal growth at 30°C on 2% MEA, reaching 12 mm in diameter in 8 days. No growth below 15°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 81% reduction in growth on 0.5 g/l cycloheximide after 8 days at 25°C in the dark. Colonies olivaceous (21"m). *Colony margin* sinuate. *Hyphae* submerged on agar with no aerial mycelium, hyaline to light olivaceous (21"k), smooth, straight, occasionally clumped together, not constricted at the septa, 4.0 - 10 µm diam.

Specimens examined: Holotype: from the original strain of H. Grosmann, deposited at CBS, 1952, DAOM 29111 (note: I used the same type material as Kendrick (1962) to characterise this species). **Paratype:** Sweden, Oland, Boda, *Picea sp.*, July 1958, collected: A Mathiesen-Käärik, DAOM 63691. **Cultures**: Germany, Reinbeck, isolated from European spruce infested with bark beetles, CMW 453 (same as CBS 441.69 and RWD 783); Norway, Namskogen, Trondelag, *Picea abies* infested by *Ips typographus*, 1990, collected: H. Solheim, CMW 2644, CMW 2645; Sweden, *Picea abies,* 1958, collected: A. Käärik, CMW 2642; Norway, *Picea abies,* 1980, collected: H. Solheim, CMW 2644.

Known distribution: Europe, Japan.

Hosts/substrate: *Abies lasiocarpa* (Davidson, 1958), *Picea* sp. (Mathiesen, 1951;

Mathiesen-Käärik, 1960; Aoshima, 1965), *Picea abies* (Goidanich, 1936; Siemaszko, 1939; Solheim, 1986; 1992a, 1993), *Picea abies* (Grosmann, 1931; Kendrick, 1962; Goidanich, 1936), *Picea jezoensis* (Yamaoka *et al.*, 1997), *Pinus* sp. (Mathiesen, 1951; Mathiesen-Käärik, 1960; Aoshima, 1965), *Pinus contorta* (Mielke, 1979), *Pinus monticola* (Kulhavy *et al.*, 1978; Mielke, 1979), *Pinus strobus* (Morelet, 1986), *Pinus sylvestris* (Mielke, 1979; Morelet, 1986), *Pinus pinaster* (Morelet, 1986), *Pinus ponderosa* (Mielke, 1979).

Associated insects: *Dendroctonus rufipennis* (Perry, 1991), *Hylastes ater* (Mathiesen, 1950; Mathiesen-Käärik, 1953; Harrington, 1988), *Hylastes cunicularis* (Mathiesen-Käärik, 1953; Harrington, 1988), *Hylurgus ligniperda* (Mathiesen-Käärik, 1953; Harrington, 1988), *Hylurgops porosus* (Wagner, 1977), *Hylurgops palliatus* (Mathiesen, 1950; Mathiesen-Käärik, 1953; Harrington, 1988), *Dryocoetus confusus* (Davidson, 1958), *Ips typographus* f. *japonicus* (Yamaoka *et al.*, 1997), *Ips typographus* (Goidanich, 1936; Kendrick, 1962, Mathiesen, 1950; Grosmann, 1931; Rennerfelt, 1950; Mathiesen-Käärik, 1953; Solheim, 1986, 1992a; Harrington, 1988; Furniss *et al.*, 1990; Solheim, 1993b; Krokene, 1996; Krokene & Solheim, 1996; Viiri, 1997), *Ips duplicatus* (Valkama, 1995; Krokene, 1996; Krokene & Solheim, 1996), *Pityogenes chalcographus* (Goidanich, 1936; Mathiesen, 1950; Grosmann, 1931; Mathiesen-Käärik, 1953), *Pityogenes quadridens* (Harrington, 1988), *Polygraphus poligraphus* (Krokene, 1996; Krokene & Solheim, 1996), *Tetropium* sp (Mathiesen, 1950; Mathiesen-Käärik, 1953), *Trypodendron lineatum* (Harrington, 1988).

Notes: *Ophiostoma penicillatum* can easily be distinguished from other *Ophiostoma* spp. based on its distinct anamorph structure. *Leptographium penicillatum* is characterized by large allantoid conidia, unike the conidia of any other *Leptographium* spp. Davidson *et al.* (1967) re-examined this species and concluded that the large allantoid conidia are an important diagnostic character. Solheim (1986) established a neotype with allantoid ascospores for this species in contrast to the occasional hat-shaped ascospores described by other authors (Hunt, 1956). This replaced the original type that has been lost during the war (Solheim, 1986).

Griffin (1968) and Hunt (1956) considered *L. penicillatum* to be similar to *L. piceaperdum* and *L. serpens*. *Leptographium piceaperdum* and *L. serpens* are distinguished from *L. penicillatum* based on their obovoid conidia, in contrast to the allantoid conidia of *O. penicillatum* (Davidson, Francke-Grosmann & Käärik, 1967; Horntvedt *et al.*, 1983). Mathiesen (1950) described four *formae specialis* for this species namely *f.sp. typica, f.sp. chalcographi, f.sp. palliati, f.sp. pini*. Mathiesen-Käärik (1960) found this fungus to be common in Sweden on spruce as well as pine. She found that *L. penicillatum* was highly variable in teleomorph and anamorph characters. These isolates most probably represented more than one taxon (Jacobs *et al.* 1999).

Ophiostoma penicillatum is predominantly a European species and is commonly found on *Picea* spp. and *Pinus* spp. (Grosmann, 1931; Goidanich, 1936; Siemaszko, 1939; Mathiesen, 1951; Mathiesen-Käärik, 1960; Kendrick, 1962; Aoshima, 1965; Kulhavy *et al.*, 1978; Mielke, 1979; Morelet, 1986; Solheim, 1986; 1992a, 1993; Yamaoka *et al.*, 1997). This species is found in association with blue-stain (Grosmann, 1931; Siemaszko, 1939; Solheim, 1986; 1992a, 1993; Yamaoka *et al.*, 1997). Horntvedt *et al.* (1983) found that *O. penicillatum,* together with *C. polonica,* can kill healthy Norway spruce trees and stain the sapwood. *Ophiostoma penicillatum,* on the other hand, is not capable of primary infection of healthy trees, or blue-stain of timber (Mielke, 1981). Solheim (1992a,b) considered this species a secondary invader of Norway spruce following invasion of the sapwood by *Ceratocystis polonica* after attack by *Ips typographus.*

Ophiostoma penicillatum is has been recorded to be associated with various insects (Grosmann, 1931; Goidanich, 1936; Mathiesen, 1950; Mathiesen-Käärik, 1953; Davidson, 1958; Wagner, 1977; Harrington, 1988; Perry, 1991; Krokene, 1996; Krokene & Solheim, 1996), but is most commonly found in association with *Ips* spp. and especially *I. typographus* (Goidanich, 1936; Kendrick, 1962; Mathiesen, 1950; Grosmann, 1931; Rennerfelt, 1950; Mathiesen-Käärik, 1953; Solheim, 1986, 1992a; Harrington, 1988; Furniss *et al.*, 1990; Solheim, 1993b; Valkama, 1995; Krokene, 1996; Krokene & Solheim, 1996; Yamaoka *et al.*, 1997; Viiri, 1997).

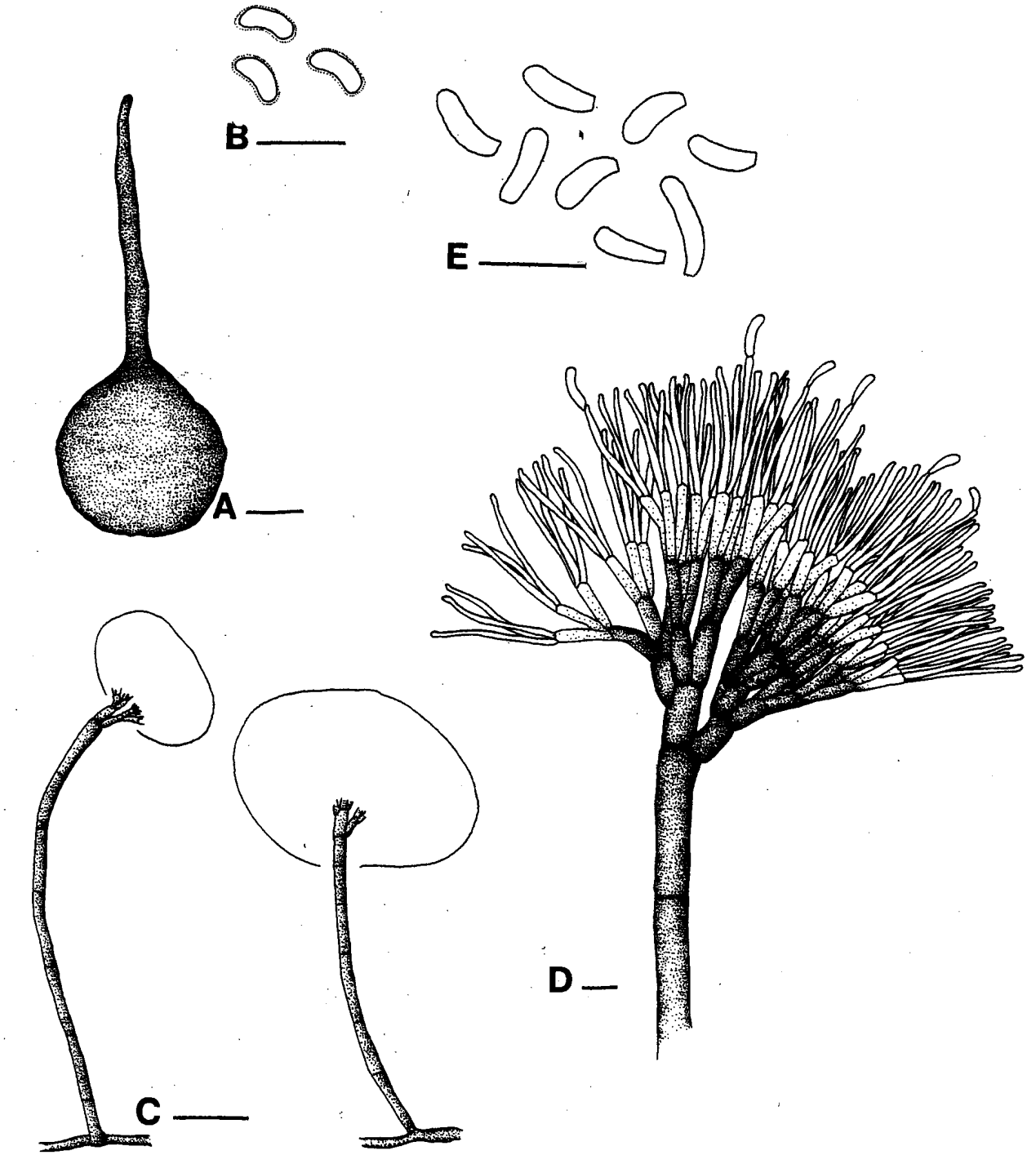

Fig. 99. Teleomorph and anamorph structures of *O. penicillatum* (CMW 453). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

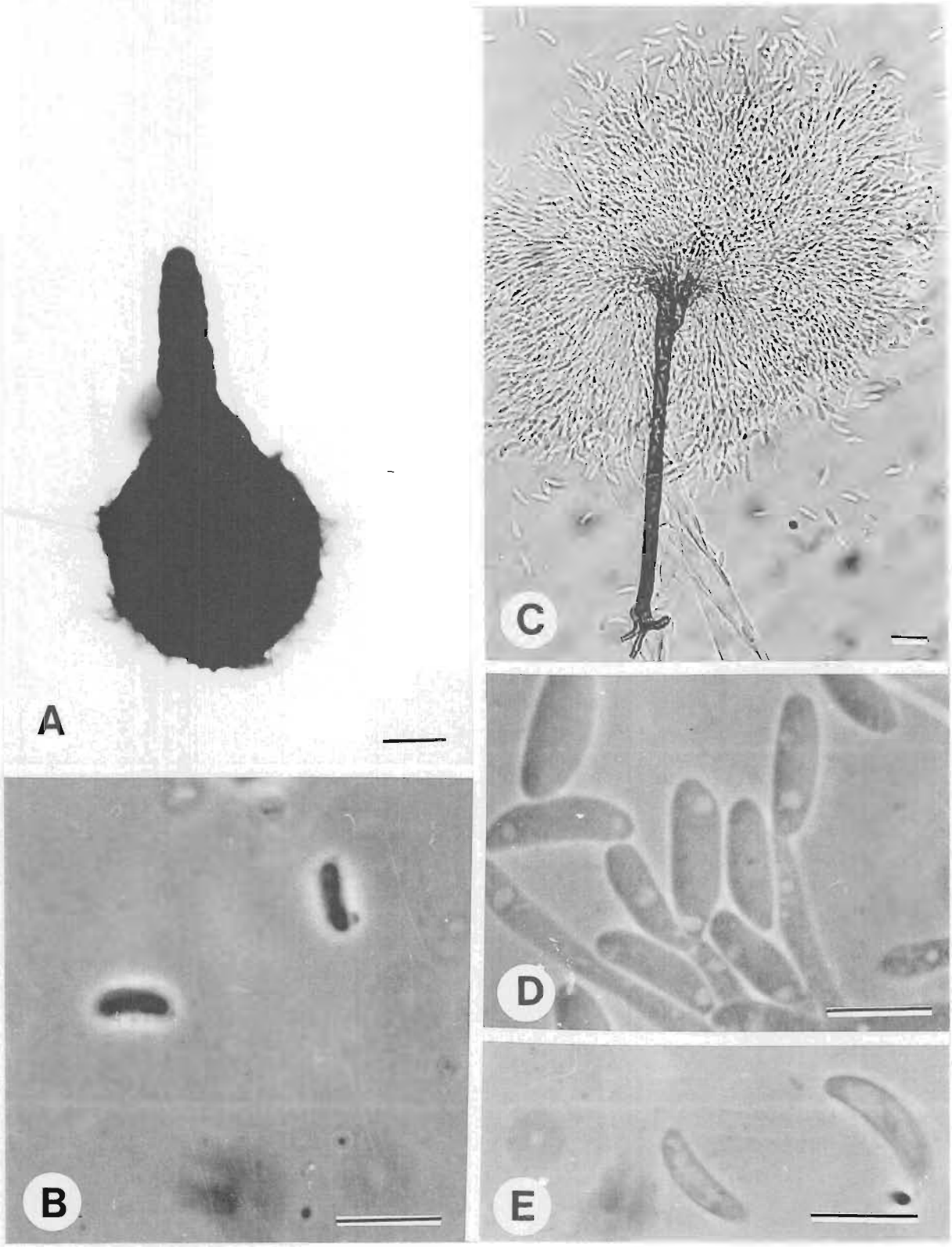

Fig. 100. Light micrographs of the teleomorph and anamorph structures of *O. penicillatum* (CMW 453). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 10 µm). **D.** Conidiogenous cells (Bar = 5 µm). **E.** Conidia (Bar = 5 µm).

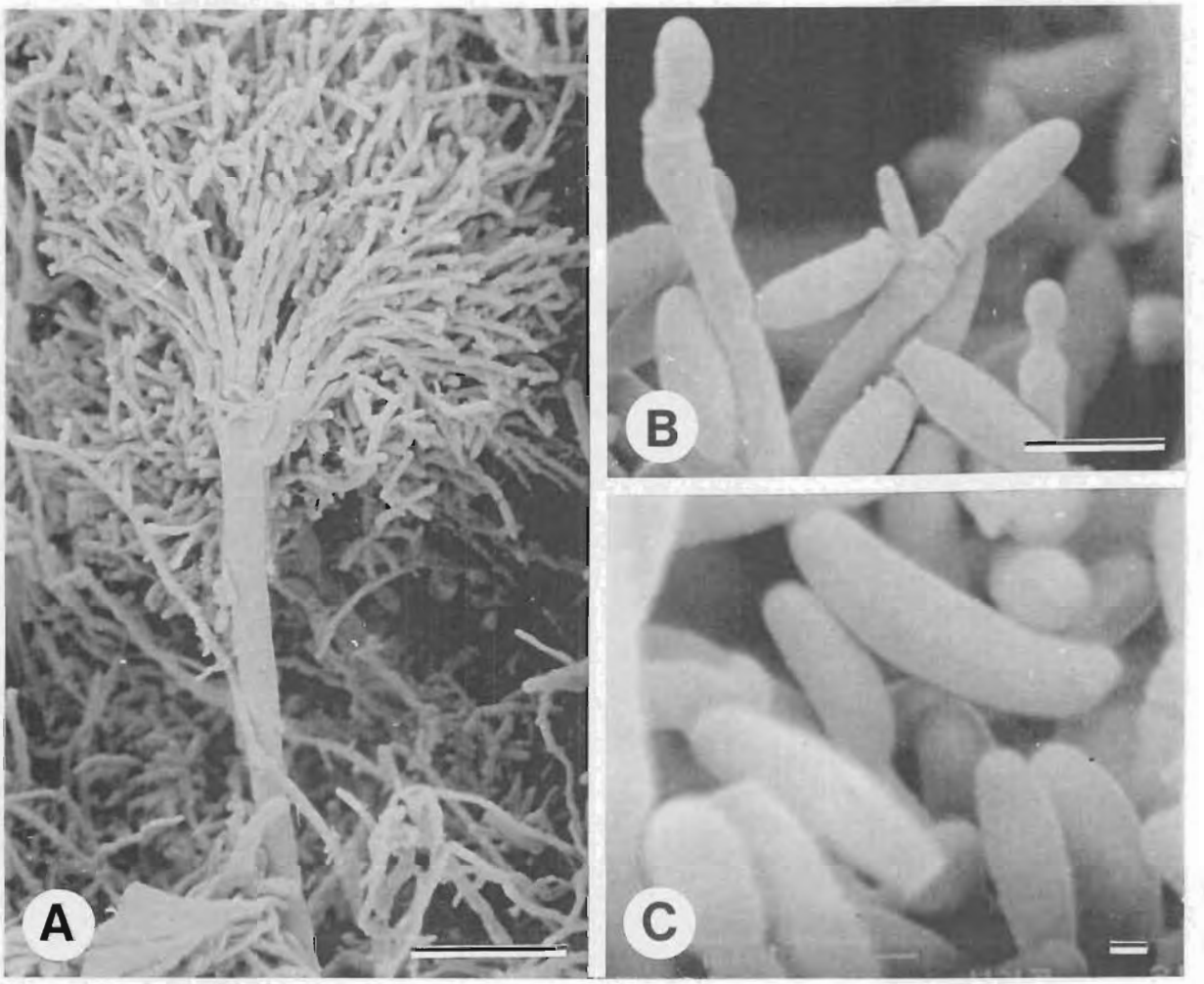

Fig. 101. Scanning electron micrographs of the conidiophores and conidia of *O. penicillatum* (CMW 453). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

29. *Leptographium peucophilum* K. Jacobs & M.J. Wingf., ***Mycological Research*** 1999. **(Figs. 102-104).**

Teleomorph state: Not known.

Etymology: peu-co-phí-lum: spruce-loving. From the Greek noun πευκε: spruce and Greek adjective φιλος: loving. This species refers to *Picea,* which is the host of this fungus.

Conidiophores occurring singly or in groups of two, arising directly from the mycelium, erect, macronematous, mononematous, (230-) 310 - 352 (-520) µm in length, rhizoid-like structures present. *Stipes* dark olivaceous, smooth, cylindrical, simple, 3-7 septate, (170-) 269.5 - 255.5 (-420) µm long (from first basal septum to below primary branches), 3.0 - 8.0 µm wide below primary branches, apical cell not swollen; 4.5 - 11 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (40-) 39.5 - 96.5 (-120) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches; 2 - 3 primary branches, arrangement of the primary branches - type B, olivaceous, smooth, cylindrical, aseptate, 9.0 - 25 µm long and (3.0-) 4.0 - 6.5 (-8.0) µm wide, secondary branches light olivaceous to hyaline, aseptate, (7.0-) 8.0 - 16.5 (-17) µm long, 2.0 - 5.0 µm wide; tertiary branches hyaline, aseptate, (7.0-) 8.0 - 13.5 (-15) µm long, 2.0 - 4.0 µm wide, quaternary branches aseptate, hyaline, (7.0-) 8.0 - 10.5 (-13) µm long, 2.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (8.0-) 9.0 - 17.5 (-20) µm long and 2.0 µm wide. *Conidia*, hyaline, aseptate, obovoid, (3.0-) 3.5 - 4.5 (-6.0) x 2.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

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. Colonies 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.

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

Known distribution: Western North America.

Hosts/substrate: *Picea rubra* (Jacobs *et al.*, 1999).

Associated insects: *Korscheltellus gracilus* (Jacobs *et al.*, 1999).

Notes: *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 than those of *L. peucophilum*.

Leptographium peucophilum has both been isolated from the roots of its host tree (*Picea rubra*), and is associated with the feeding activities of larvae of the conifer swift moth. This makes it similar to *L. abicolens*. In addition, these species have been isolated from high elevation sites, which is consistent with their low optimal temperatures for growth in culture. The larval stage of this moth feeds on the roots of *P. rubra* and the fungi appear to enter through the wounds caused by this feeding. 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.

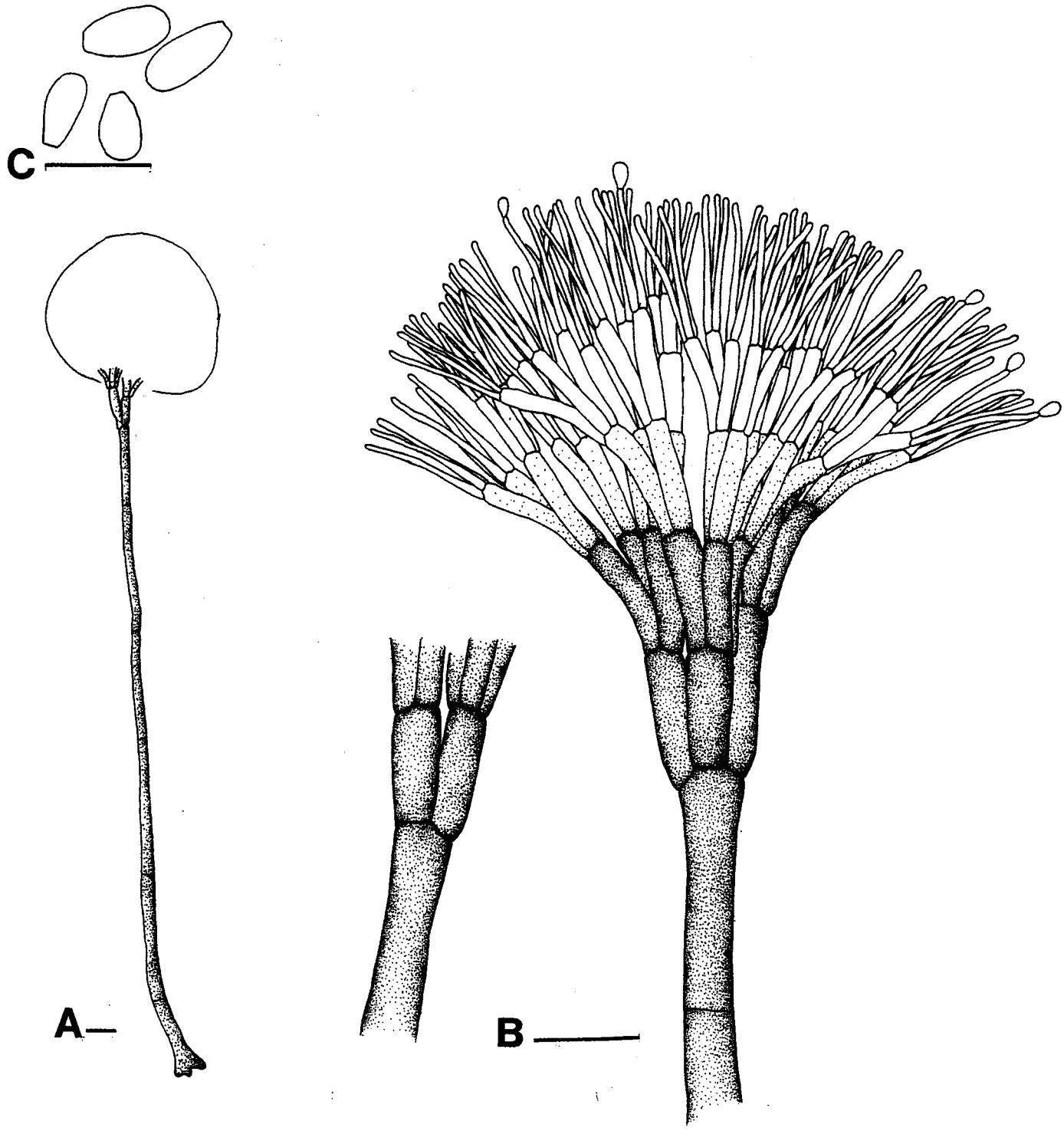

Fig. 102. Conidiophores and conidia of *L. peucophilum* (CMW 2876). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

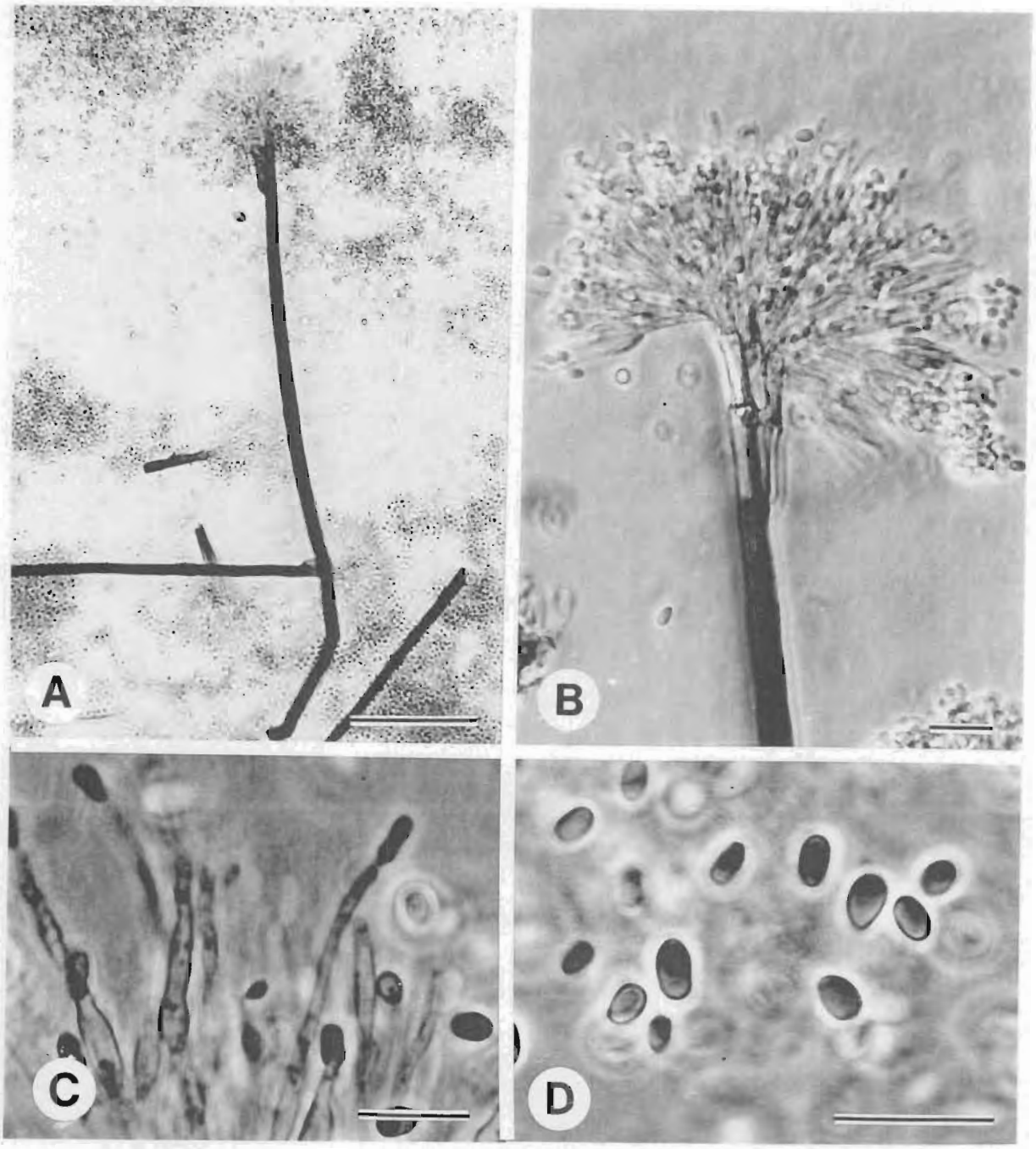

Fig. 103. Light micrographs of the conidiophores and conidia of *L. peucophilum* (CMW 2876). **A.** Conidiophore (Bar = 50 µm). **A.** Conidiogenous apparatus (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

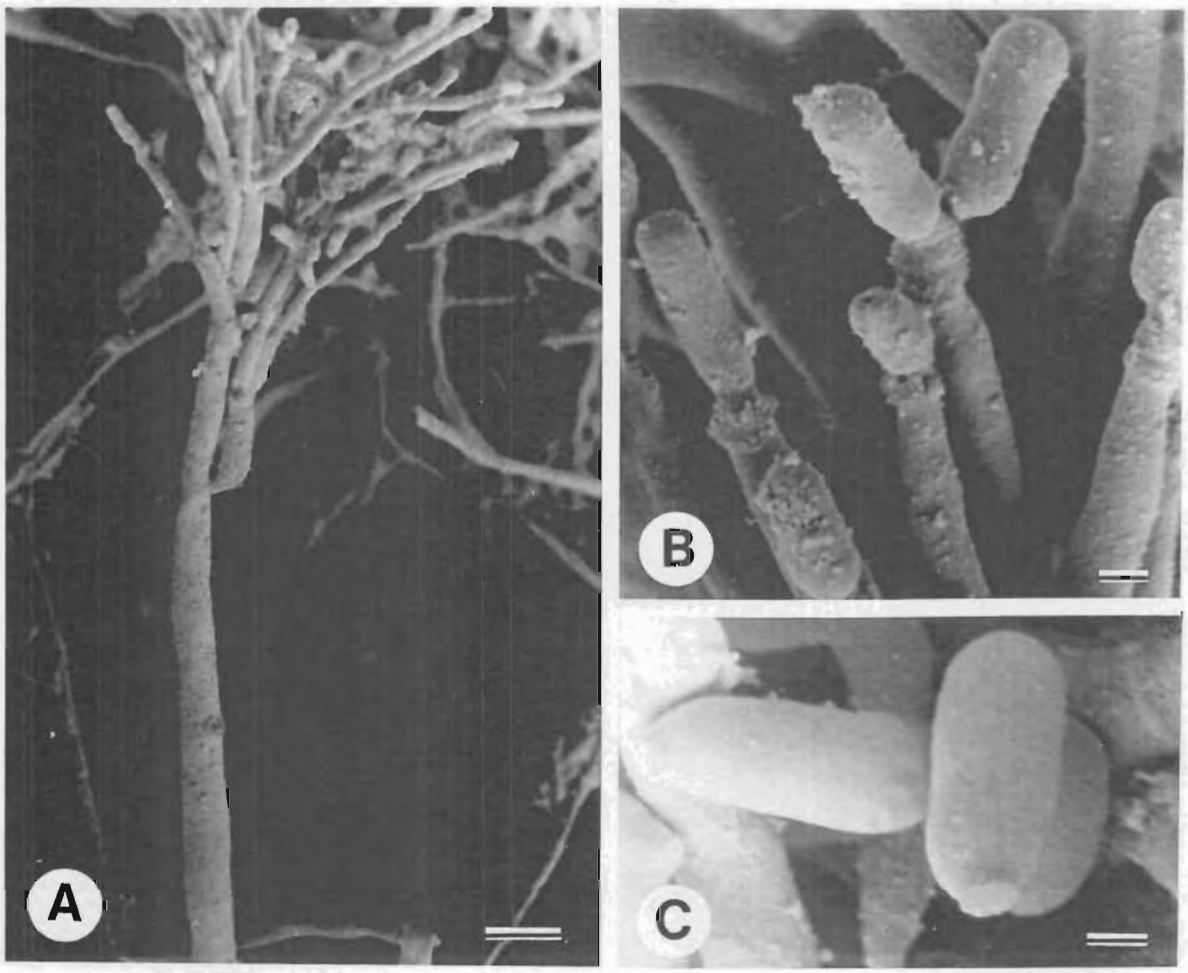

Fig. 104. Scanning electron micrographs of the conidiophores and conidia of *L. peucophilum* (CMW 2876). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 1 µm). **C.** Conidia (Bar = 1 µm).

30. *Ophiostoma piceaperdum* (Rumbold) Arx, *Antonie van Leeuwenhoek* **18**, 211. 1952. **(Figs. 105-107).**

≡*Ceratostomella piceaperda* Rumbold, *Journal of Agricultural Research.* **52**, 436. 1936.

= *Ophiostoma europhioides* (E.F. Wright & Cain) H. Solheim, *Nordic Journal of Botany* **6**, 203. 1986.

≡ *Ceratocystis europhioides* E.F. Wright & Cain, *Canadian Journal of Botany* **39**, 1222. 1961.

= *Ceratocystis pseudoeurophioides* Olchow. & J. Reid, *Canadian Journal of Botany* **52**, 1700. 1974.

Anamorph: *Leptographium piceaperdum* K. Jacobs, M.J. Wingf. & Crous *Mycological Research*. 1999.

Etymology: pi-ce-a-pér-dum: destroying the spruce. This specific epithet refers to the association of this fungus with the bark beetle, *Dendroctonus piceaperda* on spruce.

Perithecial bases black, globose and smooth walled, little ornamentation, (170-) 199 - 312 (-370) µm in diam. *Perithecial neck* dark brown to black, cylindrical with a slight apical taper, smooth, (280-) 503 - 603 (-850) µm long, (30-) 32 - 60 µm above globose base, 20 - 30 µm wide at the apex, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* cucullate in side view, aseptate, hyaline, invested in a sheath, (3.0-) 4.5 - 5.0 (-5.5) x 2.0 - 3.0 µm.

Conidiophores occurring singly or in groups of 2 to 7, arising directly from the mycelium with smaller conidiophores on aerial mycelium, erect, macronematous, mononematous, (140-) 200.5 - 207.5 (-300) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), smooth, cylindrical, simple, 3-8 septate, (70-) 117.5 - 124.5 (-195) µm long, 5.0 - 9.0 µm wide below primary branches, apical cell not swollen, 6.0 - 12.5 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* 55 - 120 µm long, excluding the conidial mass, with 2 to 5 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical, 0-1 septate (15.5-) 18.5 - 23 (-39) µm long and 3.0 - 8.0 µm wide,

arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21"k), aseptate, (11-) 14 - 18.5 (-23) µm long, (2.0-) 3.0 - 5.0 (-6.0) µm wide, tertiary branches light olivaceous (21"k), aseptate, (9.0-) 12.5 - 17 (-22) µm long, (2.0-) 2.5 - 4.0 (-5.0) µm wide, quaternary branches, light olivaceous (21"k), aseptate, (7.0-) 9.5 - 13.5 (-16) µm long, 2.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (11-) 14 - 21 (-26) µm long and 1.5 - 3.0 µm wide. *Conidia* light gray olivaceous (19""), aseptate, obovoid with truncate ends and rounded apices, (3.0-) 4.5 - 5.5 (-9.0) x 1.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 34 mm in diam. in 8 days. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.5 g/l cycloheximide after 4 days at 25°C in the dark. Colonies dark olive (21"'m). *Colony margin* smooth. *Hyphae* submerged on agar with little aerial mycelium, hyaline to light olivaceous (21"k), smooth, occasionally roughened by granular deposits, straight, not constricted at the septa, 1.5 - 6.0 µm diam.

Specimens examined: Holotype: Canada, St. Peters, Cape Breton, Nova Scotia, *Picea glauca*, June 1930, collected: C.T. Rumbold, BPI 595981. **Paratypes**: Canada, Nova Scotia, St. Peters, Cape Breton, *Picea glauca*, June 1930, collected: R.E. Balch, BPI 595982, BPI 595980; Canada, Ontario, Shabotik River, Algoma district *Picea mariana*, 20 June 1961, collected: R.F. Cain, TRTC 45762; Canada, Ontario, Sudbury district Challenen Lake, *Picea mariana*, 20 June 1961, collected: R.F. Cain, J. Reid and W. Obust, TRTC 36263; Canada, Ontario, Shabotik River, Algoma district *Picea mariana*, 20 June 1961, collected: R.F. Cain, MFB 7439; Canada, Manitoba, Sandilands Forest Reserve, *Picea mariana*, 20 June 1961, collected: A. Olchowecki, WIN(M) 71-18. **Cultures**: *Picea abies*, collected: A.M. Hallaksela, CMW 660 (same as CBS 366.75), U.S.A., Nippletop Mountain, *Picea rubens*, 1987, collected: T.C. Harrington, C274 (same as CMW 2811), collected: E.F.

Wright, CMW 479 (same as CBS 444-69), Austria, Nasswald, *Picea abies*, 1993, collected: T. Kirisits, 1993, CMW 3314.

Known distribution: Europe, Canada, Japan.

Hosts/substrate: *Picea abies* (Solheim, 1986, 1992a; 1993), *Picea glauca* (Rumbold, 1936), *Picea mariana* (Wright & Cain, 1961), *Picea jezoensis* (Yamaoka *et al.*, 1997), *Pinus glauca* (Wright & Cain, 1961), *Pinus nigra* (Hutchison & Reid, 1988), *Pinus radiata* (Hutchison & Reid, 1988), *Pinus resinosa* (Wright & Cain, 1961; Griffin, 1968), *Pinus strobus* (Wright & Cain, 1961; Griffin, 1968), *Pinus sylvestris* (Wright & Cain, 1961; Griffin, 1968), *Pinus taeda* (Hutchison & Reid, 1988), *Pinus banksiana* (Olchowecki & Reid, 1974), *Pseudotsuga menziesii* (Davidson & Robinson-Jeffrey, 1965).

Associated insects: *Dendroctonus ponderosae* (Perry, 1991), *Dendroctonus rufipennis* (Harrington, 1988; Perry, 1991), *Dendroctonus pseudostugae* (Solheim & Krokene, 1998); *Dendroctonus valens* (Perry, 1991), *Dryocoetus* sp. (Davidson & Robinson-Jeffrey, 1965; Harrington, 1988), *Hylurgops palliatus* (Harrington, 1988; Krokene & Solheim, 1996), *Ips typographus* f. *japonicus* (Yamaoka *et al.*, 1997), *Ips typographus* (Solheim, 1986, 1992a, 1993; Harding, 1995; Harrington, 1988; Viiri, 1997), *Ips duplicatus* (Krokene, 1996; Krokene & Solheim, 1996), *Pityogenes chalcographus* (Harrington, 1988), *Polygraphus poligraphus* (Krokene, 1996; Krokene & Solheim, 1996).

Notes: Griffin (1968) proposed that *O. europhioides* (= *O. piceaperdum*) might be a possible synonym of *O. penicillatum,* a suggestion also made by Wright and Cain (1961). These two species, however, show significant differences in their ascospore shapes. *Ophiostoma piceaperdum* is characterized by cucullate ascospores, whereas *O. penicillatum* has allantoid ascospores (Grosmann, 1932; Rumbold, 1936). Robinson-Jeffrey and Grinchenko (1964) distinguished between *O. piceaperdum* and *O. huntii* based on differences in their teleomorphs. *Ophiostoma piceaperdum* has ascospores that are almost twice as large as those of *O. huntii.* The perithecia of *O. piceaperdum* is also smaller than those of *O. huntii* (Robinson-

Jeffrey & Grinchenko, 1964). Although the anamorphs of these two species are morphologically very similar, they can be distinguished based on a few differences. *Ophiostoma piceaperdum* is characterized by hyphae with a serpentine growth pattern. This character is not observed in isolates of *O. huntii.* These species can also be distinguished based on the homothallic nature of *O. piceaperdum,* in contrast to the strict heterothallic mating system of *O. huntii* (Jacobs *et al.,* 1998).

Ophiostoma piceaperdum also closely resembles *O. aenigmaticum.* These species can be separated based on the elongated brims of the ascospores of *O. aenigmaticum.* These long brims are not found in *O. piceaperdum. Ophiostoma piceaperdum* and *O. trinacriforme* were also resemble each other, but can be distinguished based on the larger perithecia of *O. trinacriforme* (Wright & Cain, 1961). Solheim (1986) commented on the possible synonymy of *O. europhioides* and *O. piceaperdum.* As he had not seen material of the latter species, he did not make any conclusions regarding their synonymy. Harrington (1988) also suggested that these species may be the same species. Hutchison and Reid (1988) treated *O. piceaperdum* and *O. europhioides* as synonyms in their survey of ophiostomatoid fungi from New Zealand. Jacobs *et al.* (1999) formally synonomised *O. europhioides* with *O. piceaperdum* and provided a formal name for the anamorph of this species in *Leptographium.*

Olchowecki and Reid (1974) described *O. pseudoeurophioides* which is similar to *O. europhioides.* They distinguished between the species based on their different anamorph states, *Verticicladiella* in the case of *O. pseudoeurophioides,* and *Leptographium* in the case of *O. europhioides.* Upadhyay (1981) treated this fungus as a synonym of *O. penicillatum.* These two species do, however, have distinctly different ascospore shapes. *Ophiostoma pseudoeurophioides* and *O. europhioides,* however, became indistinguishable when Wingfield (1985) synonomised *Verticicladiella* and *Leptographium*, eliminating the only difference between *O. europhioides* and *O. pseudoeurophioides.* Harrington (1988) treated *O. europhioides* and *O. pseudoeurophioides* as synonyms. *Ophiostoma pseudoeurophioides* was formally reduced to synonymy with *O. piceaperdum* by

Jacobs *et al.* (1998). *Ophiostoma shikotsuensis,* invalidly described by Aoshima (1965), is most probably the same as *O. piceaperdum* (Yamaoka *et al.,* 1997).

Ophiostoma piceaperdum is associated with staining of the sapwood of conifers (Rumbold, 1936). Solheim (1992a) considered this species to be a tertiary invader of Norway spruce. It was also found to be highly pathogenic to Norway spruce and could replace *Ceratocystis polonicum* in attacks on healthy trees (Harding, 1995). Krokene & Solheim (1995), however, found that this species was not very pathogenic. *Ophiostoma piceaperdum* is associated with several species of insects (Davidson & Robinson-Jeffrey, 1965; Solheim, 1986, 1992a, 1993; Perry, 1991; Harding, 1995; Harrington, 1988; Krokene, 1996; Krokene & Solheim, 1996; Viiri, 1997; Yamaoka *et al.*, 1997). However, its occurrence and association with *Ips typographus* is the best documented (Solheim, 1986, 1992a, 1993; Harrington, 1988; Harding, 1995; Viiri, 1997).

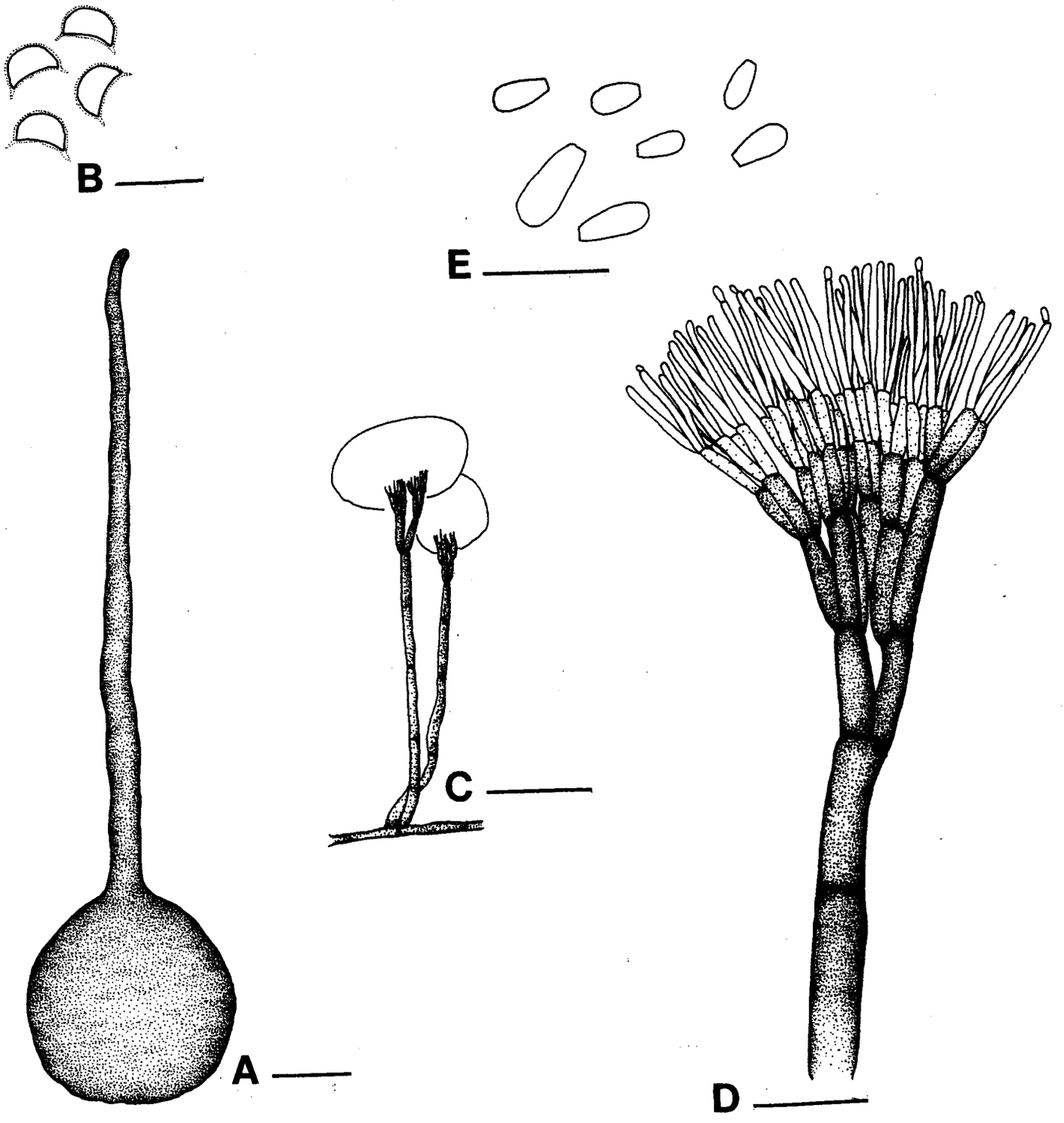

Fig. 105. Teleomorph and anamorph structures of *O. piceaperdum* (CMW 2811). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 50 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

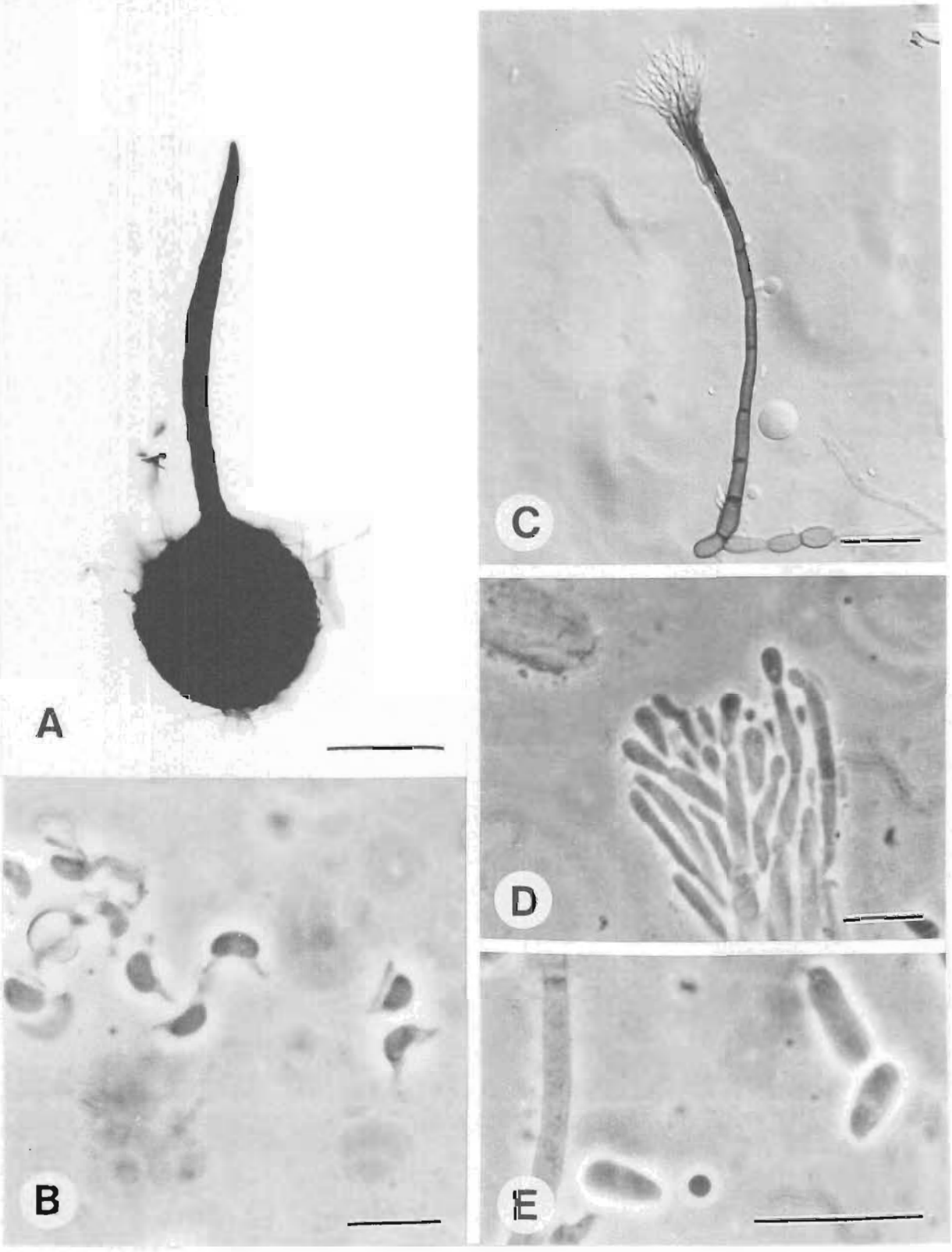

Fig. 106. Light micrographs of the teleomorph and anamorph structures of *O. piceaperdum* (CMW 2811). **A.** Perithecium (Bar = 100 µm). **B.** Ascospore (Bar = 10 µm). **C.** Conidiophore (Bar = 50 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

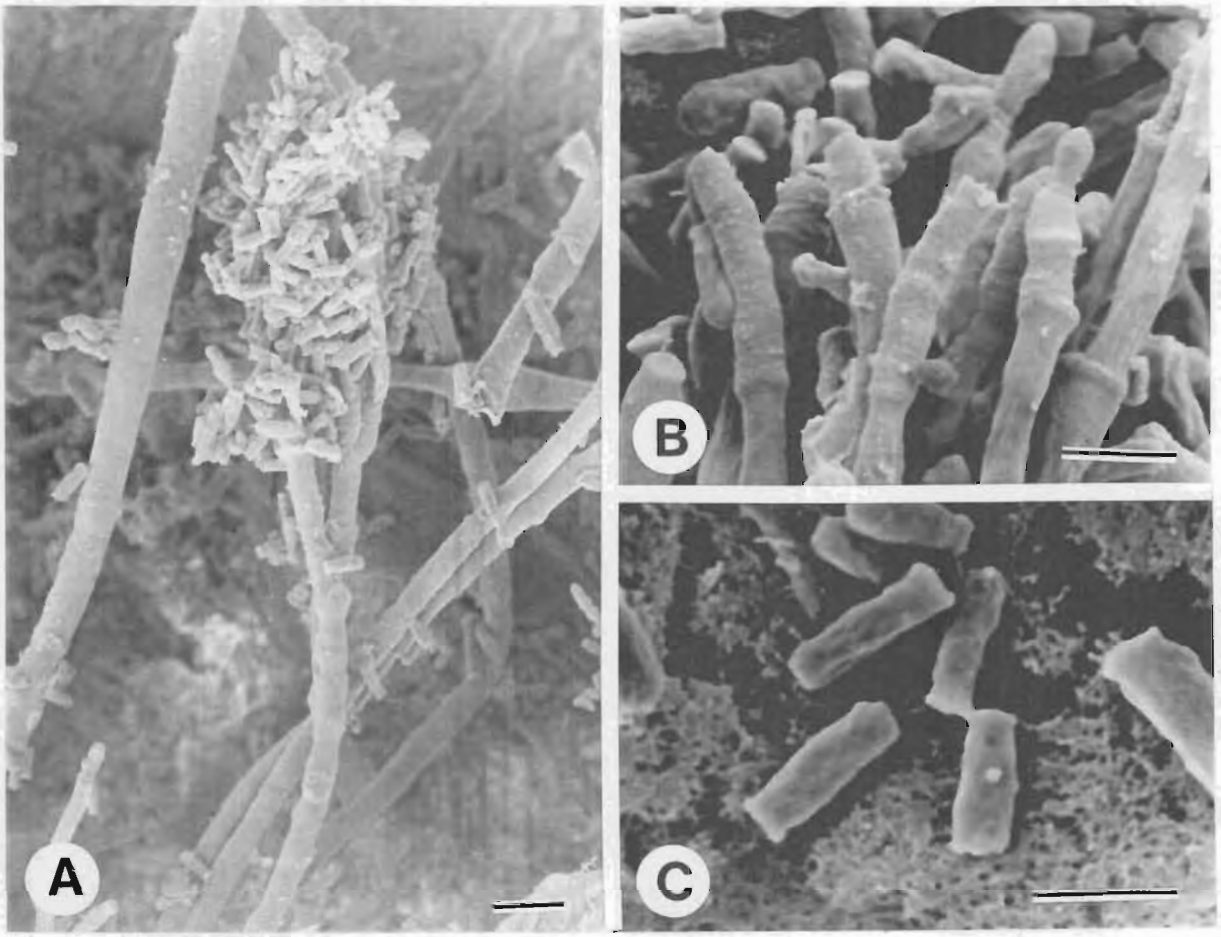

Fig. 107. Scanning electron micrographs of the conidiophores and conidia of *O. piceaperdum* (CMW 2811). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 5 µm).

31. *Leptographium pinidensiflorae* Masuya & M.J. Wingf., *Mycological Research*, 1999. **(Figs. 108-110).**

Teleomorph: Not known.

Etymology: pi-ni-den-si-fló-rae: of *Pinus densiflora*. This specific epithet refers to *Pinus densiflora* which is the host of this fungus.

Conidiophores occurring singly or occasionally in groups, arising directly from the mycelium, erect, macronematous, mononematous, 54 - 170 µm in length, rhizoid-like structures present. *Stipes* olivaceous, smooth, cylindrical, simple, 1-6 septate, 32 - 190 µm long, 5 - 7.5 µm wide below primary branches, apical cell not swollen, 3.0 - 10.5 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* 22 - 80 µm long, excluding the conidial mass, with 3 to 5 series of cylindrical branches, 2 - 4 primary branches, light olivaceous to hyaline, smooth, cylindrical, aseptate, 6.0 - 24 µm long and 2.0 - 6.5 µm wide, arrangement of the primary branches on the stipe - type B. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 5.5 - 16 µm long and 1.0 - 2.5 µm wide. *Conidia* aseptate, oblong to ellipsoid, 2.5 - 13 x 1.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Colonies with optimal growth at 20°C on 2% MEA, reaching 36 mm in diameter in 6 days. No growth below 5°C or above 35°C Able to withstand high concentrations of cycloheximide with a 65% reduction in growth on 5.0 g/l cycloheximide after 6 days at 20°C in the dark. Colonies hyaline to light olivaceous (19"f). *Colony margin* smooth. *Hyphae* submerged or on top of agar with abundant aerial mycelium, light olivaceous to hyaline, smooth, not constricted at the septa, 1.5- 12 µm diameter.

Specimens examined: Holotype: Japan, Tsukuba, Ibaraki, *Pinus densiflora* infested with *Tomicus piniperda,* 27 May 1996, collected: H. Masuya, MCC 071. **Paratypes:** Japan, Ichinoseki, Iwate, *P. densiflora* infested with *T. piniperda,* collected: H. Masuya, 19 June 1996, (MCC 193), Japan, Masuho, Yamanashi, *P. densiflora* infested with *T. minor,* collected: H. Masuya, 15 May 1996, (MCC194),

Japan, Metgi, Saitama, *P. densiflora* infested with *Xyleborus validus,* collected: H. Masuya, 14 July 1996, (MCC205). **Cultures:** Japan, *Pinus densiflora,* 1996, collected: H. Masuya, CMW 5157 (PREM 56393), CMW 5158, CMW 5159 (PREM 56394), CMW 5160, CMW 5161, CMW 5162.

Known distribution: Japan

Hosts/substrate: *Pinus densiflora* (Masuya *et al.,* 1999).

Associated insects: *Tomicus piniperda, T. minor, Xyleborus validus* (Masuya *et al.,* 1999).

Notes: This fungus was found to resemble *L. lundbergii,* but could be distinguished based on different conidial shapes and optimal growth temperature. This species also showed no pathogenicity to *Pinus densiflora* seedlings. It is speculated to be accidental associate of *T. piniperda* (Masuya *et al.,* 1999).

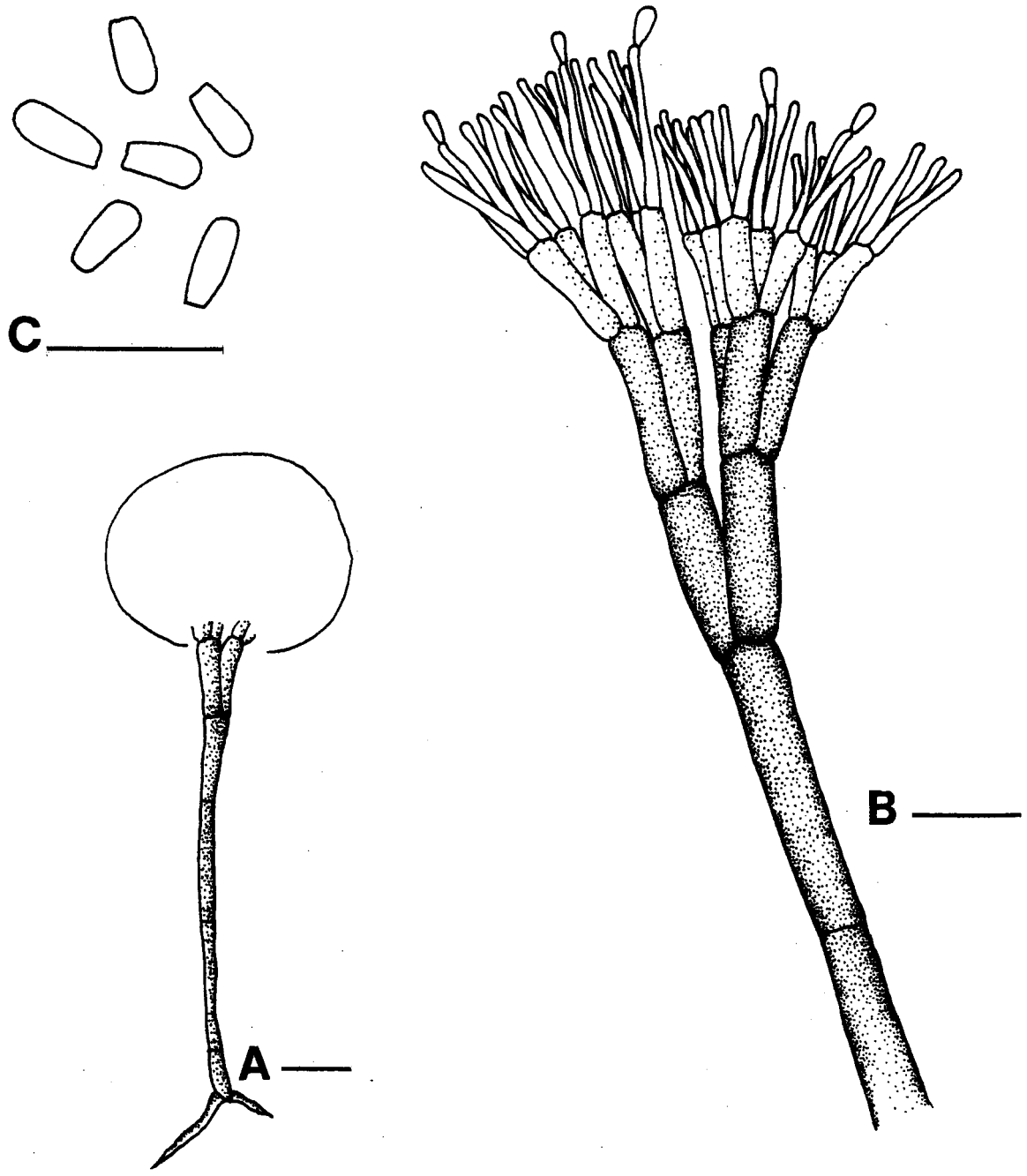

Fig. 108. Conidiophores and conidia of *L. pinidensiflorae* (CMW 5157). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

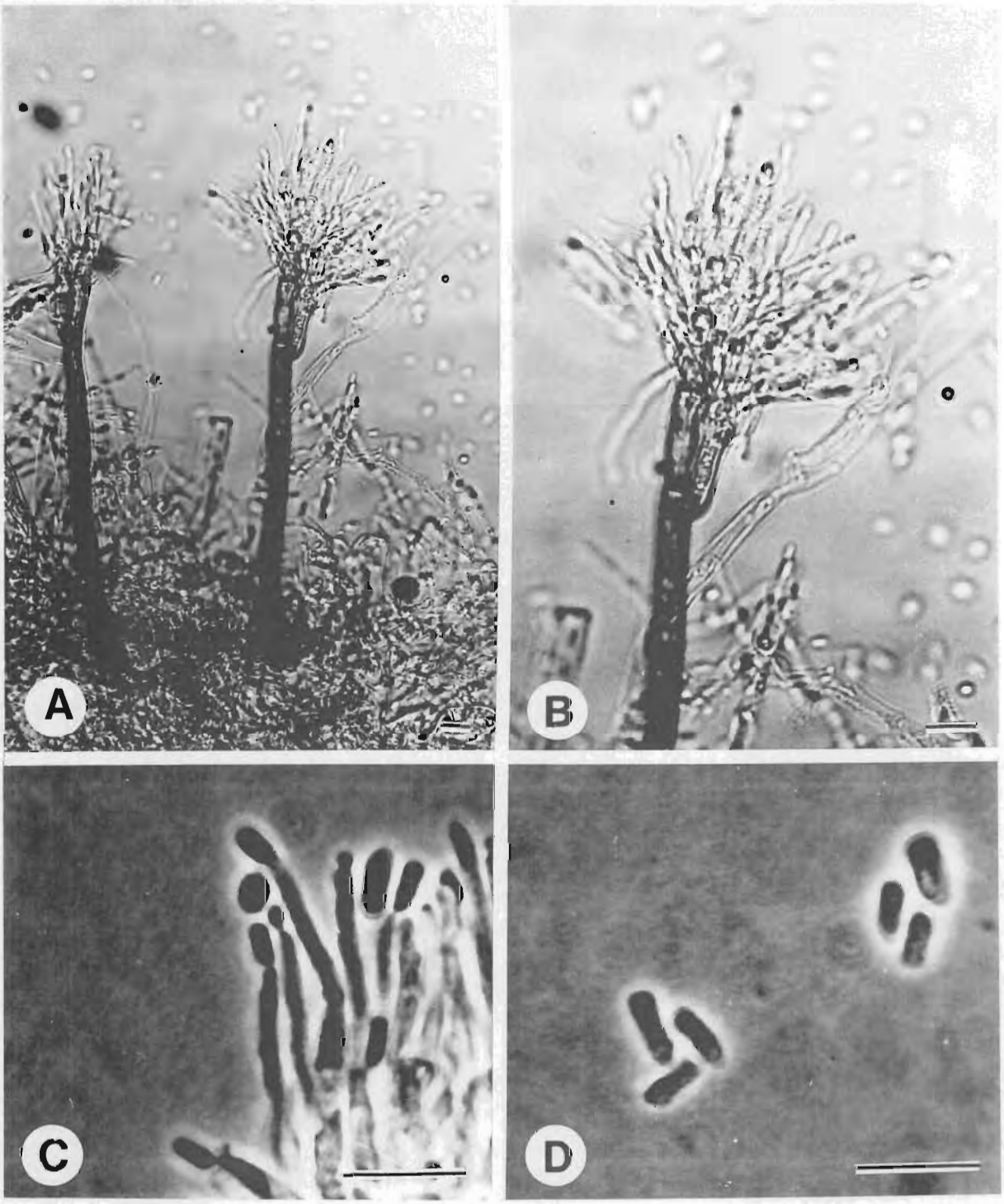

Fig. 109. Light micrographs of the conidiophores and conidia of *L. pinidensiflorae* (CMW 5157). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

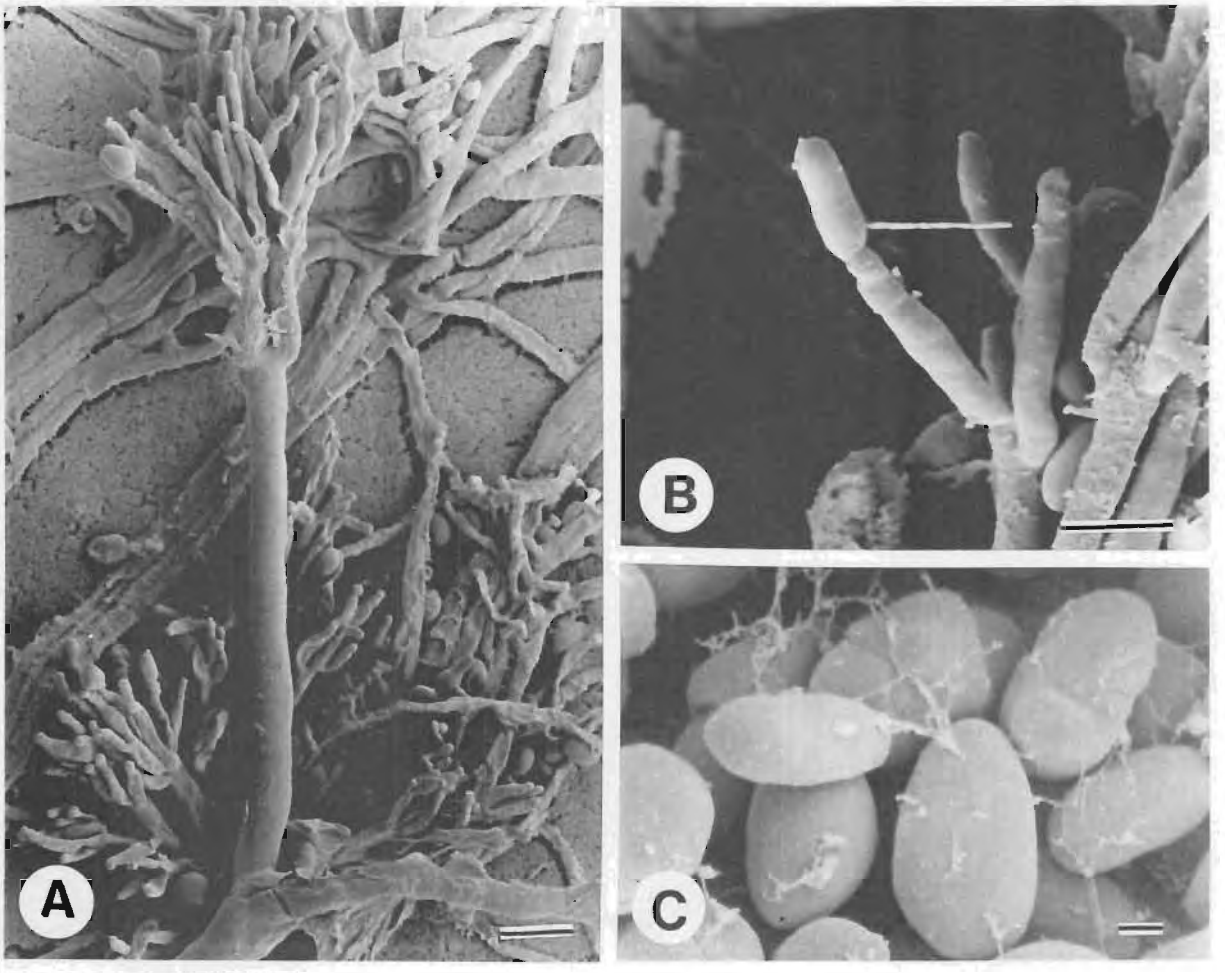

Fig. 110. Scanning electron micrographs of the conidiophores and conidia of *L. pinidensiflorae* (CMW 5157). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

32. *Leptographium pineti* K. Jacobs & M.J. Wingf., *Mycological Research*, 1999. **(Figs. 111-113).**

Teleomorph state: Not known

Etymology: pi-né-ti: of pine plantation. From the Latin noun pinetum: a pine stand. This specific epithet refers to the habitat, of the fungus on *Pinus* spp.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (100-) 89 -202 (-210) µm in length, rhizoid-like structures absent. *Stipes* olivaceous, smooth, cylindrical, simple, 2-4 septate, (50-) 71 - 127.5 (-150) µm long, 5.0 - 7.5 µm wide below primary branches, apical cell not swollen, (5.0-) 6.0 - 9.5 (-10) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (30-) 17.5 - 74 (-70) µm 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-) 13 - 17 (-20) µm long and (3.0-) 3.5 - 5.0 (-6.0) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, aseptate, (7.0-) 9.0 - 12 (-15) µm long, (2.0-) 2.5 - 4.0 µm wide, tertiary branches hyaline, aseptate, (5.0-) 7.5 - 9.0 (-15) µm long, 2.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 6.0 - 16) µm long and 2 µm wide. *Conidia*, aseptate, obovoid, 2.0 - 3.0 x 1 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

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. Colonies dark olivaceous (19"f). *Colony margin* smooth. *Hyphae* submerged or on top of agar with no aerial mycelium, light olivaceous to hyaline, smooth, not constricted at the septa, (2.0-) 2.5 - 4.0 (-6.0) µm diameter.

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

galleries of *Ips* sp. under the bark of *P. merkusii*, March 1996, collected by M.J. Wingfield, PREM 56351, PREM 56354, PREM, 56392, PREM 56355, PREM 56353, PREM 56352, PREM 56351. **Cultures:** Indonesia, Samosir Island, Sumatra, from galleries of *Ips* sp. under the bark of *P. merkusii*, March 1996, collected by M.J. Wingfield, CMW 3832, CMW 3831, CMW 3833, CMW 3834, CMW 3835, CMW 3836, CMW 3837.

Known distribution: Indonesia.

Host/substrate: *Pinus merkusii* (Jacobs *et al.*, 1999).

Associated insects: *Ips* spp. (Jacobs *et al.*, 1999).

Notes: *Leptographium pineti* closely resembles the *Leptographium* anamorph of *O. robustum.* It can, however, easily be distinguished from this and other *Leptographium* spp. based on its characteristic short, robust conidiophores and small conidia. 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 (100 -210 µm) conidiophores of the latter species. *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 is another *Leptographium* spp. that is morphologically similar to *L. pineti* (Webber *et al.*, 1999). *Leptographium calophylli* is 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 longer (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). *Leptographium pineti* is not known to be pathogenic and appears to be saprophytic and is believed to be restricted to *Pinus,* whereas *L. calophylli* is a pathogen of *Calophyllum* species.

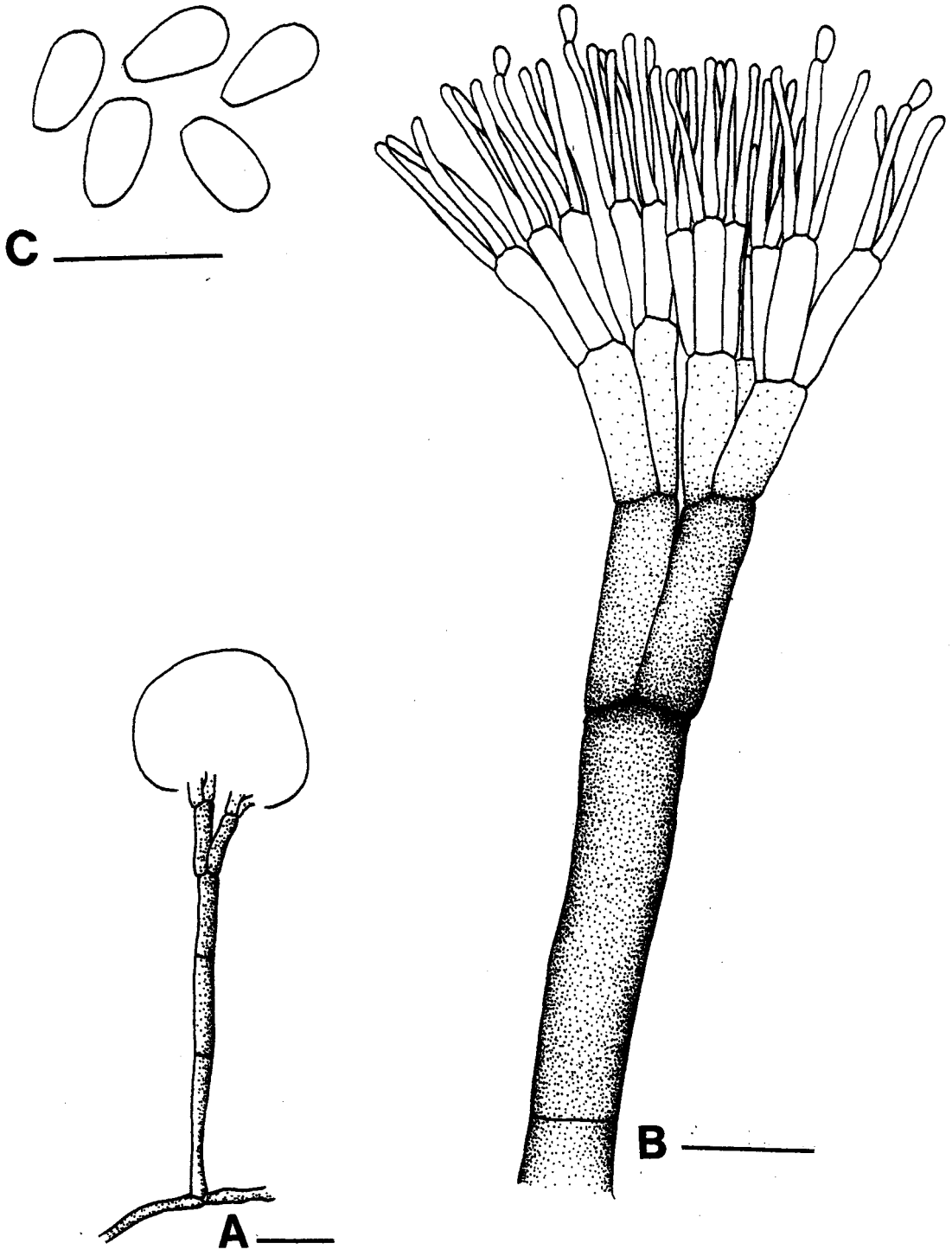

Fig. 111. Conidiophores and conidia of *L. pineti* (CMW 3832). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

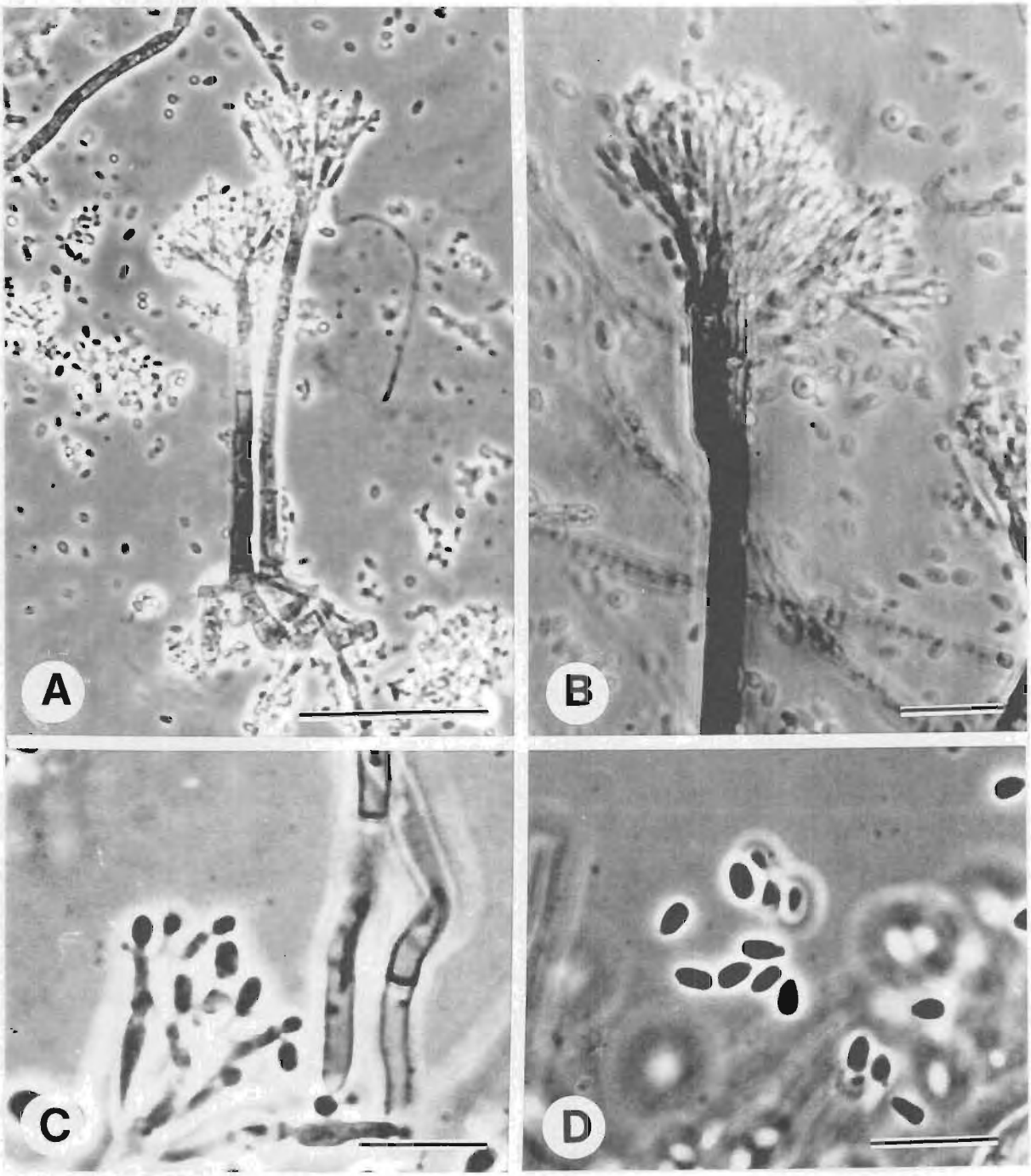

Fig. 112. Light micrographs of the conidiophores and conidia of *L. pineti* (CMW 3832). **A.** Conidiophore (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

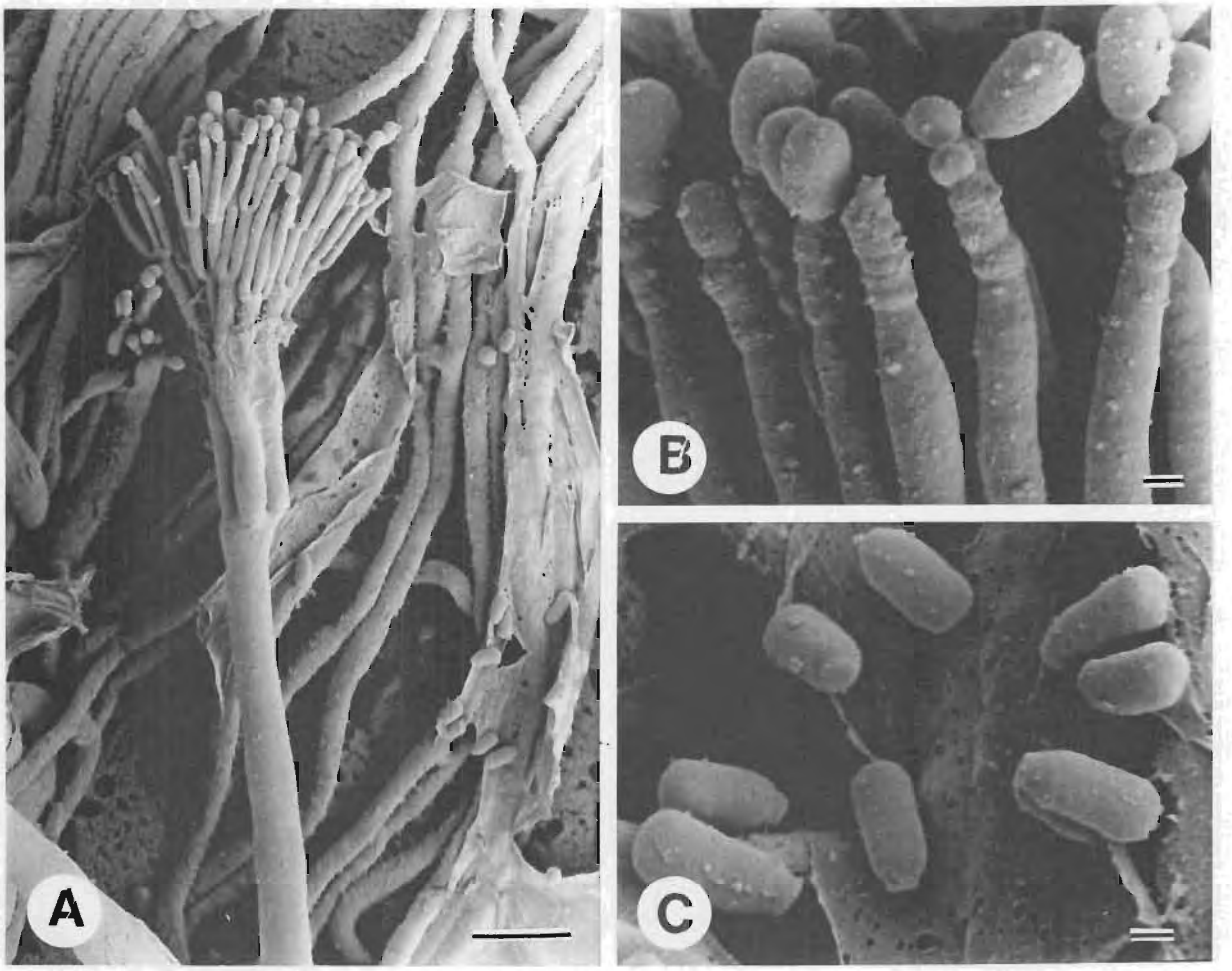

Fig. 113. Scanning electron micrographs of the conidiophores and conidia of *L. pineti* (CMW 3832). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 1 µm). **C.** Conidia (Bar = 1 µm).

33. *Leptographium pityophilum* K. Jacobs & M.J. Wingf., *Mycological Research,* 1999. **(Figs. 114-116).**

Teleomorph: Not known.

Etymology: pi-ty-o-phí-lum: pine-loving. From the Greek noun πιτυς: a pine tree and Greek adjective φιλος: loving. This specific epithet refers to *Pinus,* which is the host of this fungus.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (142-) 350.5 - 412 (-626) µm in length, rhizoid-like structures absent. *Stipes* dark olivaceous, smooth, cylindrical, simple, 3 - 9 septate, (105.5-) 294.5 - 338.5 (-564) µm long, 7.5 - 12.5 µm wide below primary branches, apical cell not swollen, 7.5 - 12.5 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (37-) 57 - 74.5 (-99) µm long, excluding the conidial mass, with 3 to 4 series of cylindrical branches, 2 - 5 primary branches, olivaceous, smooth, cylindrical to barrel-shaped, aseptate, (11-) 13.5 - 20 (-25) µm long and (5.0-) 5.5 - 8.0 (-11) µm wide, arrangement of the primary branches on the stipe - type C, secondary branches light olivaceous to hyaline, aseptate, (8.0-) 9.0 - 15.5 (-17) µm long, 3.0 - 8.0 µm wide, tertiary branches hyaline, aseptate, (7.0-) 8.5 - 12.7 (-16) µm long, 2.0 - 5.0 µm wide, quaternary branches aseptate, hyaline, 8.0 - 12 µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (14-) 16.0 - 17.5 (-21) µm long and 1.5 - 3.0 µm wide. *Conidia*, aseptate, obovoid, 4.0 - 6.0 x 2.0- 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Colonies with optimal growth at 20°C on 2% MEA, reaching 25 mm in diameter in 6 days. No growth below 5°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 20°C in the dark. Colonies dark olivaceous (19"f). *Colony margin* laciniate. *Hyphae* submerged or on top of agar with no aerial mycelium, light olivaceous to dark olivaceous, surrounded by rough granular layer, not constricted at the septa, 2.0 - 3.0 µm diameter.

Specimens examined: Holotype: Italy, isolated from *Pinus nigra,* collected: S. Frisullo, PREM 56365. **Paratypes**: Italy, isolated from *Pinus nigra,* collected: S. Frisullo, PREM 56367, PREM 56366, PREM 56396, PREM 56395. **Cultures:** Italy, isolated from *Pinus nigra,* collected: S. Frisullo, CMW 2840, CMW 2892, CMW 3047, CMW 2838, CMW 3063, CMW 2874.

Known distribution: Europe (Italy).

Host/substrate: *Pinus nigra* (Jacobs *et al.,* 1999).

Associated insects: not known.

Notes: *Leptographium pityophilum* is similar to *L. procerum* but can be distinguished based on the absence of rhizoids as well as by the distinct arrangement of its primary branches. *Leptographium procerum* is characterized by 2 to 3 primary branches of almost equal size. In contrast, *L. pityophilum* is characterized by 2 to 5 primary branches with one central branch that is almost twice the size of the others. In this respect, *L. pityophilum* is more similar to species such as *L. serpens* and *L. wageneri* than to *L. procerum.* It can be distinguished from *L. wageneri* based on its optimal growth temperature at 20 °C, compared with 15 °C for *L. wageneri. Leptographium pityophilum* can be distinguished from *L. serpens* based on its straight uncurved hyphae, compared to the distinctly serpentine hyphae of *L. serpens.* No specific insects have been recorded as associates of *L. pityophilum.* The pathogenicity of *L. pityophilum* is unknown and it is most probably a saprophyte (Jacobs *et al.,* 1999).

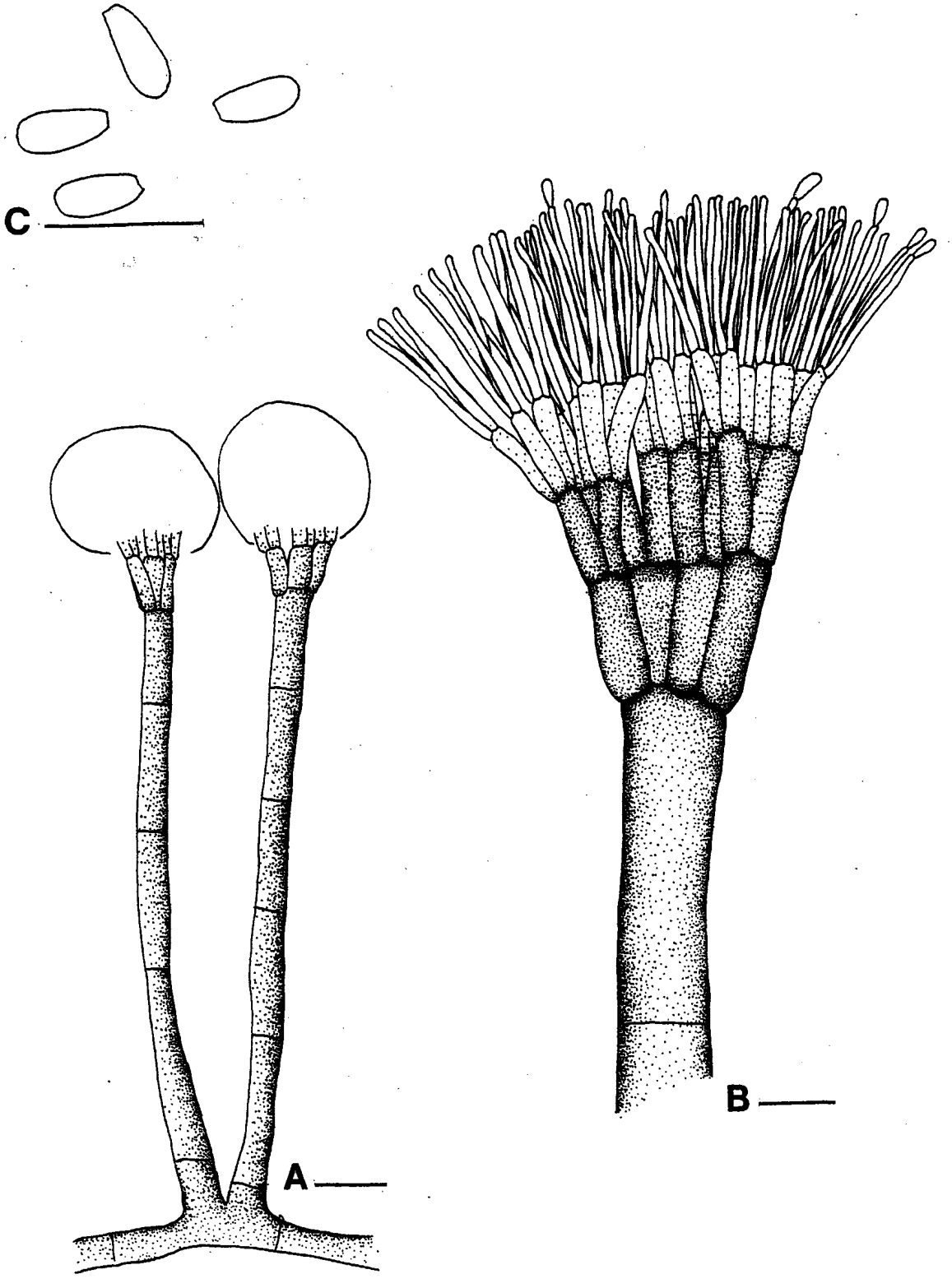

Fig. 114. Conidiophores and conidia of *L. pityophilum* (PREM 56365). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

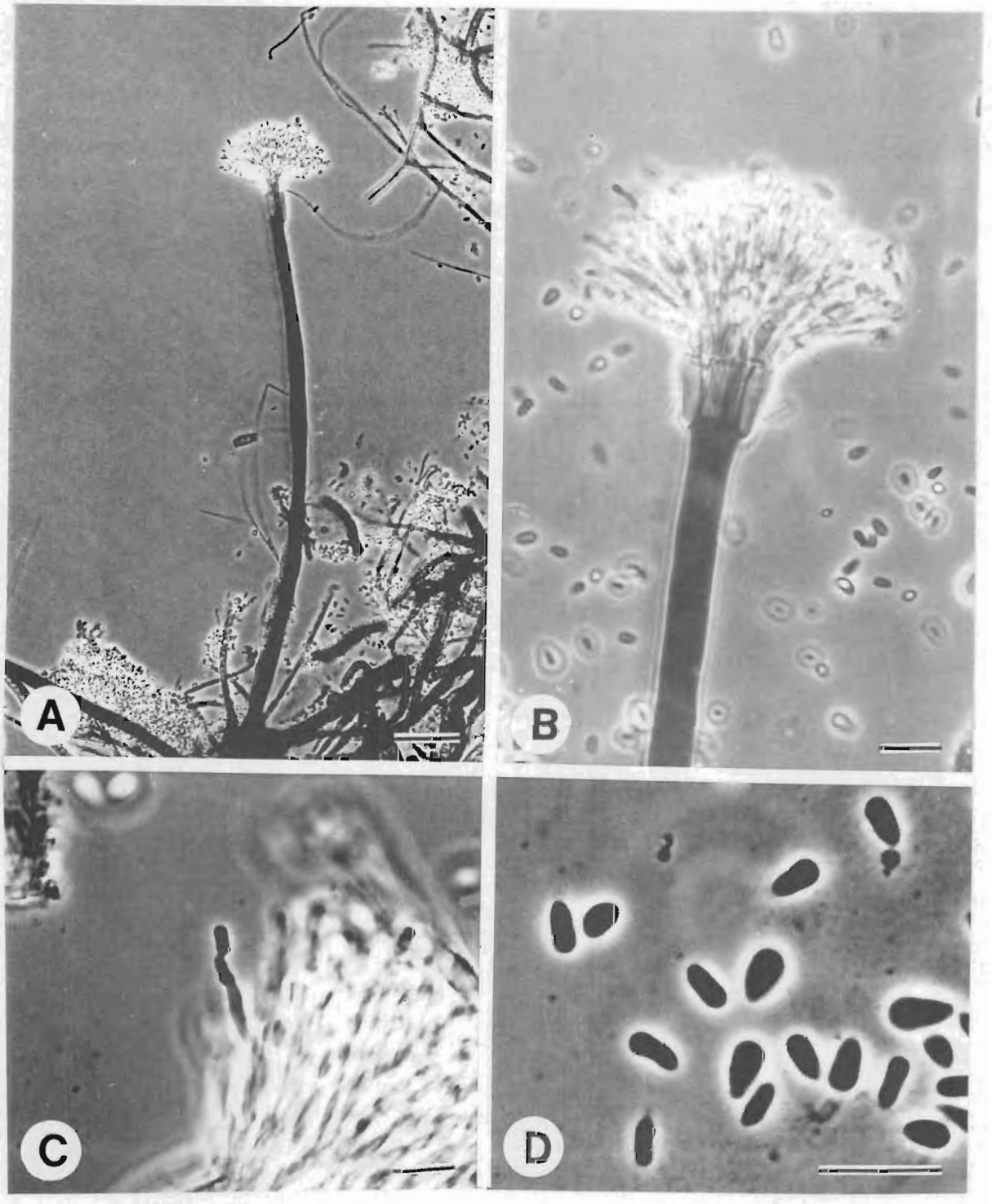

Fig. 115. Light micrographs of the conidiophores and conidia of *L. pityophilum* (PREM 56365). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

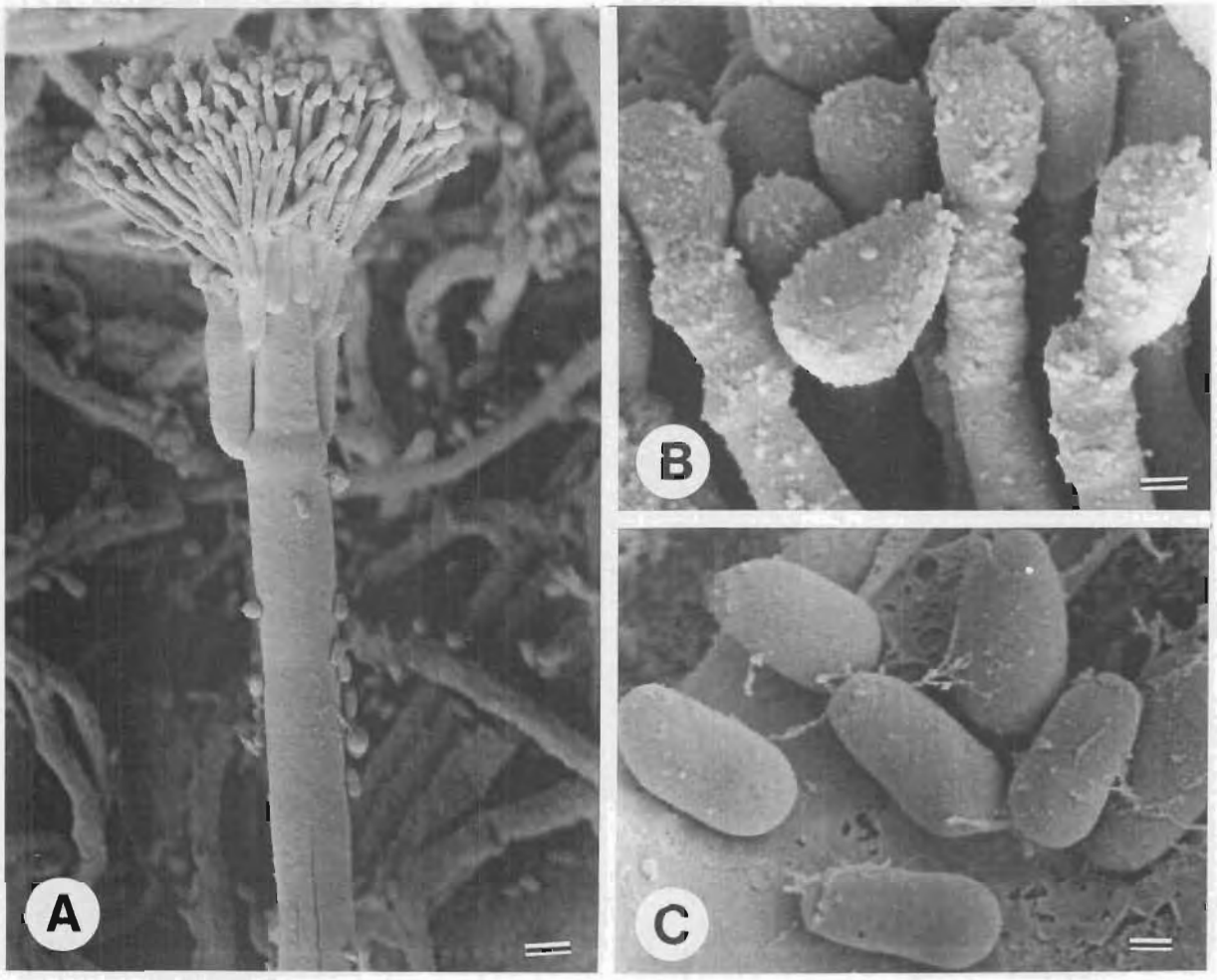

Fig. 116. Scanning electron micrographs of the conidiophores and conidia of *L. pityophilum* (PREM 56365). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 1 µm). **C.** Conidia (Bar = 1 µm).

34. *Leptographium procerum* (W.B. Kendr.) M.J. Wingf., *Trans Br. Mycol. Soc.* **85**, 92. 1985. **(Figs. 117-119).**

≡ *Verticicladiella procera* W.B. Kendr. *Canadian Journal of Botany* **40**, 783. 1962.

Teleomorph: Not known.

Etymology: pro-cé-rum: long. From the Latin adjective procerus: tall, long. This specific epithet refers to the long conidiophores which is characteristic of this fungus.

Conidiophores occurring singly or in groups of up to three, arising directly from the mycelium, erect, macronematous, mononematous, (150-) 245.4 - 570.5 (-760) µm in length, rhizoid-like structures present. *Stipes* olivaceous, smooth, cylindrical, simple, 3 - 10 septate, (125-) 206 - 496 (-690) µm long, 3.0 - 9.0 µm wide below primary branches, apical cell not swollen, 3.0 - 15 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (25-) 39.4 - 75 (-90) µm long, excluding the conidial mass, with 2 to 5 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical, aseptate (11-) 16.5 - 22.5 (-34) µm long and (3.0-) 4.5 - 5.5 (-7.5) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21"k), aseptate, (8.5-) 11 - 12 (-15.5) µm long, (2.0-) 3.5 - 4.5 (-7.5) µm wide, tertiary branches hyaline, aseptate, (7.0-) 7.0 - 13.5 (-14) µm long, 2.0 - 6.0 µm wide, quaternary branches aseptate, (7.5-) 8.0 - 12.5 (-13.5) µm long, 2.0 - 5.0 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, cylindrical, tapering slightly at the apex, (11-) 15 - 18 (-22) µm long and 1.0 - 2.5 µm wide. *Conidia* hyaline, aseptate, obovoid to broadly ellipsoid with truncate ends and rounded apices, 3.0 - 5.0 x 1.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 23 mm in diam. in 9 days. No growth below 10°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 21% reduction in growth on 0.5 g/l cycloheximide after 8

days at 20°C in the dark. Colonies dark mouse gray (15""k) to olivaceous (21"m) towards the edge with darker concentric rings in the colony. *Colony margin* smooth or slightly effuse. *Hyphae* submerged on agar with no aerial mycelium, hyaline to light olivaceous (21"k), smooth, straight, occasionally constricted at the septa, (3.0-) 3.5 - 4.5 (-8) µm diam.

Specimens examined: Holotype: Canada, St. Paul, Quebec, *Pinus banksiana*, 4 September 1959, collected: W.B. Kendrick, DAOM 63700. **Paratypes**: New York, Montgomery County, *Pinus resinosa* (interior of roots with resinous lesions), February 1959, collected: D.S. Welch, DAOM 62093; New York, Newfield, *Pinus resinosa* (interior of roots with resinous lesions), Feb. 1959, collected: D.S. Welch, DAOM 62094; New York, Columbia County, Conoan, *Pinus resinosa* (interior of roots with resinous lesions), collected: Feb. 1959, D.S. Welch, DAOM 62095; New York Stockton, Chatauqua County, *Pinus resinosa* (interior of roots with resinous lesions), Feb. 1959, collected: D.S. Welch, DAOM 62096; Sweden, Järna, Södermanland, *Pinus* sp., Aug. 1959, collected: A. Mathiesen-Käärik, DAOM 63686; Sudbury, *Pinus strobus*, Nov. 1952, collected: S.N. Linszon, DAOM 33940. **Cultures**: New York Stockton, Chatauqua County, *Pinus resinosa* (interior of roots with resinous lesions), Feb. 1959, collected: D.S. Welch CMW 13 (same as DAOM 62096), Poland, *Pinus spp.,* collected: T. Kawalski, CMW 2460; USA, *P. strobus,* collected: J. Altman, CMW 3; USA, *P. strobus*, collected: M.J. Wingfield, CMW 12; USA, *P. monticola,* collected: P. Kulhavy, CMW 1831; England, *Hylastes opacus,* collected: J.N. Gibbs, CMW 825; England, *Hylurgops palliatus,* collected: J.N. Gibbs, CMW 828; England, *Hylobius* sp., collected: J.N. Gibbs, CMW 2172; South Africa, *Pinus* infested with *Hylastes* sp, collected: G. Tribe, CMW 522; Italy, *P. pinea,* collected: P. Capretti, CMW 699; Norway, *Picea* sp., collected: M.J. Wingfield, CMW 3797; Yugoslavia, *P. strobus,* collected: M. Halambek, CMW 25; France, *Picea abies,* collected: M. Morelet, CMW 747; New Zealand, *P. strobus,* collected: M. Dick, CMW 261; Canada, *P. strobus,* collected: Lincar, CMW 20.

Known distribution: Canada, Europe (England, France, Norway, Italy, Sweden), New Zealand.

Hosts/substrate: *Abies grandis* (Lane & Goheen, 1979), *Picea abies* (Hallaksela, 1977; Alexander *et al.*, 1988), *Picea fraseri* (Alexander *et al.*, 1988), *Pinus banksiana* (Kendrick, 1962; Wingfield, 1982, 1983; Alexander *et al.*, 1988), *Pinus contorta* (Mielke, 1979; Alexander *et al.*, 1988), *Pinus clausa* (Barnard *et al.*, 1985; Alexander *et al.*, 1988), *Pinus echinata* (Horner & Alexander, 1983a; Alexander *et al.*, 1988), *Pinus elliotii* (Horner & Alexander, 1983a; Alexander *et al.*, 1988), *Pinus monticola* (Alexander *et al.*, 1988), *Pinus nigra* (Lackner & Alexander, 1982; Wingfield, 1982; Alexander *et al.*, 1988), *Pinus pinaster* (Morelet, 1986), *Pinus ponderosa* (Mielke, 1979; Wingfield, 1982; Alexander *et al.*, 1988), *Pinus radiata* (mackenzie & Dick, 1984), *Pinus resinosa* (Kendrick, 1962; Towers, 1977; Sinclair & Hudler, 1980; Halambek, 1981; Wingfield, 1982; Harrington, 1988; Alexander *et al.*, 1988), *Pinus strobus* (Kendrick, 1962; Houston, 1969; Towers, 1977; Shaw & Dick, 1980; Sinclair & Hudler, 1980; Livingston & Wingfield, 1982; Wingfield, 1982; Lackner & Alexander, 1982; Horner & Alexander, 1983a, b; Lackner & Alexander, 1984; mackenzie & Dick, 1984; Alexander *et al.*, 1988; Smith, 1991), *Pinus sylvestris* (Wingfield & Gibbs, 1991; Wingfield, 1982; Lackner & Alexander, 1984; Horner & Alexander, 1983b; Harrington, 1988; Alexander *et al.*, 1988), *Pinus taeda* (Horner & Alexander, 1983a; Alexander *et al.*, 1988), *Pinus virginia* (Horner & Alexander, 1983a; Alexander *et al.*, 1988), *Pseudotsuga menziesii* (Mielke, 1979; Morrison & Hunt, 1988; Alexander *et al.*, 1988).

Associated insects: *Dendroctonus frontalis* (Otrosina *et al.*, 1997), *Dendroctonus valens* (Wingfield, 1983; Harrington, 1988), *Dendroctonus terebrans* (Harrington, 1988; Perry, 1991), Hylastes sp. Lewis & Alexander, 1986; Alexander *et al.*, 1988), *Hylastes ater* (mackenzie & Dick, 1984), *Hylastes opacus* (Wingfield & Gibbs, 1991), *Hylobius abietis* (Lévieux *et al.*, 1994), *Hylobius pales* (Lackner & Alexander, 1982; Wingfield, 1983; Lewis & Alexander, 1986; Alexander *et al.*, 1988; Nevill & Alexander, 1992a, b), *Hylobius radicis* (Wingfield, 1982; Wingfield, 1983; Alexander *et al.*, 1988), *Hylobius rhizophagus* (Wingfield, 1982; Wingfield, 1983; Alexander *et al.*, 1988), *Hylurgus ligniperda* (mackenzie & Dick, 1984), *Hylurgops palliatus* (Wingfield & Gibbs, 1991), *Hylurgops porosus* (Wagner, 1977), *Ips typographus*

(Harrington, 1988), *Orthotomicus spp.* (Lewis & Alexander, 1986; Alexander *et al.*, 1988), *Pachylobius picivorus* (Wingfield, 1983; Alexander *et al.*, 1988), *Pissodes* spp. (Lewis & Alexander, 1986), *Pissodes approximatus* (Lackner & Alexander, 1982; Alexander *et al.*, 1988), *Pissodes nemorensis* (Nevill & Alexander, 1992a, b), *Pissodes pini* (Kendrick, 1962; Livingston & Wingfield, 1982), *Pityokteines* sp. (Lackner & Alexander, 1984; Alexander *et al.*, 1988), *Pityogenes* sp. (Lackner & Alexander, 1984; Lewis & Alexander, 1986; Harrington, 1988; Alexander *et al.*, 1988), *Pityophthorus* sp. (Lackner & Alexander, 1984; Alexander *et al.*, 1988), *Tomicus piniperda* (Gibbs & Inman, 1991), *Xyleborus* sp. (Lewis & Alexander, 1986; Alexander *et al.*, 1988).

Notes: Kendrick (1962) considered *L. procerum* to be similar to *L. abietinum.* He distinguished these species based on the broader, uncurved conidia and longer sporogenous apparatus and primary branches of *L. procerum.* *Leptographium procerum* is also similar to *L. alethinum, L. pityophilum* and *L. euphyes.* These species were initially identified as *L. procerum.* They were, however, separated from *L. procerum* based on clear morphological differences. The most prominent distinguishing character is probably the characteristic concentric rings formed by the mycelium in culture. Horner *et al.* (1986), hypothesized that the teleomorph of *Leptographium procerum* is possibly a species of *Ophiostoma.* This was based on the findings that *L. procerum* is resistant to cycloheximide and possess cellulose in its cell walls, characteristic of *Ophiostoma* spp.

Leptographium procerum is associated with a root disease of pines, especially white pine (*Pinus strobus*) (Gill *et al.,* 1951; Hubert, 1953; Dochinger, 1967). It was also found on many *Pinus* spp. infested with root and root collar feeding insects. The pathogenicity of *L. procerum* has been extensively debated for many years. Some authors suggested that the fungus is pathogenic and can cause severe disease (Halambek, 1981; Lackner, 1981; Lackner & Alexander, 1982). In other cases *L. procerum* was found to be weakly pathogenic and unable to kill wounded or unwounded host trees (Towers, 1977; Livingston & Wingfield, 1982; Wingfield, 1982; Harrington & Cobb, 1983; Wingfield, 1986; Wingfield *et al.,* 1988; Wingfield, 1993).

Leptographium procerum is known to be spread by weevils (Wingfield, 1983; Lewis, 1985; Lewis & Alexander, 1986; Lévieux *et al.,* 1994). These insects are mainly root and root collar feeding insects. Wingfield *et al.,* (1988) proposed that the association of *L. procerum* with these insects, explains the occurrence of the fungus in trees other that those dying from white pine root decline. The feeding and breeding activities of the insects appears to have an effect on the severity of the disease (Alexander *et al.,* 1988).

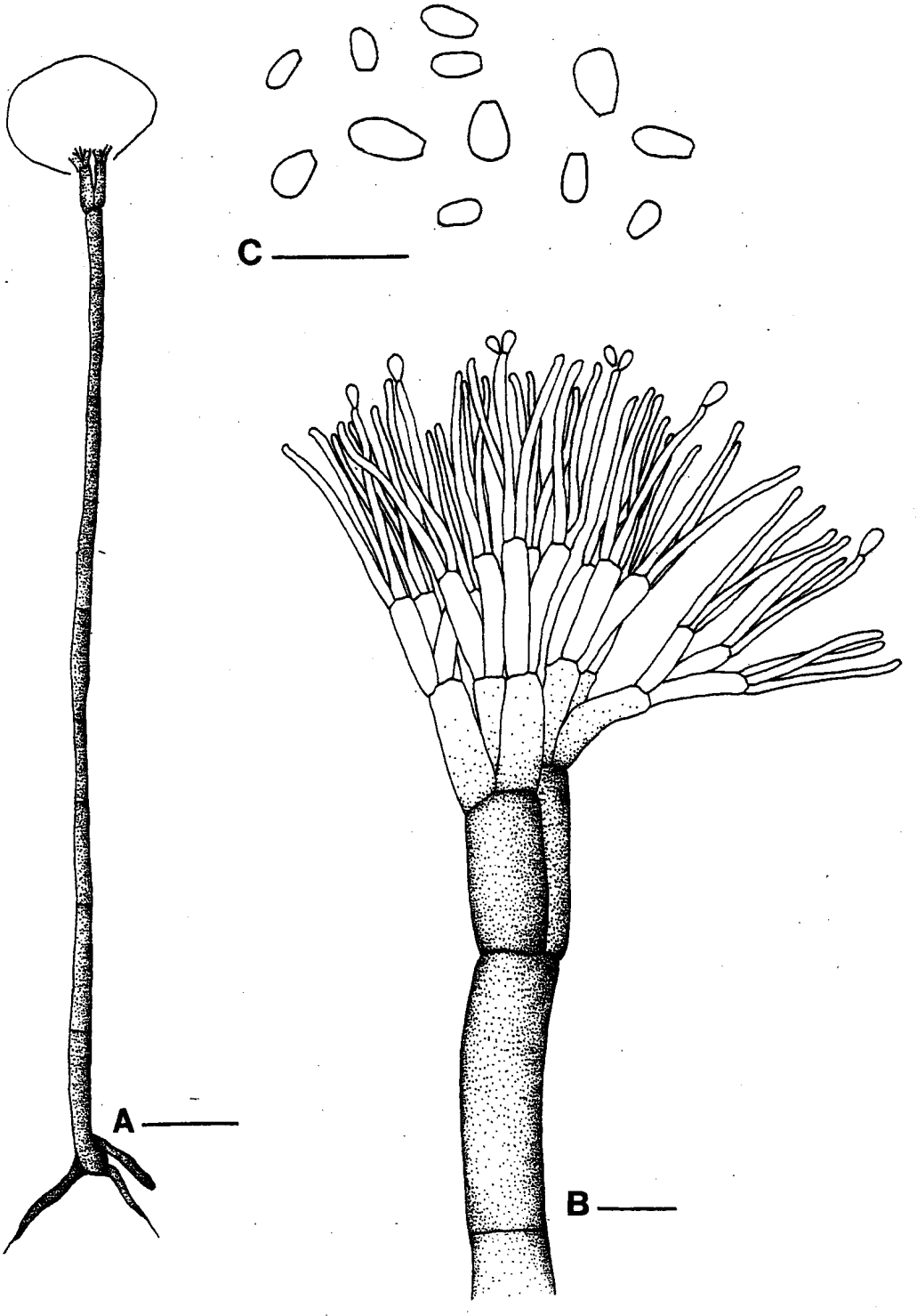

Fig. 117. Conidiophores and conidia of *L. procerum* (CMW 12). **A.** Habit sketch (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

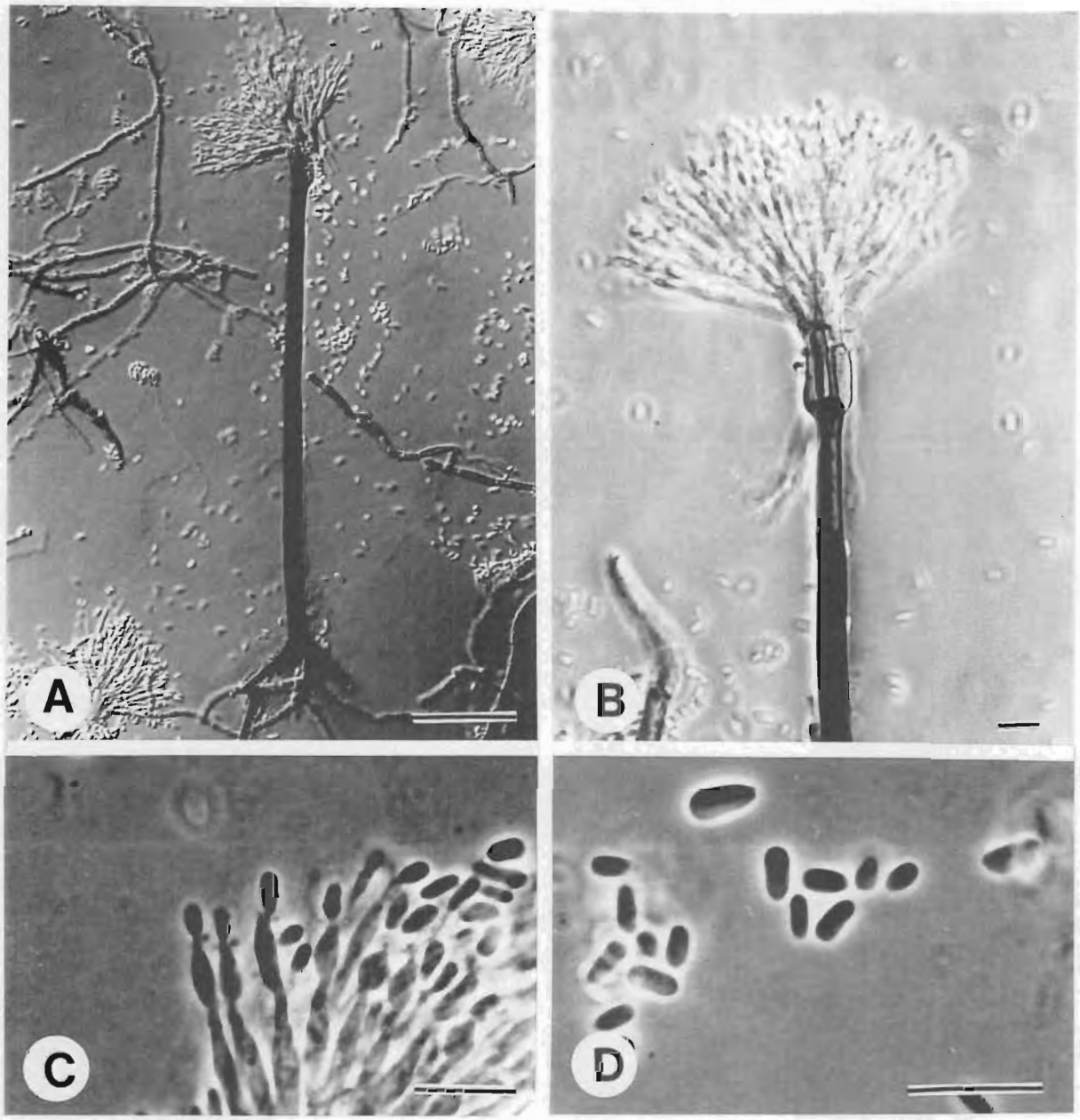

Fig. 118. Light micrographs of the conidiophores and conidia of *L. procerum* (CMW 12). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

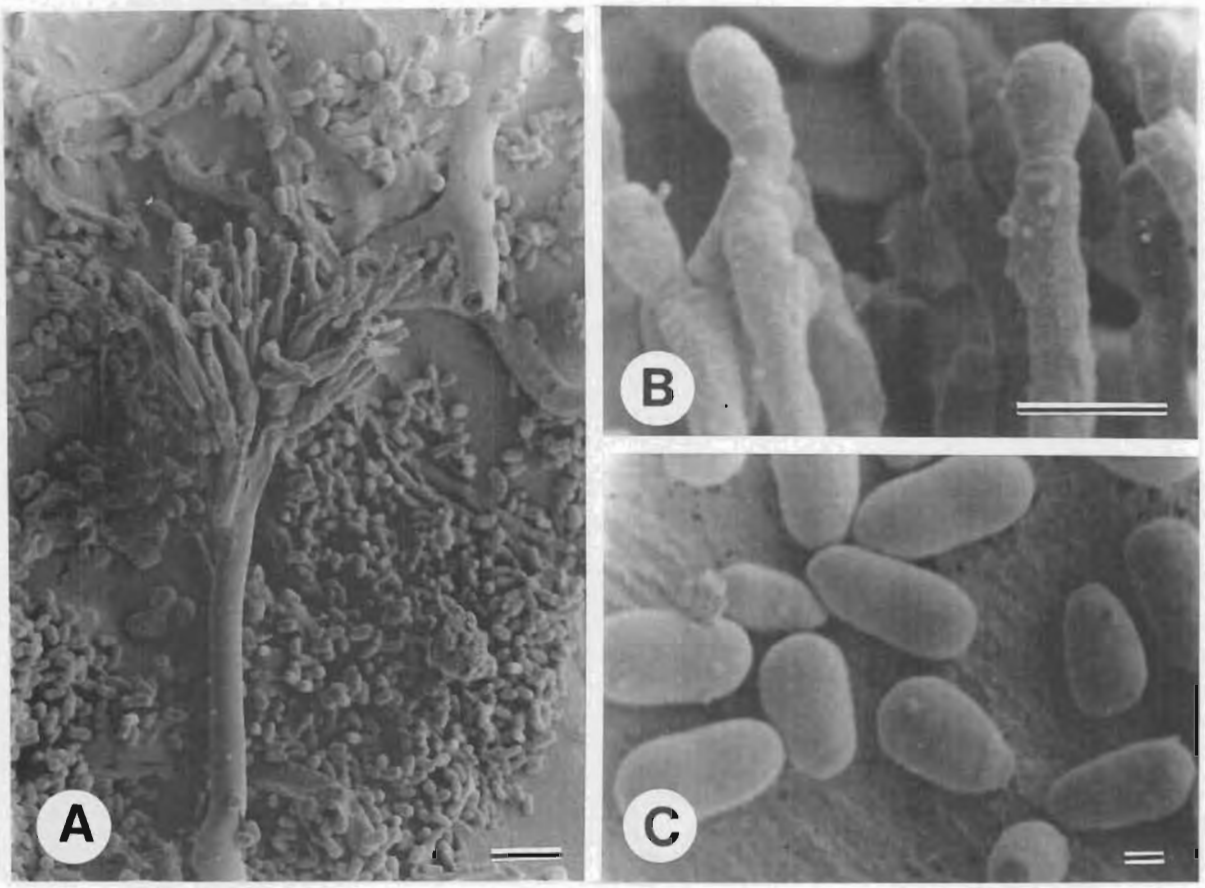

Fig. 119. Scanning electron micrographs of the conidiophores and conidia of *L. procerum* (CMW 12). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

35. *Leptographium pyrinum* R.W. Davidson, *Mycologia* **70**, 39. 1978. **(Figs. 120-122).**

Teleomorph: Not known.

Etymology: py-rí-num: pear-shaped. From the Latin noun pirum: a pear. This specific epithet refers to the pear-shaped conidia that are characteristic of this species.

Conidiophores occurring singly or in groups, mostly on aerial mycelium, erect, macronematous, mononematous, (117.5-) 215 - 236.5 (-392.5) µm in length, rhizoid-like structures present. *Stipes* light olivaceous (21''k), smooth, cylindrical, simple, 1-7 septate, (35-) 119 - 147 (-302.5) µm long, 7.5 - 15 µm wide below primary branches, apical cell occasionally swollen, 7.5 - 15 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (57.5-) 75 - 99.5 (-132.5) µm long, excluding the conidial mass, with 1 to 3 series of cylindrical branches, 2-4 primary branches, light olivaceous (21''k), smooth, cylindrical, aseptate (14-) 18.5 - 31.2 (-40) µm long and 5.0 - 12) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, aseptate, (12-) 19 - 20.5 (-33) µm long, 4.0 - 10 µm wide, tertiary branches hyaline, aseptate, (12-) 15 - 32.5 (-30) µm long, 3.0 - 10 µm wide, quaternary branches aseptate, 14 - 24 µm long, 3.0 - 8.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (11-) 18 - 23.5 (-32) µm long and 2.0 - 5.0 µm wide. *Conidia* hyaline, aseptate, oblong, 5.0 - 12 x 4.0 - 6.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 27 mm in diam. in 8 days. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 9% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15''''k). *Colony margin* laciniate. *Hyphae* submerged on agar with abundant aerial mycelium, hyaline, smooth,

straight, not constricted at the septa, 3.0 - 11.0 µm diam.

Specimens examined: Holotype: *Pinus ponderosa* infested with *Dendroctonus adjunctus*, 1978, collector R.W. Davidson, ATCC 34943. **Cultures**: *Pinus ponderosa* infested with *Dendroctonus adjunctus*, 1978, collector R.W. Davidson,. CMW 169 ex type; Ponderosa pine infested with *Dendroctonus adjunctus*, 1978, collector R.W. Davidson, CMW 509; U.S.A., California, *Pinus jeffreyi,* collected: D. Six, CMW 3889, CMW 3891, CMW 3895 (PREM 56398).

Known distribution: U.S.A.

Hosts/substrate: *Pinus ponderosa* (Davidson, 1978).

Associated insects: *Dendroctonus adjunctus* (Davidson, 1978; Harrington, 1988; Perry, 1991; Six & Paine, 1996).

Notes: *Leptographium pyrinum* can readily be distinguished from other *Leptographium* spp. based on its characteristic large, obovoid conidia. The conidiophores are also unlike those of other *Leptographium* spp., in that they have a very "clumsy" and untidy appearance. The hyphae of this species are covered by granular material. *Leptographium pyrinum* has been isolated from blue-stained sapwood of ponderosa pine (*Pinus ponderosa*) associated with adult beetles in diseased trees (Davidson, 1978). Six and Paine (1996) found that *L. pyrinum* is carried in the mycangia of the bark beetle, *Dendroctonus adjunctus.*

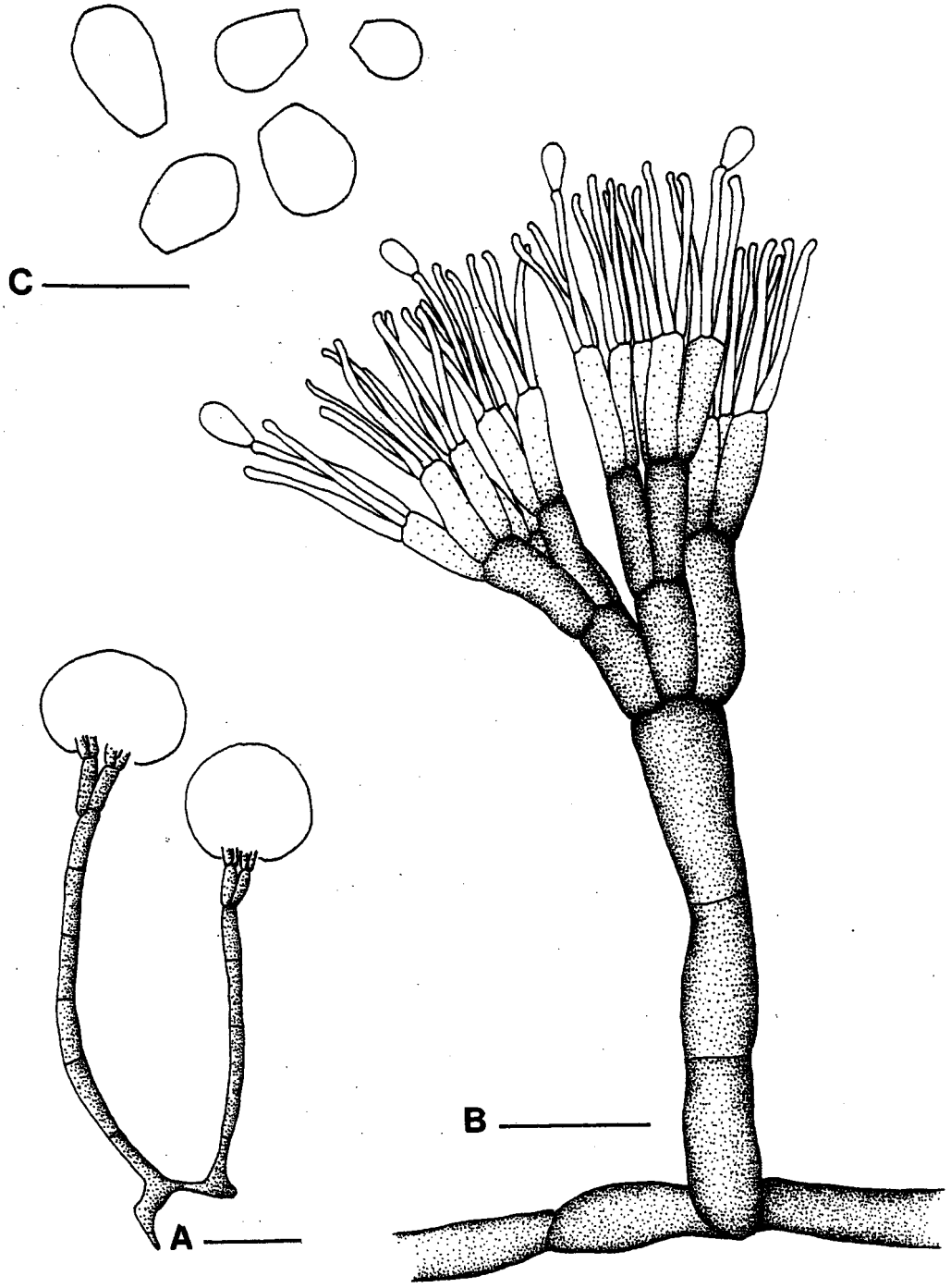

Fig. 120. Conidiophores and conidia of *L. pyrinum* (CMW 169). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

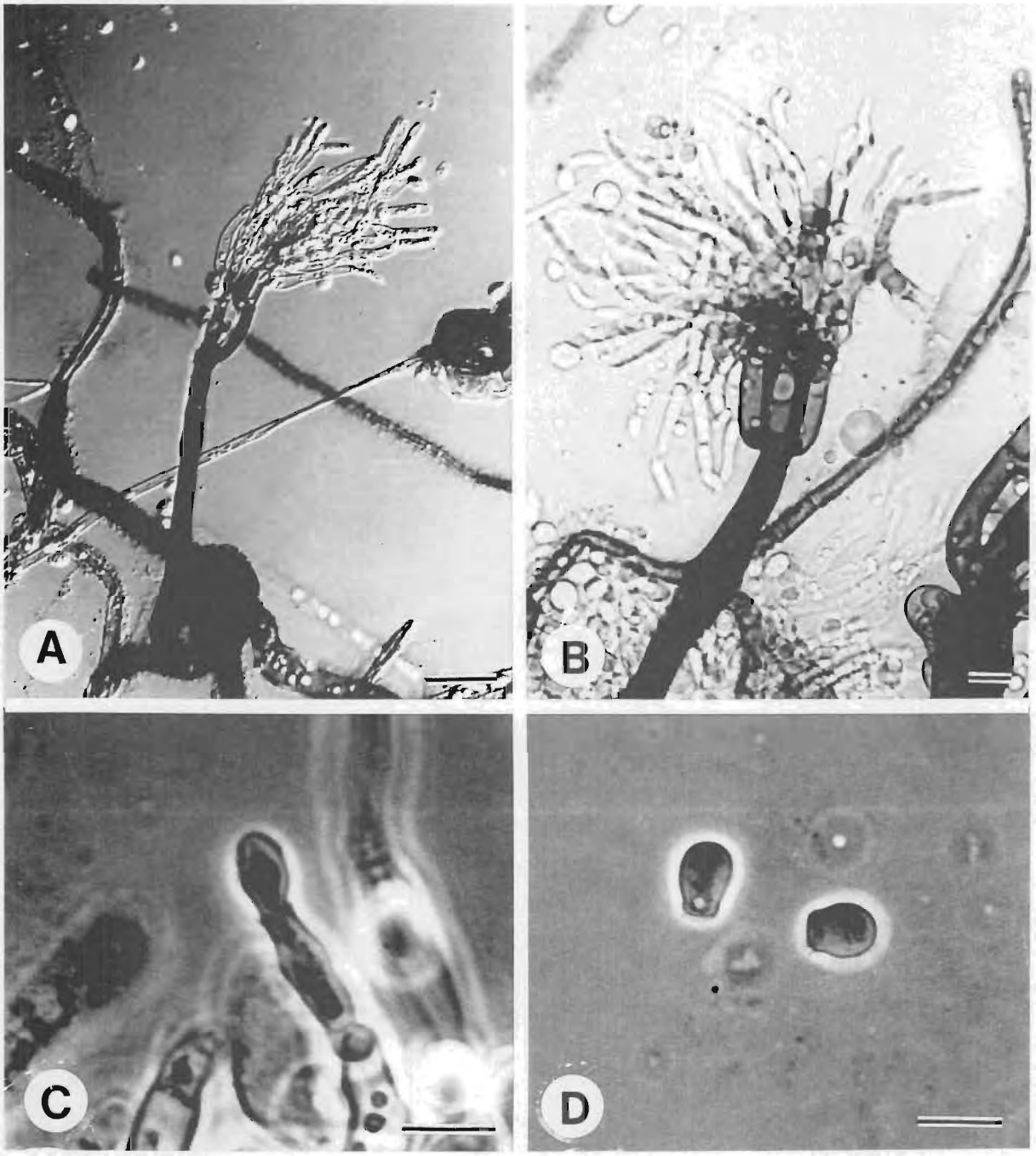

Fig 121. Light micrographs of the conidiophores and conidia of *L. pyrinum* (CMW 169). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 20 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

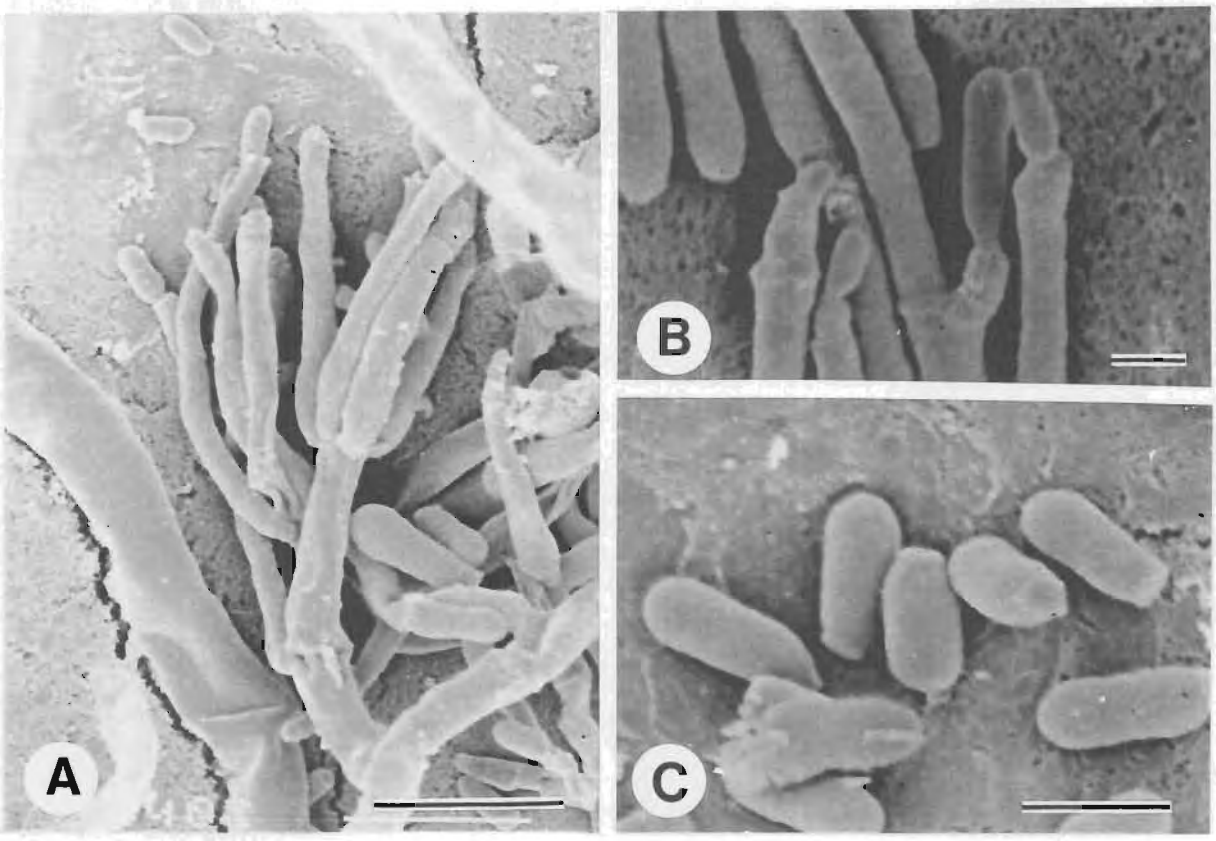

Fig. 122. Scanning electron micrographs of the conidiophores and conidia of *L. pyrinum* (CMW 169). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

36. *Leptographium reconditum* Jooste, *Transactions of the British Mycological Society* **70**, 154. 1978. **(Figs. 123-125).**

Teleomorph: Not known.

Etymology: re-con-dí-tum: concealed. From the Latin verb recondere: to conceal. This specific epithet refers to the habitat of this fungus which is the rhizosphere of *Triticum.*

Conidiophores occurring singly or in groups of up to three, arising directly from the mycelium or on aerial mycelium, erect, macronematous, mononematous, (150-) 291.5 - 440 (-725) µm in length, rhizoid-like structures present. *Stipes* olivaceous (21"m), smooth, cylindrical, simple, 4-14 septate, (110-) 238 - 386.5 (-660) µm long, 4.5 - 8.0 µm wide below primary branches, apical cell not swollen, 6.0 - 12.5 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (30-) 50 - 57 (-95) µm long, excluding the conidial mass, with 2 to 3 series of cylindrical branches, 2-5 primary branches, light olivaceous (21"k), smooth, cylindrical, aseptate (9.0-) 13 - 17.5 (-25) µm long and 3.0 - 9.0 µm wide, arrangement of the primary branches on the stipe - type C, secondary branches light olivaceous (21"k) to hyaline, aseptate, (7.5-) 9.0 - 13 (-17) µm long, (2.0-) 2.5 - 3.5 (-6.0) µm wide, tertiary branches hyaline, aseptate, (7.0-) 8.5 - 11.5 (-13.5) µm long, 2.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (8.0-) 13 - 19 (-23) µm long and 1.0 - 2.5 µm wide. *Conidia* light gray olivaceous (19""), aseptate, oblong with truncate ends and rounded apices, slightly curved at the point of attachment, (3.0-) 3.5 - 4.5 (-5.0) x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming honey yellow (19") with age. Conidial mass honey yellow when wet, turning black when dry.

Colonies with optimal growth at 20°C on 2% MEA, reaching 36 mm in diam. in 6 days. Little growth at 5°C and no growth above 30°C. Able to withstand high concentrations of cycloheximide with a 29% reduction in growth on 0.5 g/l cycloheximide after 4 days at 20°C in the dark. Colonies dark mouse gray (15""k). *Colony margin* laciniate. *Hyphae* submerged on agar with abundant aerial mycelium,

light olivaceous (21"k) to olivaceous (21"m), smooth, straight, not constricted at the septa, occasionally roughened by granules, (1.5-) 3.0 - 5.5 (-8.0) µm diam.

Specimens examined: Holotype: RSA, Mooinooi, Rustenburg, Triticum roots, 1975, collected: W.J. Jooste, PREM 45016. **Cultures**: RSA, Potchefstroom, *Zea Mays*, 1976, collected: W Jooste, CMW 15.

Known distribution: South Africa.

Hosts/substrate: *Triticum* rhizosphere (Jooste, 1978).

Associated insects: Not known.

Notes: This is one of the few species of *Leptographium* that is not associated with a coniferous host and its association with wheat roots is unusual. *Leptographium reconditum* is considered to be similar to *L. lundbergii, L. procerum*, and *L. abietinum.* Comparison of these fungi indicated that the conidiogenous apparatuses of *L. reconditum* are more complex than those of *L. lundbergii. Leptographium reconditum* was distinguished from *L. procerum* and *L. abietinum* based on the mode of conidium development (Jooste, 1978). Because this character was shown to be invalid (Van Wyk *et al.*, 1987), other characters are required to distinguish between these species. The most important distinguishing character is the unique habitat of the fungus (Jooste, 1978). In addition, the short, stout conidiogenous cells of *L. reconditum,* distinguish it from other closely related species.

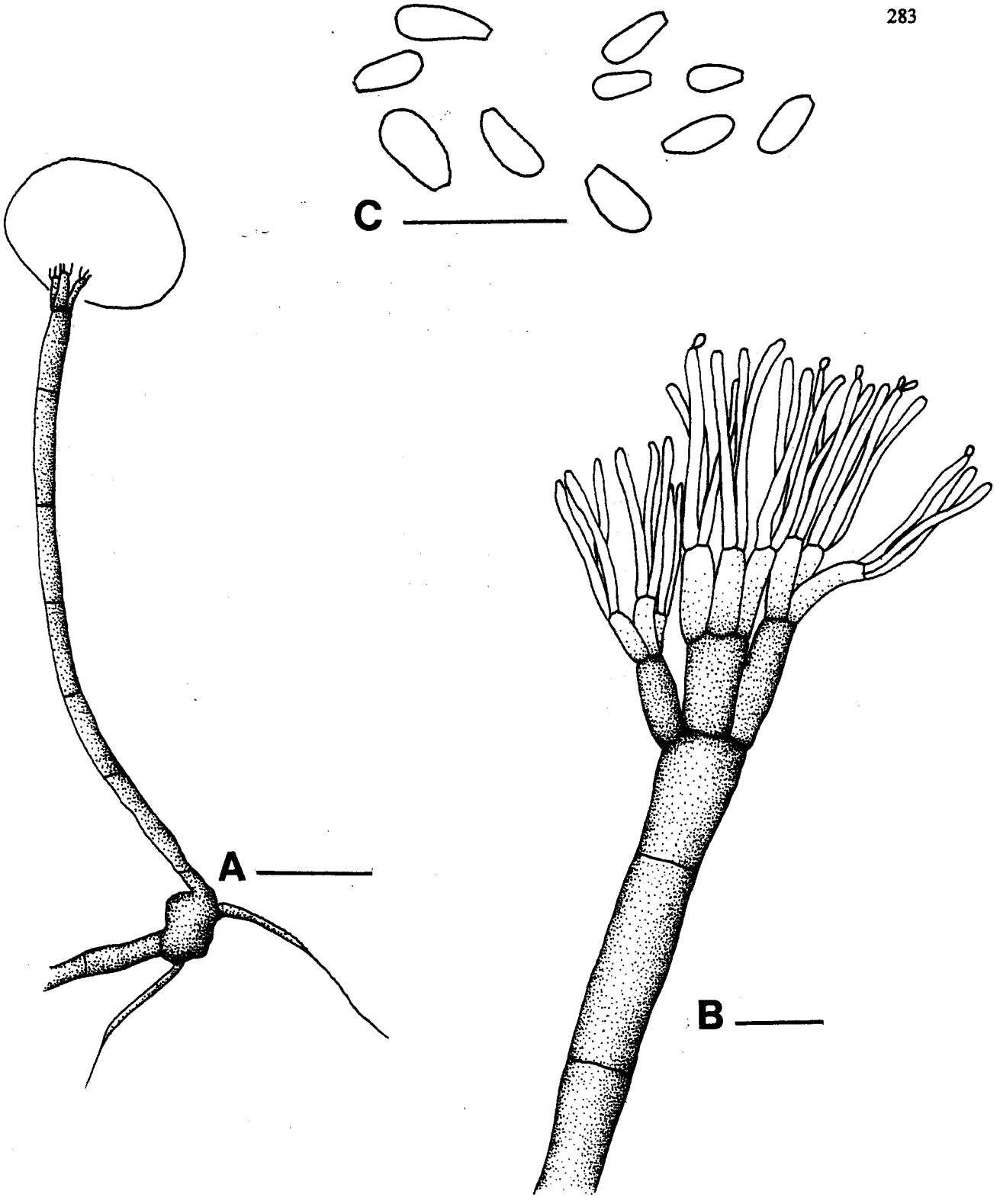

Fig. 123. Conidiophores and conidia of *L. reconditum* (CMW 15). **A.** Habit sketch (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

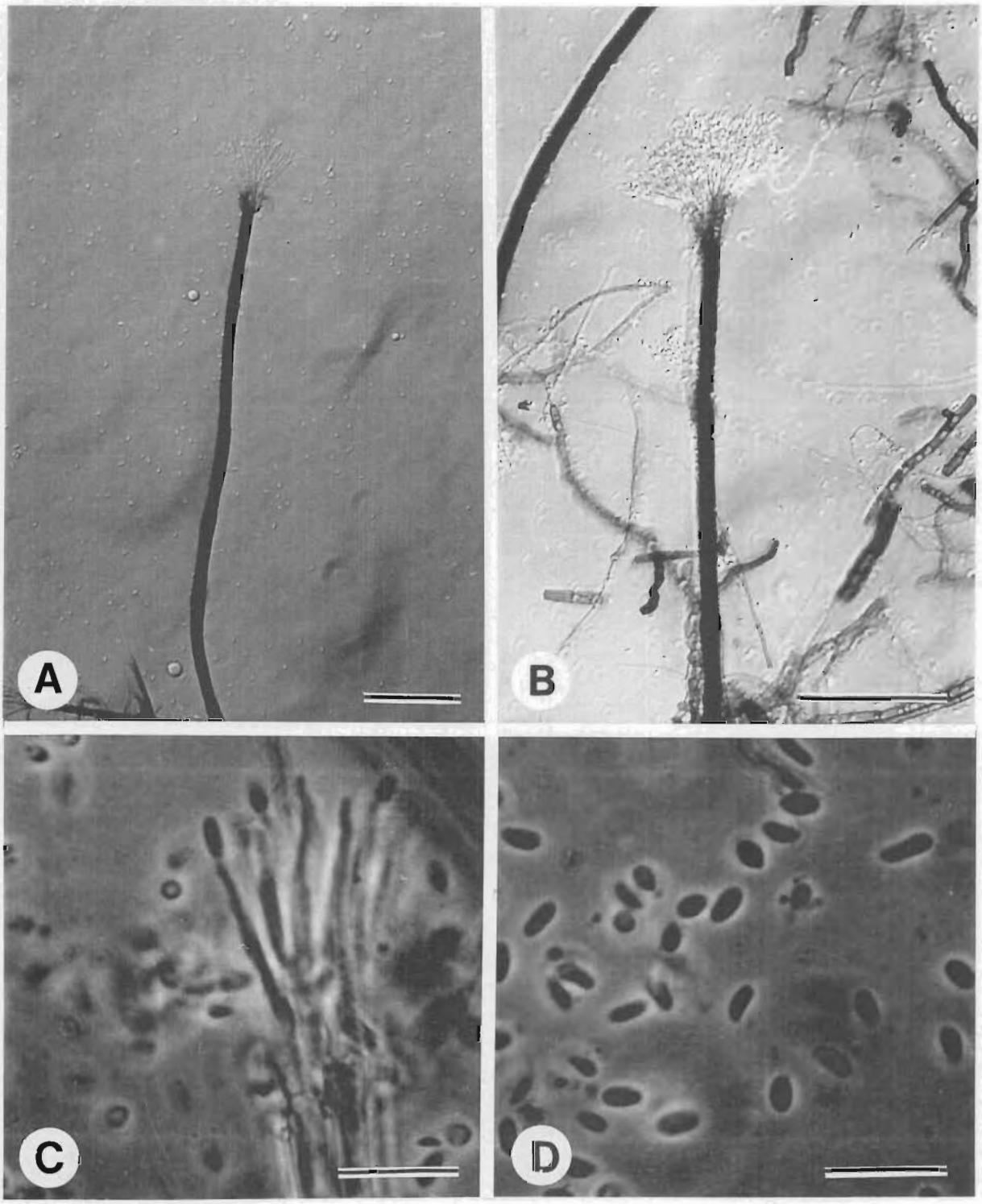

Fig 124. Light micrographs of the conidiophores and conidia of *L. reconditum* (CMW 15). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 50 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

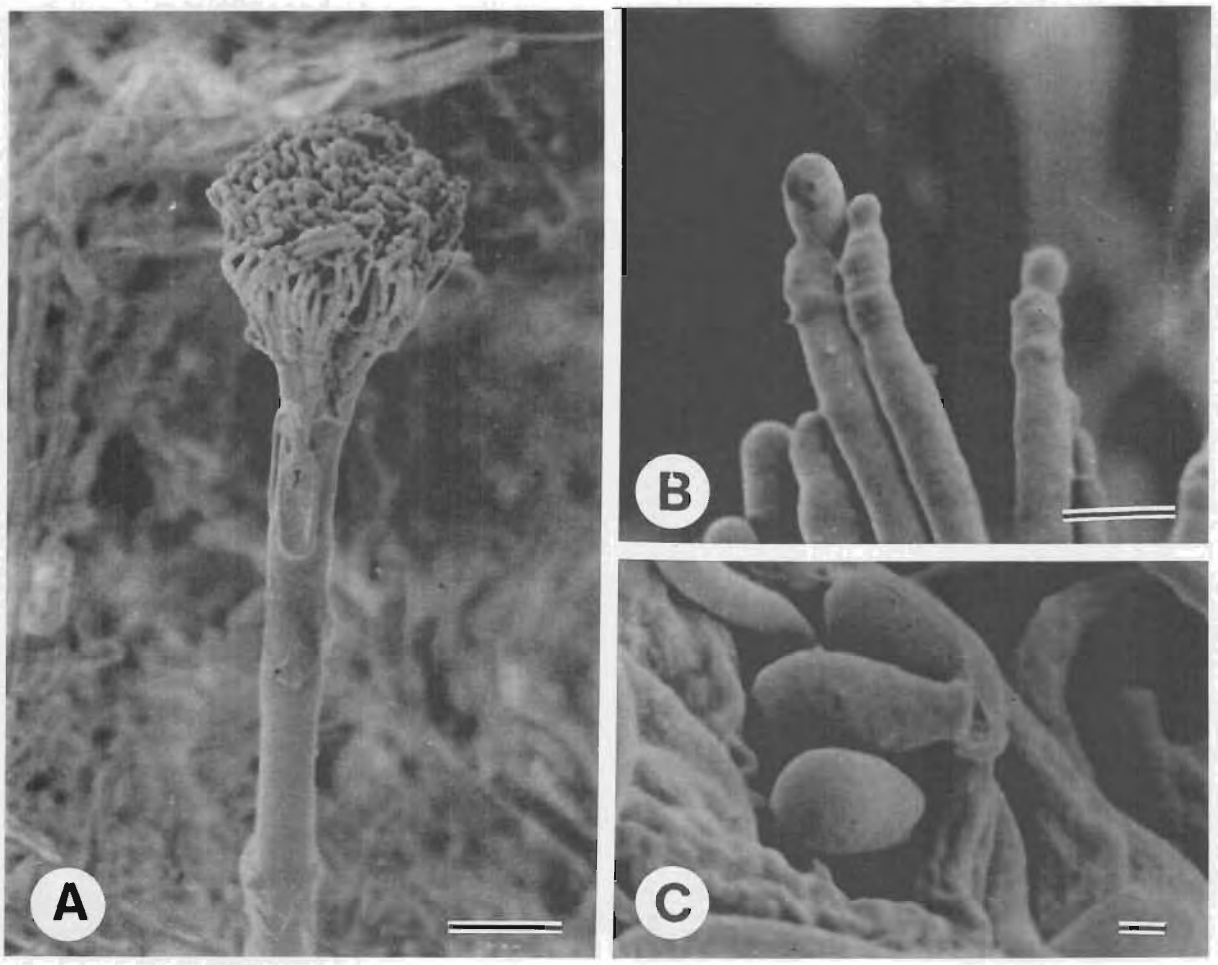

Fig. 125. Scanning electron micrographs of the conidiophores and conidia of *L. reconditum* (CMW 15). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

37. *Ophiostoma robustum* (Rob.-Jeffr. & R.W. Davidson) T.C. Harr., *Mycotaxon* **28**, 42. 1987. **(Figs. 126-128).**

≡ *Ceratocystis robusta* (Rob.-Jeffr. & R.W. Davidson) H.P. Upadhyay, A monograph of *Ceratocystis* and *Ceratocystiopsis*. 1981.

≡ *Europhium robustum* Robinson-Jeffrey, *Canadian Journal of Botany* **46**, 1525. 1968.

Anamorph: *Leptographium robustum* (Rob.-Jeffr. & R.W. Davidson) M.J. Wingf., *Transactions of the British Mycological Society* **85**, 92. 1985.

Etymology: ro-bús-tum: strong, robust. From the Latin adjective robustus: strong, powerful, firm. This specific epithet refers to the robust nature of this fungus.

Perithecial bases black, globose and smooth walled, unornamented, 200 - 400 µm in diam. *Perithecial neck* mostly absent, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* reniform, aseptate, hyaline, invested in a sheath, 3.0 - 5.5 x 2.0 - 3.5 µm. (Robinson-Jeffrey, & Davidson 1968; Upadhyay, 1981).

Conidiophores occurring singly, mostly on aerial mycelium, erect, macronematous, mononematous, 31 - 112 (-116) µm in length, rhizoid-like structures absent. *Stipes* hyaline to light olivaceous, smooth, cylindrical, simple, 0-2 septate, 9.0 - 36 (-39) µm long, 3.0 - 8.0 µm wide below primary branches, apical cell not swollen, 3.0 - 6.0 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* 20 - 67.5 (-70) µm long, excluding the conidial mass, with 1 to 3 series of cylindrical branches, 2-3 primary branches, hyaline to olivaceous (21"m), smooth, cylindrical, occasionally crooked, aseptate 8.0 - 12.5 (-31) µm long and 3.0 - 8.0 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches olivaceous (21"m), aseptate, 8.0 - 17.5 (-18.5) µm long, 2.0 - 4.5 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 8.0 - 23 µm long and (1.5-) 2.0 - 3.0 (-4.5) µm wide. *Conidia* hyaline, aseptate, oblong, with truncate ends and rounded apices, 3.0 -7.0 x 2.0 - 6.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, turning brussels brown (15m) when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 9 mm in diam. in 8 days. No growth below 5°C and little above 35°C. Able to withstand high concentrations of cycloheximide with a 10% increase in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15''''k). *Colony margin* effuse. *Hyphae* submerged on agar with abundant aerial mycelium, hyaline to light olivaceous (21''k), smooth, straight, not constricted at the septa, 3.0 - 12.5 (-15.5) µm diam.

Specimens examined: Holotype: British Columbia, Toby Creek, Invermere, *Pinus ponderosa*, 15 July 1962, collected: R.W. Davidson, BPI 688938. **Cultures**: U.S.A., collected: T. Hinds, CMW 2805 (PREM 56338), U.S.A., Idaho, *Pinus ponderosa,* 1962, collected: R.W. Davidson, CMW 668 (ATCC 16937, CBS 439.69)

Known distribution: Canada, USA.

Host/substrate: *Pinus ponderosa* (Robinson-Jeffrey & Davidson, 1968).

Associated insects: *Dendroctonus* sp. (Robinson-Jeffrey & Davidson, 1968; Perry, 1991).

Notes: This species is one of four *Leptographium* species that had been associated with the teleomorph genus *Europhium.* This fungus can be distinguished from the other species that have resided in *Europhium* based on its smaller, broadly ovoid conidia, and the fact that it produces its teleomorph state readily in culture (Robinson-Jeffrey & Davidson, 1968). The *Leptographium* anamorph of *O. robustum* most closely resembles *L. pineti. Leptographium robustum* can be distinguished from *L. pineti* based on the considerably shorter (31-116 µm) conidiophores in the former species, compared to the relatively longer (100 - 210 µm) conidiophores of the latter species. *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). 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. Ophiostoma robustum* has been

isolated from beetle-infested, blue-stained pines (Robinson-Jeffrey & Grinchenko, 1968).

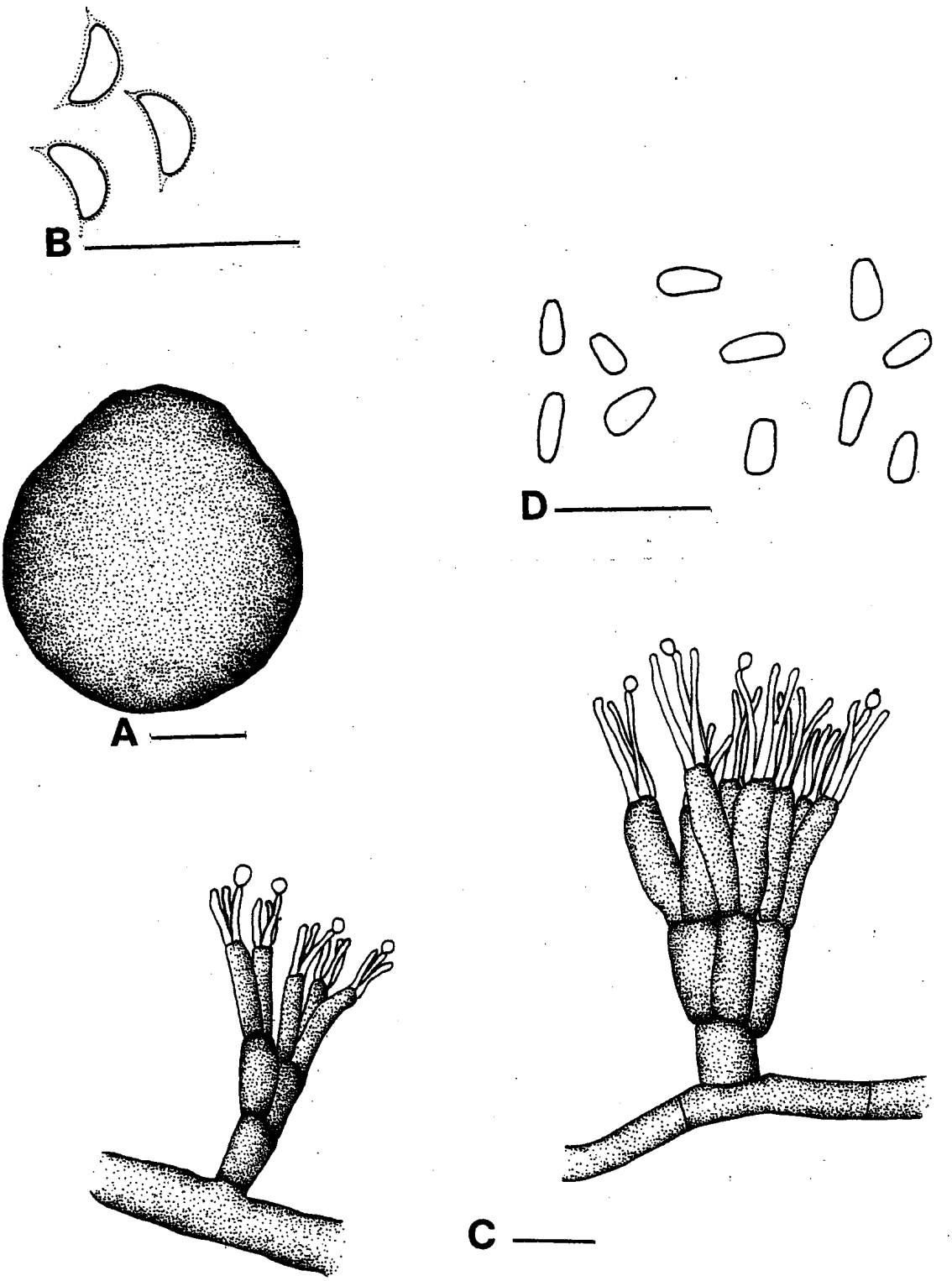

Fig. 126. Teleomorph and anamorph structures of *O. robustum* (PREM 56338). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 50 µm). **D.** Conidia (Bar = 10 µm).

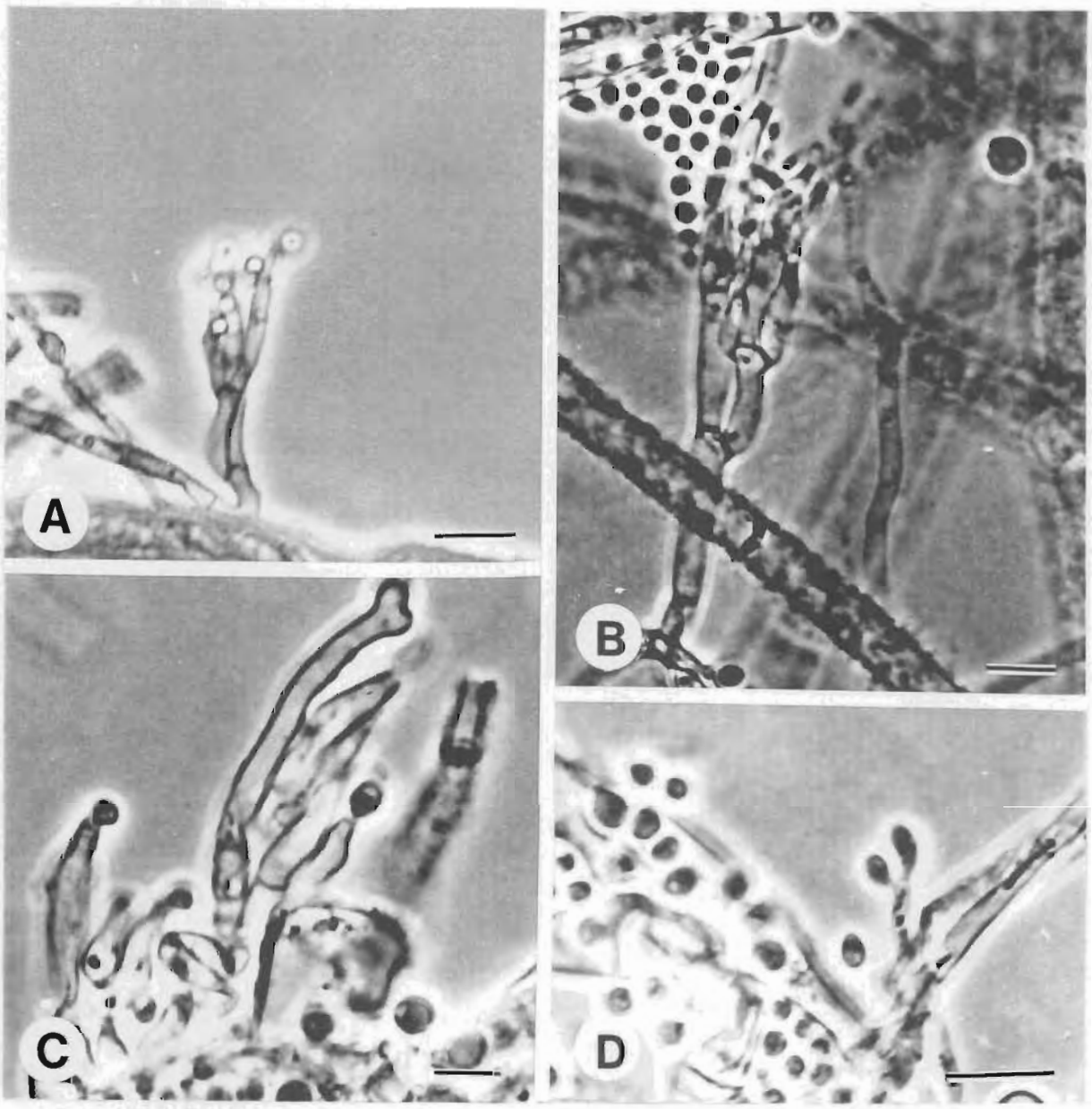

Fig 127. Light micrographs of the teleomorph and anamorph structures of *O. robustum* (PREM 56338). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

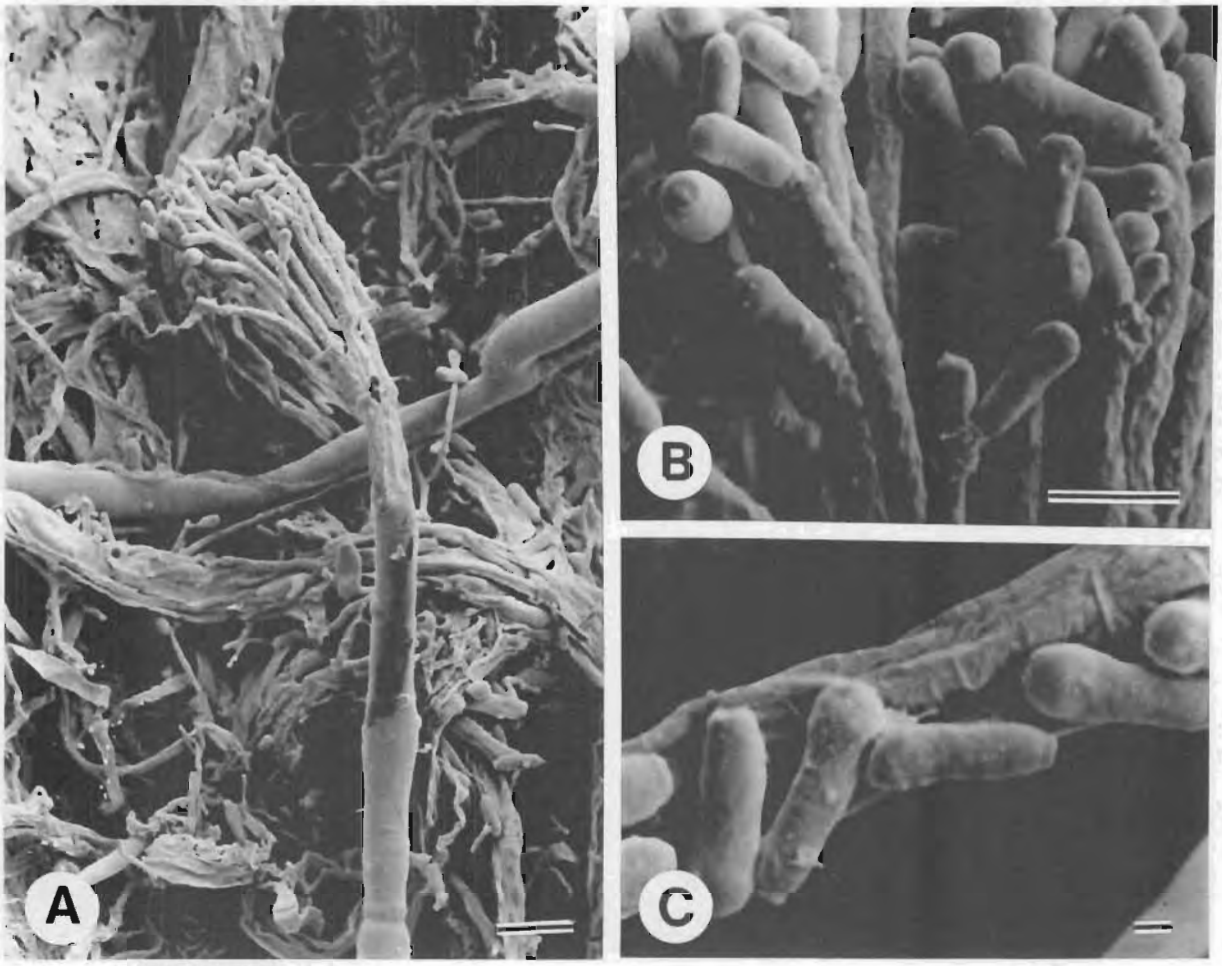

Fig. 128. Scanning electron micrographs of the conidiophores and conidia of *O. robustum* (PREM 56338). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

38. *Ophiostoma serpens* (Goid.) Arx, *Antonie van Leeuwenhoek* **18**, 211. 1952. **(Figs. 129-130).**

≡ *Grosmanniae serpens* Goid., *Boll. Staz. Patol. Roma. N.S.* **16**, 42. 1936.

≡ *Ceratocystis serpens* (Goid.) Moreau, *Rev. Myc. Suppl. Col.* **17**, 22. 1952.

Anamorph: *Leptographium serpens* (Goid.) M.J. Wingf., *Transactions of the British Mycological Society* **85**, 92. 1985.

≡ *Scopularia serpens* Goid., *Boll. Staz. Patol. Roma. N.S.* **16**, 42. 1936.

≡ *Verticicladiella serpens* (Goid.) W.B. Kendr., *Canadian Journal of Botany* **40**, 781. 1962.

= *Verticicladiella alacris* M.J. Wingf. & Marasas, *Transactions of the British Mycological Society* **75**, 22. 1980.

= *Leptographium alacre* (M.J. Wingf. & Marasas) M. Morelet, *Annales de la S.S.N.A.T.V.* **40**, 44. 1988.

= *Leptographium gallaeciae* F.Magan (nom. inval.).

Etymology: sér-pens: serpentine. From the Latin noun serpens: a snake, serpent. The specific epithet refers to the serpentine growth pattern of the mycelium in culture.

Perithecial bases black, globose and smooth walled, unornamented, 300 - 420 µm in diam. *Perithecial neck* black, cylindrical with a slight apical taper, smooth, 400 - 700 µm long, 33 - 65 µm above globose base, 21 - 39 µm wide at the apex, *ostiolar hyphae* absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* ellipsoid, aseptate, hyaline, invested in a sheath, 3.3 - 4.8 x 1.0 - 2.0 µm (Goidanich, 1936).

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (250-) 558.5 - 629.5 (-1270) µm in length, rhizoid-like structures present. *Stipes* dark olivaceous (21"m), smooth, cylindrical, simple, 4-20 septate, (200-) 493 - 592 (-1220) µm long, 7.5 - 12.5 µm wide below primary branches, apical cell occasionally swollen, 7.5 - 15 µm wide at base, basal cell not swollen. *Conidiogenous apparatus* 40 - 65.5 (-70) µm long, excluding the conidial mass, with 2 to 4 series of cylindrical branches, 2-6 primary branches, light

olivaceous (21"k), smooth, cylindrical to barrel-shaped, aseptate, 10 - 22.5 µm long and 5.0 - 12.5 µm wide, arrangement of the primary branches on the stipe - type C, secondary branches light olivaceous (21"k) to hyaline, aseptate, (6.0-) 7.0 - 14 (-17) µm long, 2.0 - 7.0 µm wide, tertiary branches hyaline, aseptate, 6.0 - 13 µm long, 2.0 - 4.0 µm wide, quaternary branches aseptate, (6.0-) 6.5 - 11 (-13) µm long, 2.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, cylindrical, tapering slightly at the apex, (7.0-) 10.5 - 13.5 (-20) µm long and 1.0 - 3.0 µm wide. *Conidia* hyaline, aseptate, oblong with truncate ends and rounded apices, 3.0 - 5.0 x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming amber to dark amber (21'b) with age. Conidial mass amber when wet, turning dark amber when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 36 mm in diam. in 8 days. Little growth below 5°C and above 35°C. Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15""k). *Colony margin* effuse with hyphae extending to the edge of the petri dish. *Hyphae* submerged on agar with no aerial mycelium, light olivaceous (21"k), smooth, serpentine, occasionally constricted at the septa, (1.5-) 3.5 - 5.5(-9.5) µm diam.

Specimens examined: Holotype: Italy, *Pinus sylvestris*, Jan. 1953, collected: G. Goidanich, DAOM 34869. The material is in a bad condition with only a small number of structures. No sexual structures has been observed in the herbarium material. **Cultures**: South Africa, Tokai State Forest, *Pinus pinaster*, collected: M.J. Wingfield, 1986, CMW 60 (same as PREM 45483, DAOM 17660, CMW 2844), Italy, *Pinus pinea,* 1986, collected: P. Capretti, CMW 193; South Africa, Jonkershoek, *Pinus radiata,* 1987; collected M.J. Wingfield, CMW 310 (PREM 56334), Italy, 1987; collected: P. Gambogi and G. Lorenzini, CMW 290 (CBS 67.76), Portugal, Serrade sta Luzia, *Pinus pinaster,* 1987, collected: Maria de Fatima Mariz, CMW 623; France, La Garde Freinet, *Pinus pinea,* 1987, collected: M. Morelet, CMW 748; England, Yateley, Heathwood Hampshire, from *Pinus* sp. infested with *Hylastes ater,* 1988, collected: J. Gibbs, CMW 1376.

Known distribution: Europe and South Africa.

Hosts/substrate: *Pinus monticola* (Gill *et al.*, 1951), *Pinus nigra* (Morelet, 1988), *Pinus taeda* (Gill *et al.*, 1951), *Pinus sylvestris* (Goidanich, 1936; Kendrick, 1962; Morelet, 1988; Wingfield, Gibbs, 1991), *Pinus pinaster* (Wingfield & Knox-Davies, 1980a; Wingfield & Marasas, 1980; Wingfield *et al.,* 1988), *Pinus pinea* (Wingfield *et al.,* 1988), *Pinus radiata* (Wingfield & Knox-Davies, 1980; Wingfield & Marasas, 1980; Wingfield *et al.,* 1988), *Pseudotsuga menziesii* (Mielke, 1979).

Associated insects: *Hylastes angustatus* (Harrington, 1988; Wingfield *et al.,* 1988), *Hylastes ater* (Wingfield & Gibbs, 1991), *Hylastes linearis* (Harrington, 1988), *Hylurgus ligniperda* (Harrington, 1988; Wingfield *et al.,* 1988; Wingfield & Knox-Davies, 1980a), *Myelophilus piniperda* (Siemaszko, 1939; Harrington, 1988), *Orthotomicus erosus* (Wingfield & Knox-Davies, 1980a).

Notes: Goidanich (1936) first described this fungus as a species of *Scopularia.* Although most authors at that time used *Leptographium,* Goidanich argued for the use of *Scopularia* and transferred several species of *Leptographium* to *Scopularia.* Hunt (1956) considered *O. penicillatum* to be a synonym of *O. serpens.* However, these fungi differ markedly in their conidial morphology and could easily be distinguished based on the small obovoid conidia of *O. serpens,* in contrast to the large allantoid conidia of *O. penicillatum.* Kendrick (1962) considered the arrangement of the branches on the conidiogenous apparatus to be distinct and unique to *L. serpens.* Gambogi and Lorenzini (1977) made a detailed study of the conidiophores produced by this species. They observed the formation of lateral branches similar to those formed in *L. brachiatum.* They also commented on the complexity of the branching in this fungus.

Wingfield and Marasas (1980) described *Verticicladiella alacris* for a species of *Leptographium* (*Verticicladiella*) associated with a root disease on *Pinus* spp. in South Africa. However, they did not compare *V. alacris* with *L. serpens* due to the fact that *L. serpens* was described as having lateral outgrowths on the conidiophores and these were absent in *V. alacris.* They considered the serpentine hyphae as

unique to *L. serpens* and the chief characteristic distinguishing this species from other *Leptographium* spp. Later, side branches found in *L. serpens* (Gambogi & Lorenzini, 1977), were also observed in isolates of *V. alacris* and based on this character as well as the serpentine hyphal patterns, *V. alacris* was reduced to synonymy with *L. serpens* (Wingfield and Marasas, 1981). *Leptographium serpens* is considered to be similar to the *pini* form of *O. penicillatum* described by Mathiesen (Harrington, 1988). Unfortunately, no records or specimens exist for the ascocarp material of *L. serpens* or the variety described by Mathiesen (1950). The only material available from the original collections is an isolate of *L. serpens,* that does not produce ascocarps (Harrington, 1988). Based on the lack of teleomorph material and the fact that this might not have been appropriately connected, Upadhyay (1981) and Harrington (1988) proposed that only the anamorph name be used.

Leptographium serpens has been associated with a root disease of *Pinus pinea* in Italy (Lorenzini & Gambogi, 1976). A similar disease was later found in on *Pinus radiata* and *P. pinaster* in South Africa (Wingfield & Knox-Davies, 1980a; Wingfield & Marasas, 1980; 1981). There have been some reports of this fungus from the USA, although these are of dubious validity (Harrington, 1988). In South Africa, *L. serpens* was reported to behave as a typical root infecting fungus, spreading to adjacent trees through root contacts. It colonizes both the ray parenchyma as well as the tracheids resulting in wedge shaped patterns of discoloration (Wingfield & Knox-Davies, 1980a; Wingfield & Marasas, 1980). Wingfield *et al.* (1988) suggested that the primary pathogenicity of this fungus has not been conclusively established and that the combined feeding activity of the insects and the subsequent colonization by the fungus could result in tree death. Two root feeding insects, *Hylurgus ligniperda* and *Hylastes angustatus,* are associated with this fungus and apparently act as vectors (Wingfield *et al.,* 1988).

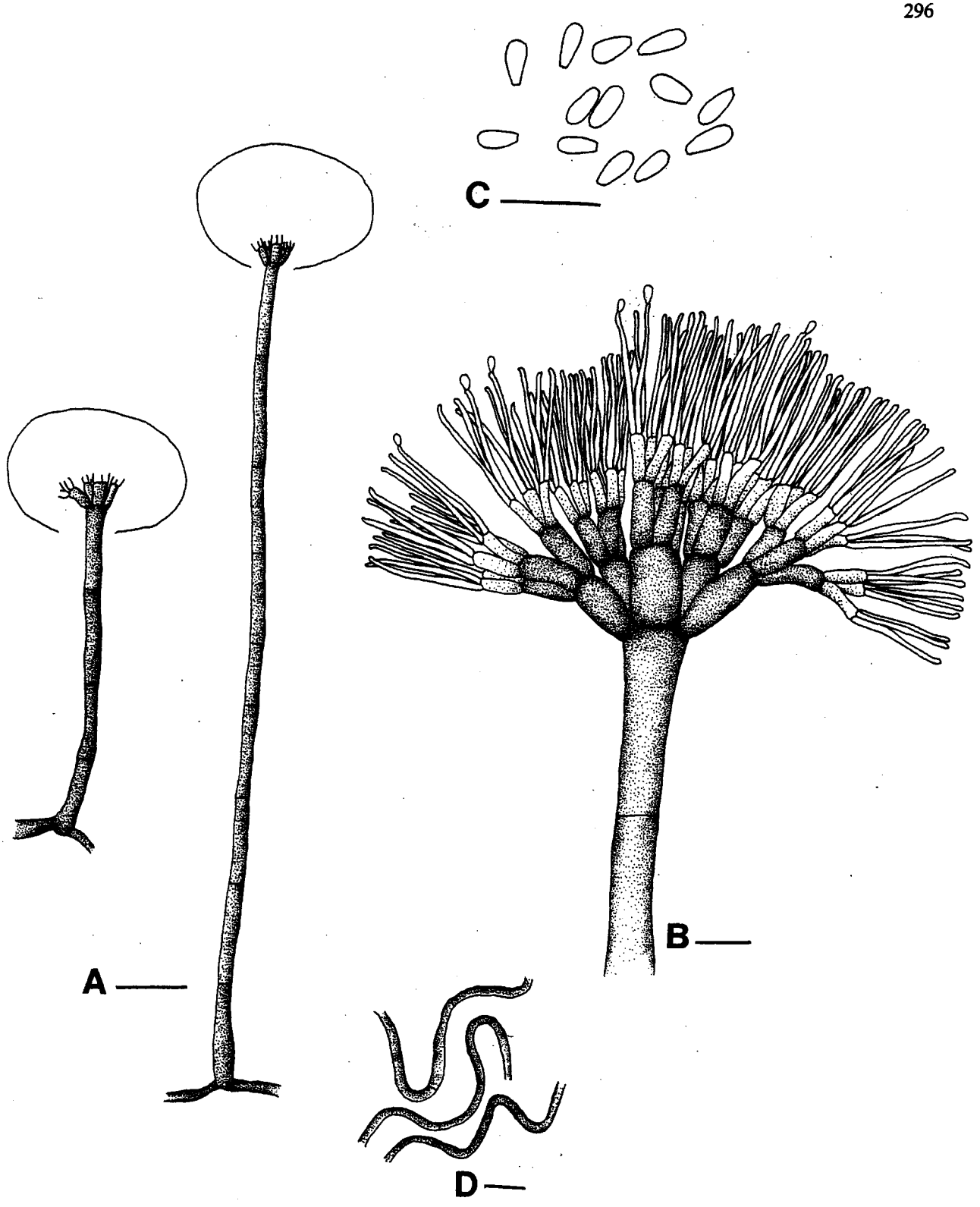

Fig. 129. Anamorph structures of *L. serpens* (PREM 45483). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm). **D.** Serpentine hyphae (Bar = 10 µm).

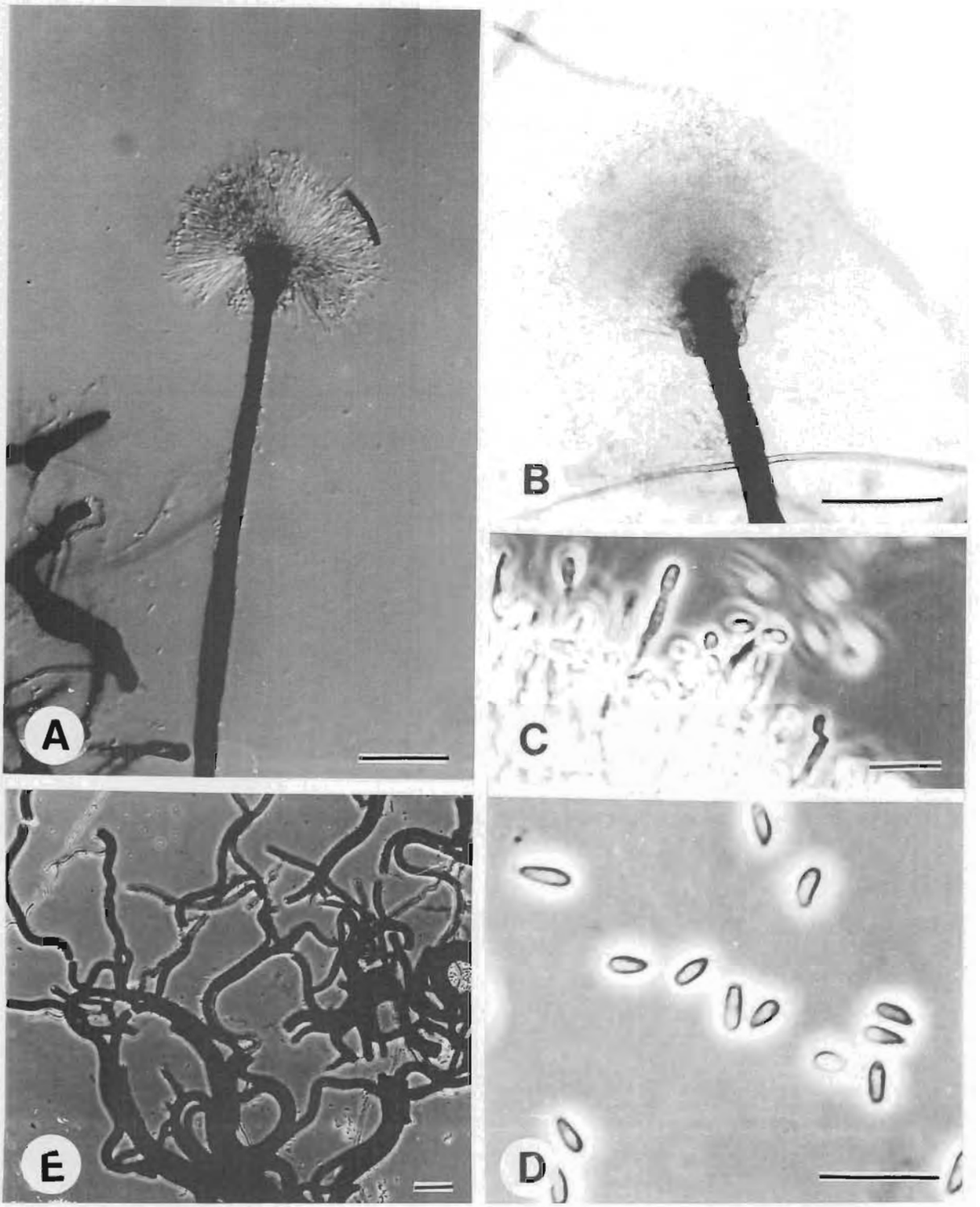

Fig 130. Light micrographs of the anamorph structures of *L. serpens* (PREM 45483). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm). **E.** Serpentine hyphae (Bar = 10 µm).

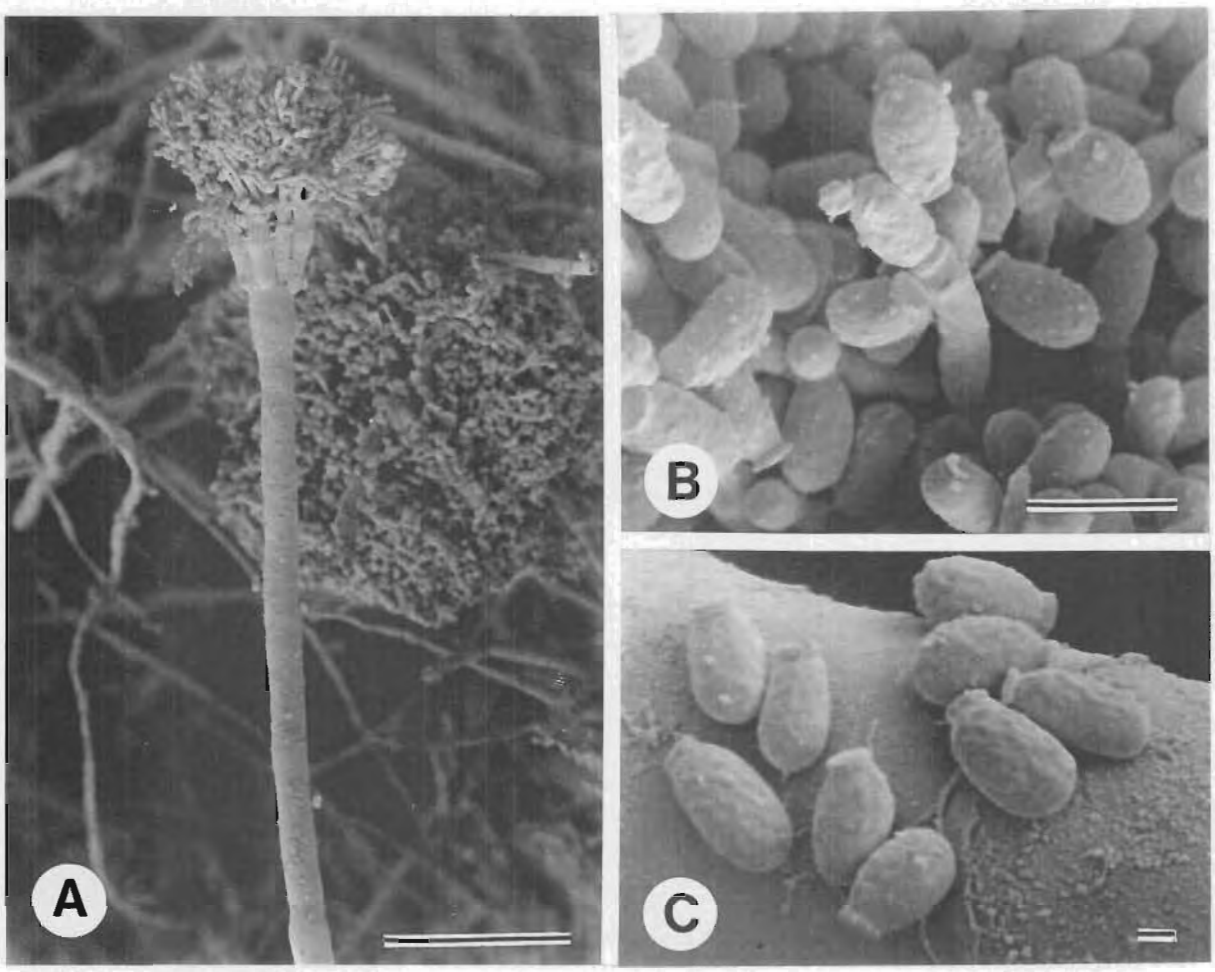

Fig. 131. Scanning electron micrographs of the conidiophores and conidia of *L. serpens* (PREM 45483). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 1 µm).

39. *Leptographium sibiricum* K. Jacobs & M.J. Wingf., *Mycological Research.* 1999. **(Figs. 132-134).**

Teleomorph: Not known.

Etymology: si-bí-ri-cum: Siberian. This specific epithet refers to Siberia, Russia, from where this fungus is derived.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (109-) 146 - 184 (-238) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous, smooth, cylindrical, simple, 2-7 septate, (68-) 104 - 153 (-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-) 5.0 - 6.0 (-8.0) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (26-) 36 - 43 (-56) long, excluding the conidial mass, with 2 to 3 series of cylindrical branches; arrangement of primary branches - type B, 2-3 primary branches, light olivaceous, smooth, cylindrical, aseptate, (8.0-) 13 - 14 (-25) µm long and (2.0-) 3.0 - 4.0 (5.0) µm wide, secondary branches hyaline, light olivaceous aseptate, (8.0-) 11 - 12 (-17) µm long, 2.0 - 3.0 µm wide, tertiary branches hyaline, aseptate, (5.0-) 6.0 - 11 (-12) µm long, 1.0 – 3.0 µm wide. Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (6.0-) 11 - 15 (-20) µm long and 1.0 - 3.0 µm wide. Conidia oblong, 2.0 - 6.0 x 1.0 – 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

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. Colonies dark olivaceous (19"f). Colony margin smooth. Hyphae submerged or on top of agar with no aerial mycelium, light olivaceous to hyaline, smooth, not constricted at the septa, 2.0 - 5.0 (-7.0) µm.

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

Krasnoyarsk Territory, (about 60 of north latitude and 90 of east longitude), isolated from a larval gallery of *M. urussovi* in phloem of *Abies sibirica*, Aug. 1993, collected: V.P. Vetrova, CMW 4484; Russia, Taseevo, Krasnoyarsk Territory, (about 57 of north latitude and 94 of east longitude), isolated from egg chambers of *M. urussovi* in the phloem of *A. sibirica* damaged by the Siberian moth, *Dendrolimus superans sibiricus* Tschetv., July.,1996, collected: V.P. Vetrova, CMW 4479 and CMW 4481, 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), July,1996, collected: V.P. Vetrova, CMW 4487

Known distribution: Siberia, Russia.

Hosts/substrate: *Abies sibirica* (Jacobs *et al.*, 1999).

Associated insects: *Monochamus urussovi* (Jacobs *et al.*, 1999).

Notes: *Leptographium sibiricum* is similar to *L. brachiatum, L. elegans, L. antibioticum* and the *Leptographium* anamorphs of *Ophiostoma grandifoliae* and *O. leptographioides* (Jacobs *et al.*, 1999). *Leptographium sibiricum* and *L. antibioticum* are both characterized by short conidiophores, although those of *L. antibioticum* can be slightly longer. 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 on the conidiophores. *Leptographium sibiricum* has two or three branches, whereas *L. antibioticum* can have up to five primary branches.

Leptographium sibiricum and *L. brachiatum* have conidiophores of similar length. They also have conidia of similar shape and size. 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. 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*.

Leptographium sibiricum, *O. grandifoliae* and *O. leptographioides* cannot be distinguished based on conidiophore length or conidial shape. 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.

Leptographium sibiricum is associated with blue-stained fir infested by *M. urussovi*. The role of *L. sibiricum* in the life cycle of the beetle, or its role as pathogen, is not known. However, it has been suggested that fungi, carried by *M. urussovi,* have a role in the desiccation of branches (Isaev et al., 1988; Jacobs *et al.,* 1999).

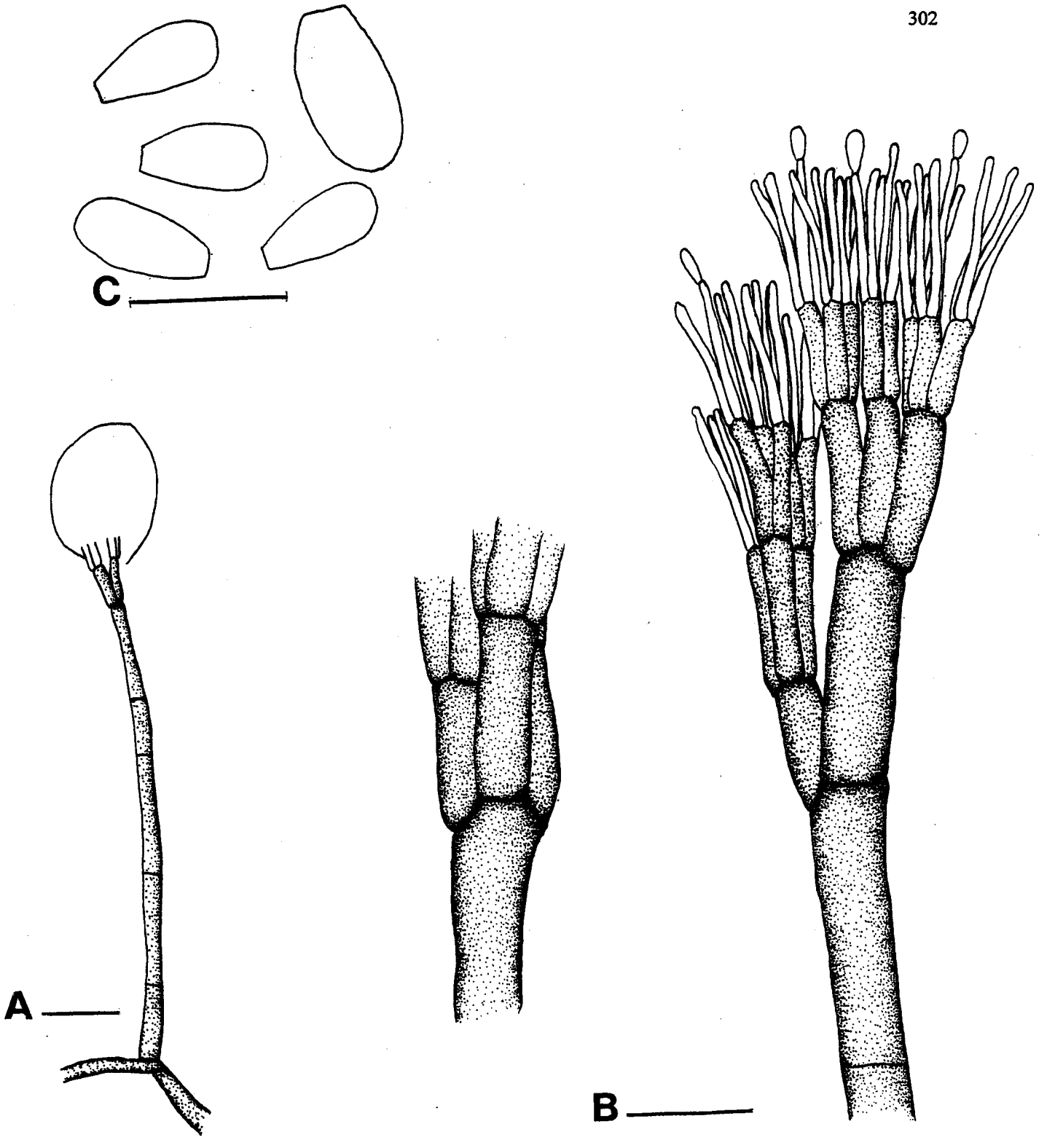

Fig. 132. Conidiophores and conidia of *L. sibiricum* (CMW 4484). **A.** Habit sketch (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

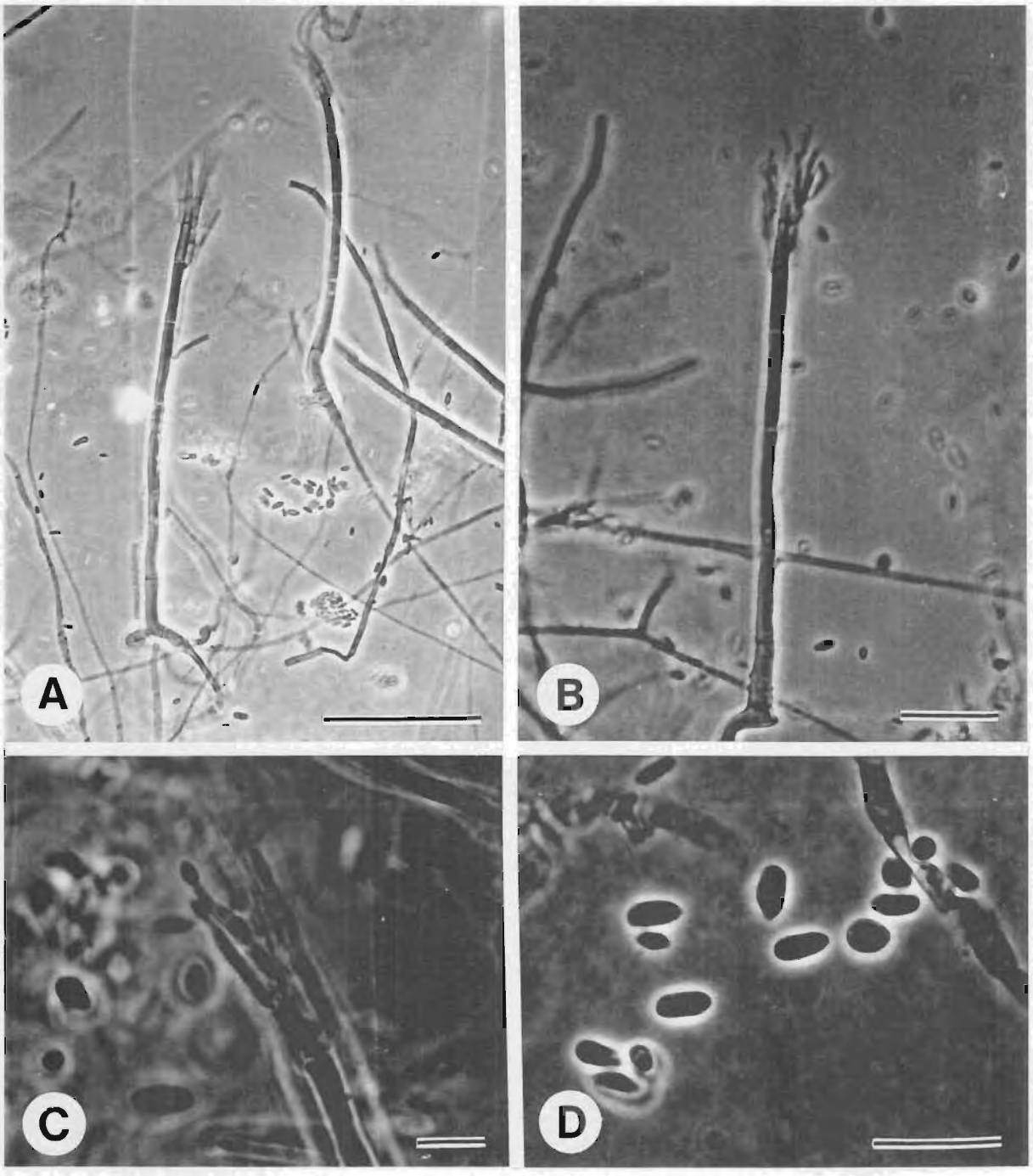

Fig 133. Light micrographs of the anamorph structures of *L. sibiricum* (CMW 4484). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiophore (Bar = 20 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

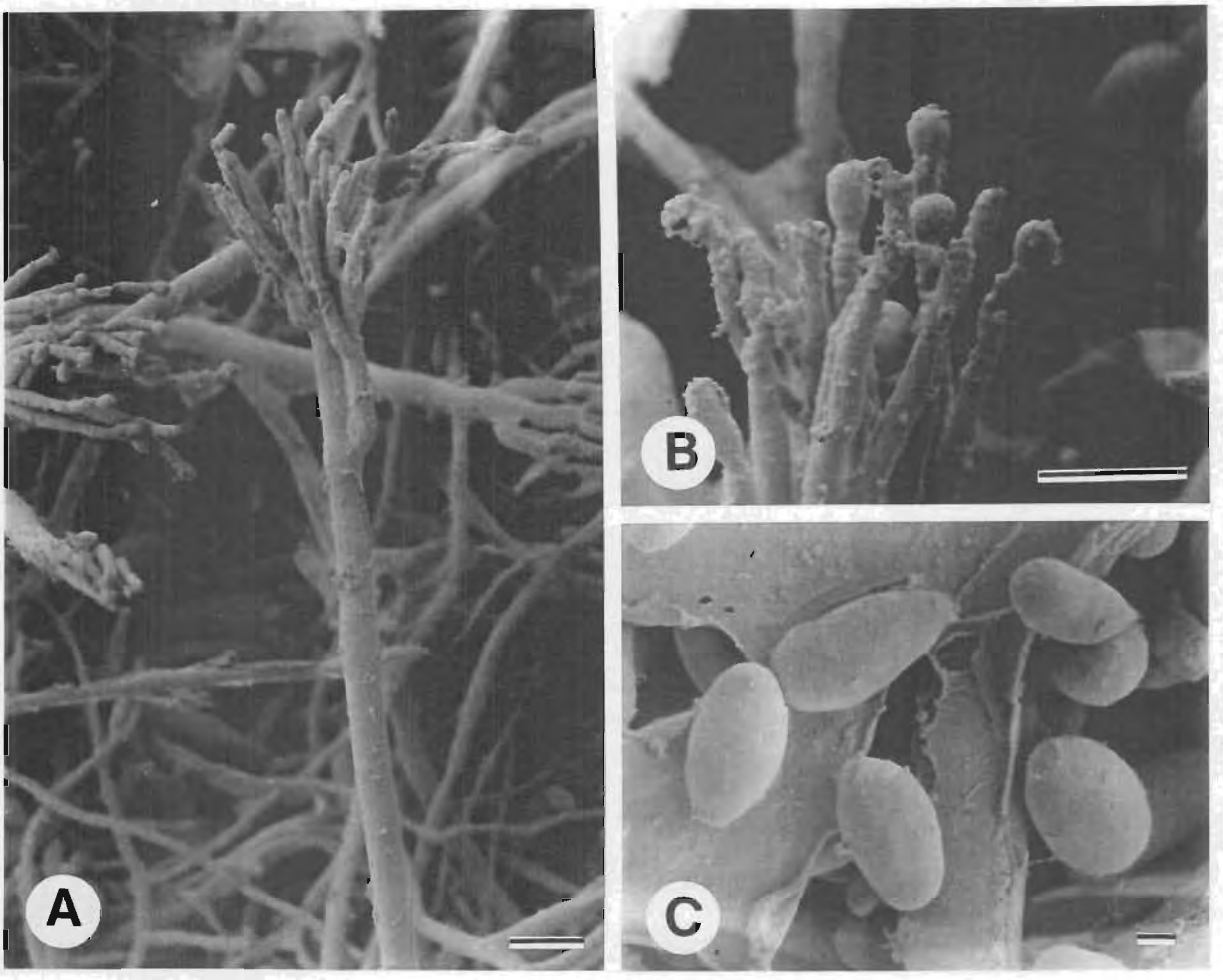

Fig. 134. Scanning electron micrographs of the conidiophores and conidia of *L. sibiricum* (CMW 4484). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 1 µm).

40. *Leptographium terebrantis* S.J. Barras & T.J. Perry, *Mycopatologia et Mycologia Applicata* **43**, 3. 1971. **(Figs. 135-136).**

Teleomorph: Not known.

Etymology: te-re-brán-tis: of *Dendroctonus terebrans*. This specific epithet refers to the association of this fungus with the bark beetle, *Dendroctonus terebrans*.

Conidiophores occurring singly or in groups of up to 4, arising directly from the mycelium, occasionally on aerial mycelium, erect, macronematous, mononematous, (142.5-) 245 - 438 (-508.5) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k) to olivaceous (21"m), smooth, cylindrical, simple, 4 - 11 septate, (87-) 238.5 - 310 (-434) µm long, 5.0 - 8.0 (-10) µm wide below primary branches, apical cell not swollen, (5.0-) 7.0 - 10.5 (-12.5) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (37-) 38 - 99.5 (-105.5) µm long, excluding the conidial mass, with 1 to 4 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k) to olivaceous (21"m), smooth, cylindrical, 0-1 septate, (13-) 20 - 20.5 (-39) µm long and 3.0 - 6.0 µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, aseptate, 9.0 - 30 µm long, 3 - 6 µm wide, tertiary branches hyaline, aseptate, 9 - 22 µm long, 2 - 5 µm wide, quaternary branches aseptate, 9 - 10 µm long, 2 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, cylindrical, tapering slightly at the apex, (9.0-) 14 - 15.5 (-22) µm long and 2.0 - 3.0 µm wide. *Conidia* hyaline, aseptate, obovoid with truncate ends and rounded apices, (4.0-) 5.5 -10 x 2.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, turning amber (21'b) when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 16 mm in diam. in 8 days. No growth below 5°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 12% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies olivaceous black (21""m). *Colony margin* laciniate. *Hyphae* submerged on agar with little aerial mycelium, hyaline to light olivaceous,

smooth, straight, occasionally constricted at the septa, walls roughened by granular material, (1.5-) 3.0 - 5.5 (-6.0) µm diam.

Specimens examined: Holotype: U.S.A., Elisabeth, LA, *Pinus taeda* bark in association with *Dendroctonus terebrans* pupa, 29 Sept 1966, collected: S.J. Barras, DAOM 128706. **Cultures:** U.S.A., Minnesota, *Pinus sylvestris*, 1986, collected: M.J. Wingfield, CMW 9 (same as ATCC 58098, CMW 2814), U.S.A., Minnesota, *Pinus sylvestris*, 1986, collected: M.J. Wingfield, CMW 45; U.S.A., California, from *Dendroctonus valens*, 1986, collected: T.C. Harrington, CMW 47; U.S.A., from *D. terebrans*, 1966, collected: S.J. Barras, CMW 663 (CBS 377.70, DAOM 128706), U.S.A., Cape Cod, *Pinus thunbergiana*, 1985, collected: T.Tattar, CMW 757 (PREM 56329), CMW 758 (PREM 56330).

Known distribution: U.S.A.

Hosts/substrate: *Pinus sylvestris* (Highley & Tattar, 1985; Highley & Tattar, 1987; Bennet & Tattar, 1988), *Pinus thunbergiana* (Highley & Tattar, 1985; Highley & Tattar, 1987; Bennet & Tattar, 1988), *Pinus taeda* (Barras & Perry, 1971a), *Pinus banksiana* (Wingfield, 1983), *Pinus ponderosa* (Harrington, 1988), *Pinus resinosa* (Wingfield, 1983; Bennet & Tattar, 1988; Harrington, 1988), *Pinus edulis* (Harrington, 1988), *Pinus strobus* (Wingfield, 1983; Harrington, 1988), *Pseudotsuga menziesii* (Harrington, 1988).

Associated insects: *Dendroctonus frontalis* (Otrosina *et al.,* 1997), *Dendroctonus valens* (Harrington, 1982; Harrington & Cobb, 1983; Harrington, 1988; Perry, 1991), *Dendroctonus terebrans* (Barras & Perry, 1971a; Wingfield, 1983; Highley & Tattar, 1985; Highley & Tattar, 1987; Bennet & Tattar, 1988; Harrington, 1988; Perry, 1991), *Dendroctonus pseudotsugae* (Lewinsohn *et al.,* 1994); *Hylurgops porosus* (Harrington, 1982; Harrington & Cobb, 1983; Harrington, 1988), *Hylobius radicis* (Wingfield, 1983), *Hylobius rhizophagus* (Wingfield, 1983), *Ips pini* (Bennet & Tattar, 1988).

Notes: *Leptographium terebrantis* can be distinguished from other *Leptographium*

spp. by its unique arrangement of the primary branches. In isolates of this species, the primary branches are all arranged on the same plane around the apex of the stipe. This is in contrast to most other *Leptographium* spp., where the primary branches are arranged on different levels.

Wingfield (1983) found *L. terebrantis* to be considerably more virulent than *L. procerum* with which it is sometimes collected. Inoculation studies with this species produced lesions in seedlings, as well as in mature trees. Infection studies by Harrington and Cobb (1983) have showed that *L. terebrantis* is capable of killing wounded and unwounded pine, but did not infect douglas-fir. This fungus is known to cause discoloration of the sapwood (Harrington & Cobb, 1983) and was found to cause heavy resinosis and death in pine seedlings (Bennet & Tattar, 1988; Nevill *et al.,* 1995). Infection of *Pinus resinosa* and *P. banksiana* with *L. terebrantis* did not prove to be lethal, but stressed trees sufficiently to be attacked by other beetle-fungus complexes (Raffa & Smalley, 1995). It was also found to be able to cause blue-stain in Japanese pine (*Pinus thunbergiana*) and Scots pine (*P. sylvestris)* after attack by *Dendroctonus terebrans* (Highley & Tattar, 1987). *Leptographium terebrantis* appears to be important in the case of beetle attack (Harrington, 1988) and has also been isolated from trees attacked by *Dendroctonus frontalis* (Highley & Tattar, 1985; 1987; Otrosina *et al.,* 1997).

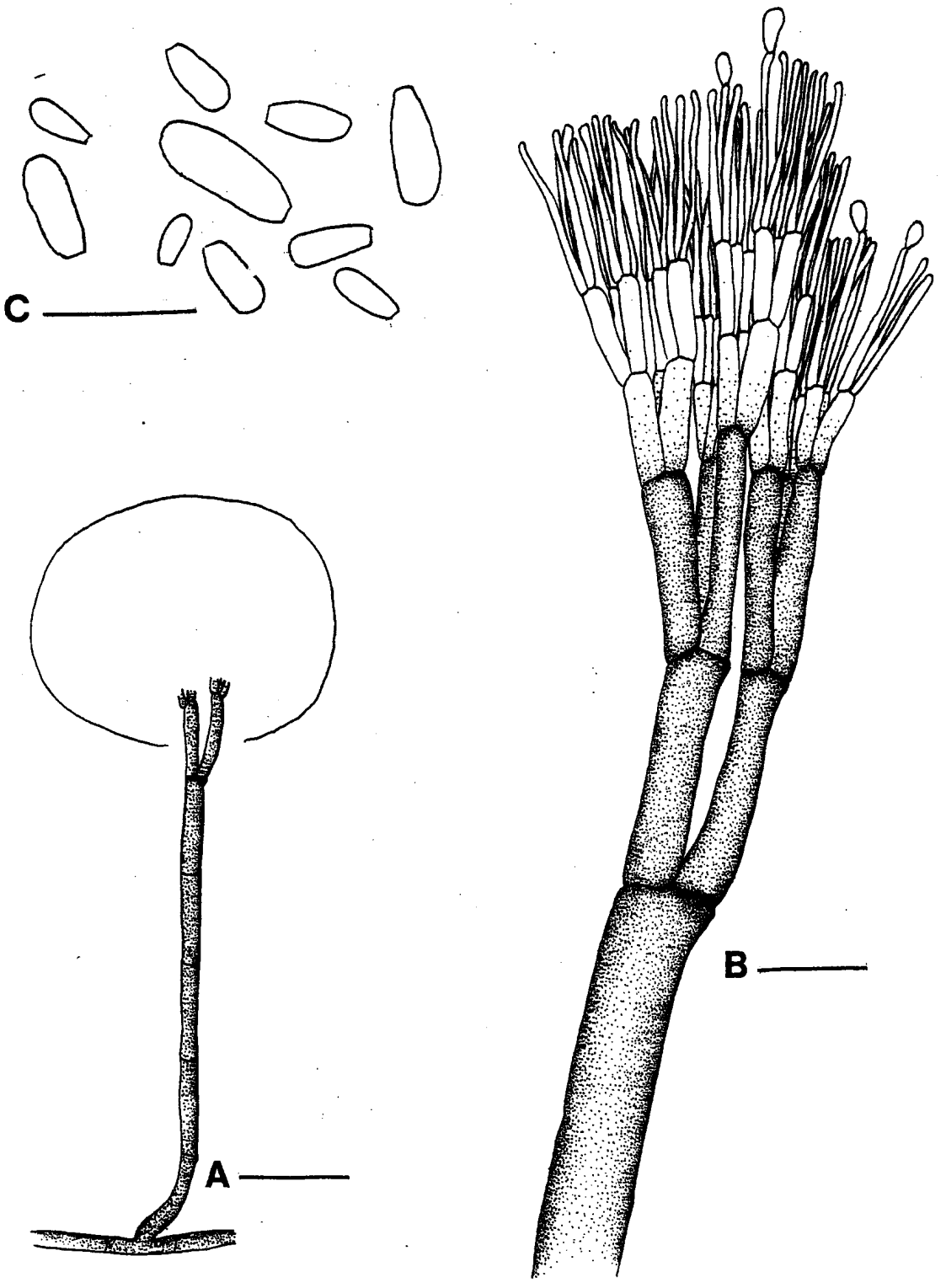

Fig. 135. Conidiophores and conidia of *L. terebrantis* (CMW 9). **A.** Habit sketch (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

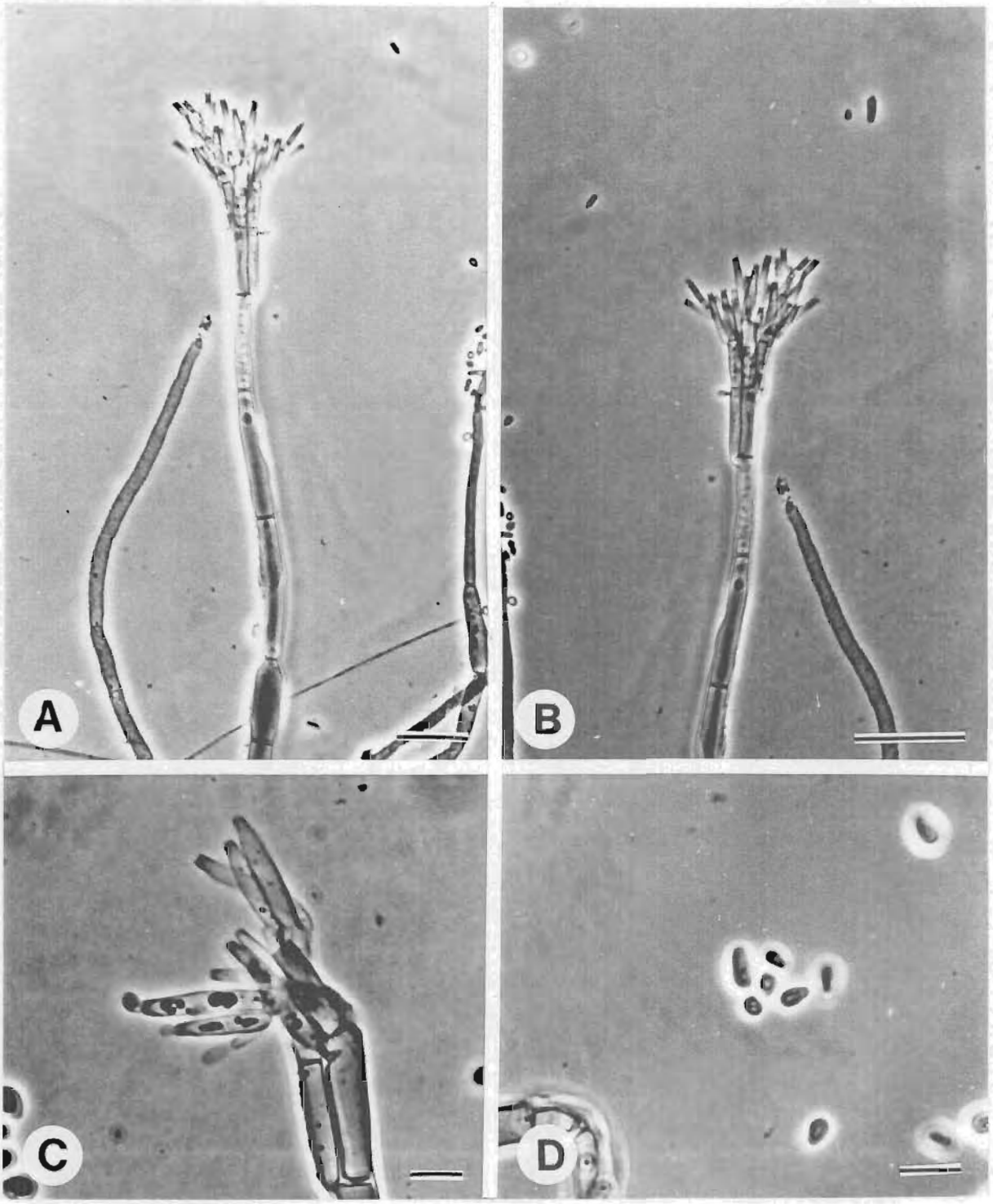

Fig. 136. Light micrographs of the conidiophores and conidia of *L. terebrantis* (CMW 9). **A**. Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 50 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

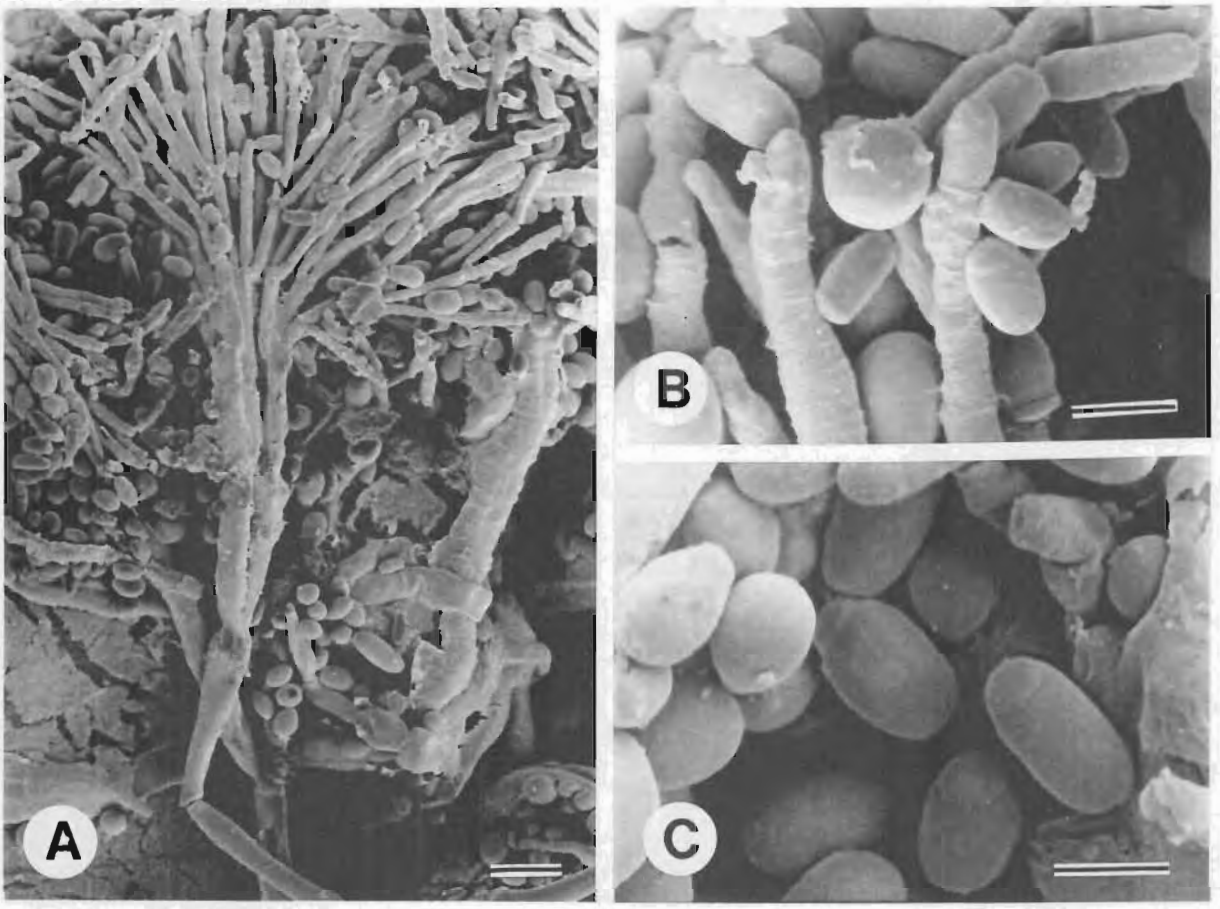

Fig. 137. Scanning electron micrographs of the conidiophores and conidia of *L. terebrantis* (CMW 9). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 5 µm).

41. *Ophiostoma trinacriforme* (A.K. Parker) T.C. Harr., *Mycotaxon* **28**, 42. 1987. **(Figs. 138-140).**

≡ *Europhium trinacriforme* A.K. Parker, *Canadian Journal of Botany* **35**, 175. 1957.

≡ *Ceratocystis trinacriforme* (A.K. Parker) H.P. Upadhyay, A monograph of *Ceratocystis* and *Ceratocystiopsis*. 1981.

Anamorph: *Leptographium trinacriforme* K. Jacobs & M.J. Wingf. sp. nov.

Etymology: trin-a-cri-fór-me: with three sharp sides. From the Latin adjectives trinus: three at a time; acriter: sharp and formo: form, shape. This specific epithet refers to the three primary branches that are found on the stipes of this fungus.

Perithecial bases black, globose, leathery, (225-) 260 (-345) µm in diam., no perithecial neck observed. *Asci* prototunicate, hyaline, evanescent. *Ascospores* cucullate, aseptate, hyaline, invested in a sheath, (3.6-) 4.8 (-5.4) x (1.8-) 2.2 (-3.1) µm (Parker, 1957a).

Conidiophores occurring in groups of up to 8, mostly on aerial mycelium, erect, macronematous, mononematous, (125-) 207 - 377 (-662.5) µm in length, rhizoid-like structures absent. *Stipes* hyaline to light olivaceous (21"k), smooth, cylindrical, simple, 3-10 septate, (70-) 145 - 297 (-587.5) µm long, 5.0 - 7.5 µm wide below primary branches, apical cell not swollen, (5.0-) 6.0 - 13.5 (-15) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (35-) 58.5 - 72.5 (-95) µm long, excluding the conidial mass, with 1 to 3 series of cylindrical branches, 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical, 0-1 septate (14-) 18.5 - 23.5 (-32) µm long and (2.5-) 4.0 - 5.0 (-7.5) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches hyaline, aseptate, (10-) 13.5 - 18.5 (-21) µm long, (2.0-) 2.5 - 3.5 (-6.0) µm wide, tertiary branches hyaline, aseptate, (9.0-) 11.5 - 15 (-19) µm long, 2.0 - 4.0 µm wide, quaternary branches aseptate, 8.5 - 12.5 µm long, 2.0 - 2.5 µm wide. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (8.0-) 10 - 23 µm long and 1.0 - 2.5 µm wide. *Conidia* hyaline, aseptate, oblong to obovoid with truncate ends and

rounded apices, 4.0 - 6.5 (-7.5) x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, cream colored (21'f) at first, becoming amber (21'b) with age. Conidial mass cream colored when wet, remaining cream colored when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 40 mm in diam. in 7 days. No growth below 5 °C or above 35°C. Able to withstand high concentrations of cycloheximide with a 29% reduction in growth on 0.5 g/l cycloheximide after 4 days at 20°C in the dark. Colonies wood brown (17"') becoming olivaceous (21"m) with age. *Colony margin* laciniate. *Hyphae* submerged on agar with abundant aerial mycelium, light olivaceous (21"k), smooth, straight, occasionally constricted at the septa, (1.5-) 4.5 - 6.5 (-11) µm diam.

Specimens examined: Holotype: Canada, British Columbia, Silverton, *Pinus monticola*, August 1952, collected: A.K. Parker, DAOM 41301. **Paratypes:** Canada, British Columbia, Silverton, *Pinus monticola*, August 1952, collected: A.K. Parker, DAOM 41307, DAOM 41306, DAOM, 41305. **Cultures**: Canada, British Columbia, Silverton, *Pinus monticola*, August 1952, collected: A.K. Parker, CMW 670.

Known distribution: Canada.

Host/substrate: *Pinus monticola* (Parker, 1957a).

Associated insects: Not known.

Notes: *Ophiostoma trinacriforme* is similar to *O. piceaperdum*. However, these species can easily be distinguished based on their teleomorph characters. *Ophiostoma trinacriforme* is characterized by perithecia without necks, whereas those of *O. piceaperdum* has long necks. Both species have cucullate ascospores. In the absence of perithecia, these species can be distinguished based on the brush-like conidiogenous apparatuses of *L. piceaperdum,* in contrast to the thinner, more delicate conidiogenous apparatuses of *L. trinacriforme*. The conidia of *L. trinacriforme* are also oblong, in comparison to the obovoid conidia of *L.*

piceaperdum.

Very little is known regarding the pathogenicity of this fungus or its ecology. In contrast to most other species of *Leptographium,* this species has never been associated with a vector. Parker (1957a) indicated that it might be the causal agent of pole blight in Western white pine *(Pinus monticola*). However, later studies indicated that *L. trinacriforme* is not capable of causing the lesions associated with pole blight (Parker, 1957b; Harrington, 1988).

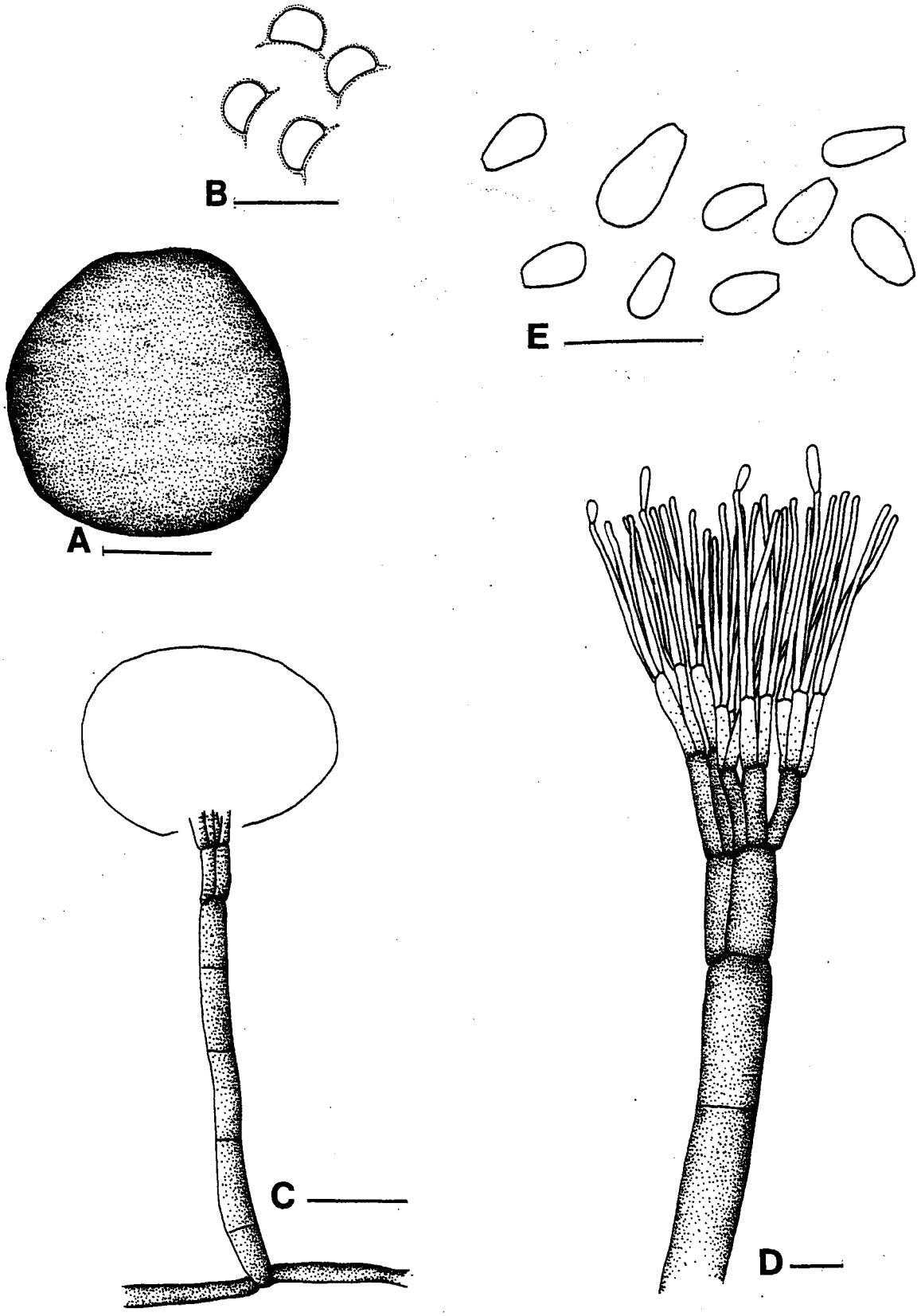

Fig. 138. Teleomorph and anamorph structures of *O. trinacriforme* (CMW 670). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 100 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

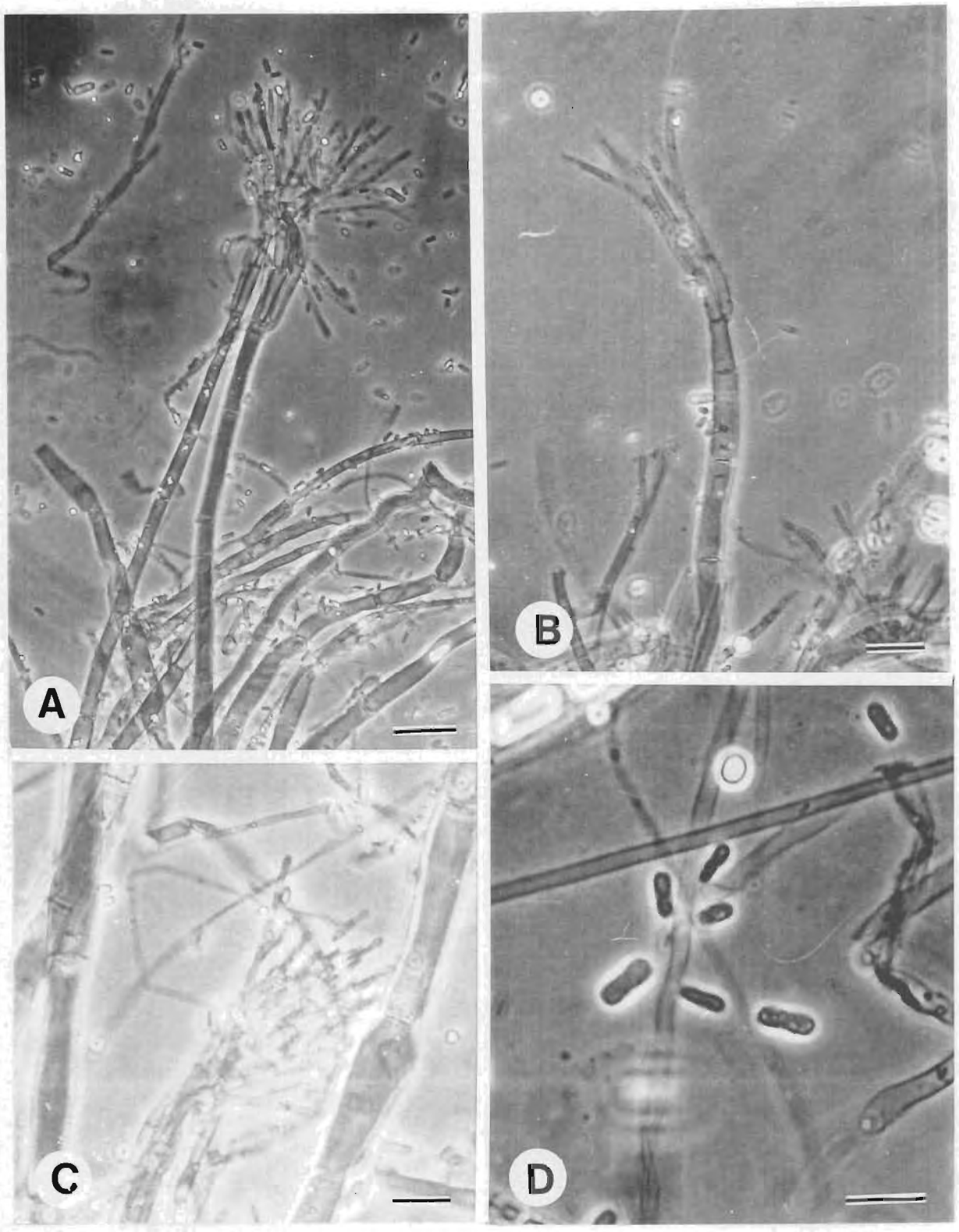

Fig. 139. Light micrographs of the anamorph structures of *O. trinacriforme* (CMW 670). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous apparatus (Bar = 20 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

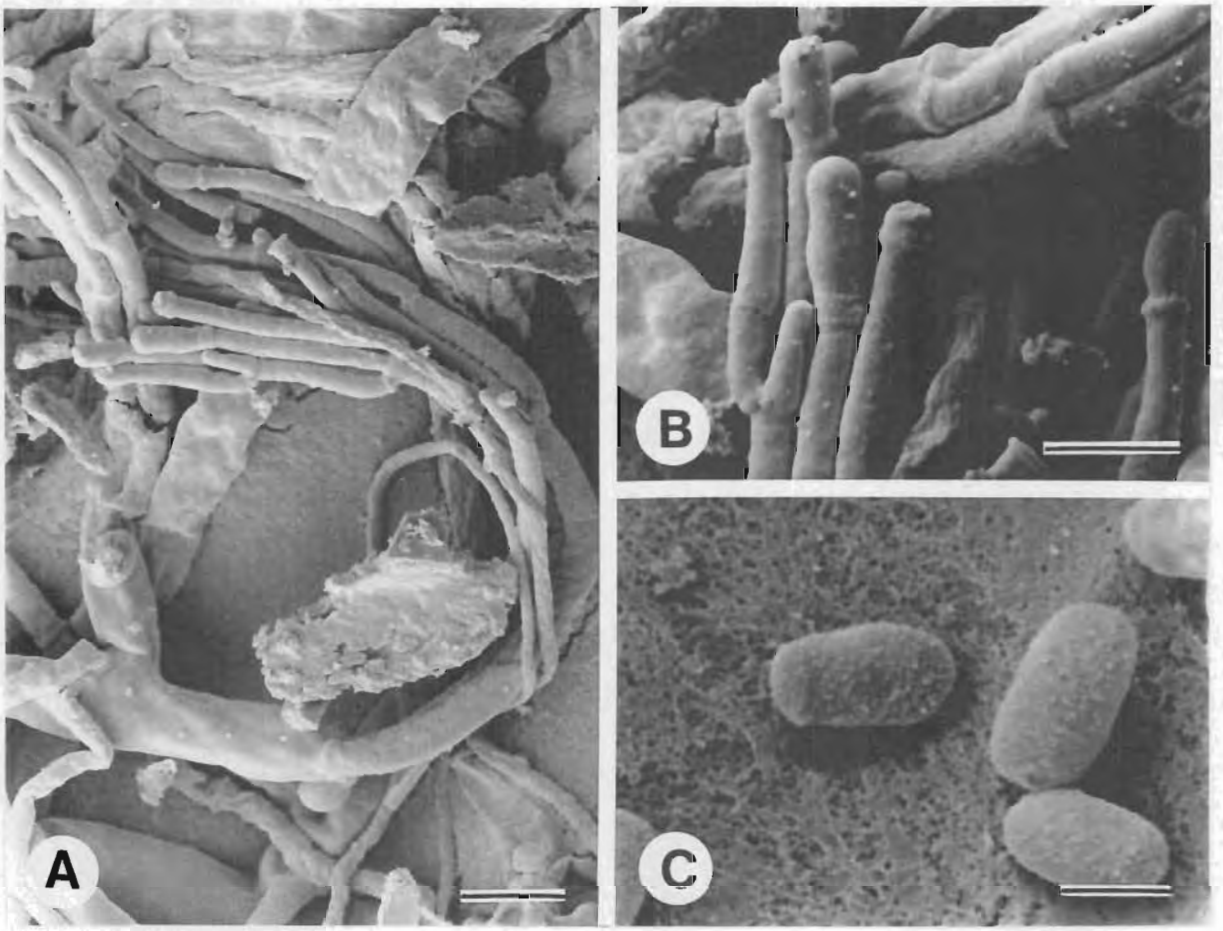

Fig. 140. Scanning electron micrographs of the conidiophores and conidia of *O. trinacriforme* (CMW 670). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 5 µm).

42. *Ophiostoma wageneri* (Goheen & F.W. Cobb) T.C. Harr., *Mycotaxon* **28**, 42. 1987. **(Figs. 141-143).**

≡ *Ceratocystis wageneri* Goheen & F.W. Cobb, *Phytopathology* **68**, 1193. 1978.

Anamorph: *Leptographium wageneri var. ponderosum* (T.C. Harr. & F.W. Cobb) T.C. Harr. & F.W. Cobb, *Mycotaxon* **30**, 505. 1987.

≡ *Verticicladiella wageneri var. ponderosa* T.C. Harr. & F.W. Cobb, *Mycologia* **78**, 568. 1986.

Etymology: wá-ge-ne-ri: genitive of Wagener. This specific epithet honors W.W. Wagener who did pioneering work on black-stain root disease.

Perithecial bases black, globose, walls smooth or occasionally roughened, 72 - 343 µm in diam., neck black becoming light brown at the apex, 345 - 786 µm long, 21 - 43 µm above globose base, 14 - 20 µm wide at the apex, ostiolar hyphae absent. Asci not seen. Ascospores subcurvate (bean-shaped) (Goheen & Cobb, 1978).

Conidiophores occurring singly or in groups of up to five, arising directly from the mycelium, erect, macronematous, mononematous, (570-) 569 - 823.5 (-880) µm in length, rhizoid-like structures absent. *Stipes* olivaceous (21"m) becoming lighter towards the apex, smooth, cylindrical, simple, 6-11 septate, (500-) 503 - 757.5 (-820) µm long, 10 - 17 (-20) µm wide below primary branches, apical cell not swollen, 10 - 17 (-20) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* 50 - 100 µm long, excluding the conidial mass, with 2 to 3 series of cylindrical branches, 2-5 primary branches, light olivaceous (21"k), smooth, cylindrical, aseptate (9.0-) 18.5 - 21 (-34) µm long and (3.0-) 6.5 - 9.0 (-15.5) µm wide, arrangement of the primary branches on the stipe - type C, secondary branches hyaline, aseptate, 7.5 -15.5 (-21) µm long, 2.0 - 5.0 (-6.0) µm wide, tertiary branches hyaline, aseptate, (6.0-) 8.0 - 9.0 (-13) µm long, 1.0 - 3.0 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, cylindrical, tapering slightly at the apex, (6.0-) 10 - 13.5 (-17) µm long and 1.0 - 2.0 µm wide. *Conidia* hyaline, aseptate, oblong with truncate ends and rounded apices, 4.0 - 7.5 x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age.

Conidial mass cream colored when wet, turning amber (21'b) when dry.

Colonies with optimal growth at 20°C on 2% MEA, reaching 34 mm in diam. in 7 days. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 30% increase in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15''''k). *Colony margin* smooth. *Hyphae* submerged on agar with little aerial mycelium, light olivaceous (21''k), smooth, straight, not constricted at the septa, 2.5 - 10 µm diam.

Specimens examined: Holotype: U.S.A., Butcher's Corral, El Dorado County, on the root of *Pinus ponderosa,* 1978; collected Goheen and Cobb, Herbarium of the University of California 1445965. **Cultures**: *Pinus ponderosa*, collected: T.C. Harrington, CAP 5 (same as CMW 2812), U.S.A., Oregon, *Pinus ponderosa,* 1988, collected J.F. Webber, CMW 1539; U.S.A., Oregon, *Pinus ponderosa,* 1988, collected: D.J. Goheen, CMW 1542; U.S.A., California, *Pinus ponderosa,* 1986, collected: T.C. Harrington, CMW 53 (56401), U.S.A., *Pinus ponderosa,* 1981; T.C. Harrington, CMW 279 (PREM 56402).

Known distribution: Western North America.

Hosts/substrate: *Pinus ponderosa; Pinus jeffreyi, Pinus contorta* (Kendrick 1962; Goheen, 1971; Mielke, 1979; Harrington, 1982; Goheen & Cobb, 1978).

Notes: There are currently three varieties of this species. These are known as *L. wageneri* var. *wageneri* (pinyon pine i.e. *Pinus monophylla, P. edulis*), *L. wageneri* var. *pseudotsugae* (*Pseudotsuga menziesii*) and *L. wageneri* var. *ponderosum* (*P. ponderosa, P. contorta* and *P. jeffreyi*). These varieties can be distinguished based on characters such as small differences in morphology (Harrington, 1982), differences in virulence (Otrosina *et al*, 1987), isozyme analysis (Otrosina, 1986; Otrosina & Cobb, 1987; Zambino & Harrington, 1987; Zambino *et al.*, 1987; Zambino & Harrington, 1989) and Random Amplified Polymorphic DNA markers (RAPD's) (Witthuhn *et al.*, 1997). It was thought to closely resemble *L. lundbergii*, with which it was initially confused. It was also thought to resemble *L. serpens* (Wagener &

Mielke, 1961). However, these species can be distinguished based on differences in optimal growth temperature. The teleomorph of *L. wageneri* has only been observed in one instance and is associated with *L. wageneri* var. *ponderosum*. This, however, represent only single collection of these structures and subsequent searches for the teleomorph have proven unsuccessful (Harrington, 1988).

Leptographium wageneri is associated with black-stain root disease, which was first recorded by Wagener and Mielke (1961) from ponderosa pine. *Leptographium wageneri* is a root pathogen and also displays symptoms characteristic of vascular wilt pathogens (Leaphart, 1960; Smith, 1967; Harrington, 1982). Infection by *L. wageneri* results in a characteristic staining of the tracheids. In addition *L. wageneri* produces Xanthone metabolites that can cause inhibition of water transport in pine seedlings (Ayer, Browne & Lin, 1989) as well as phenolic compounds that have an antibiotic effect (Ayer, Browne & Lovell, 1983). The staining pattern extends from the roots upwards through the tracheids. This is unlike the pattern associated with blue stain fungi, where the hyphae are located in the parenchyma and result in a wedge shaped staining pattern (Cobb, 1988). *Leptographium wageneri* has been isolated from trees infested with bark beetles belonging to a wide range of genera (Hansen *et al.,* 1988; Harrington *et al.*, 1985; Witcosky & Hansen, 1985; Witcosky *et al.,* 1986). However, *Hylastes nigrinus* appears to be the main vector of this species (Cobb, 1988; Harrington, 1988).

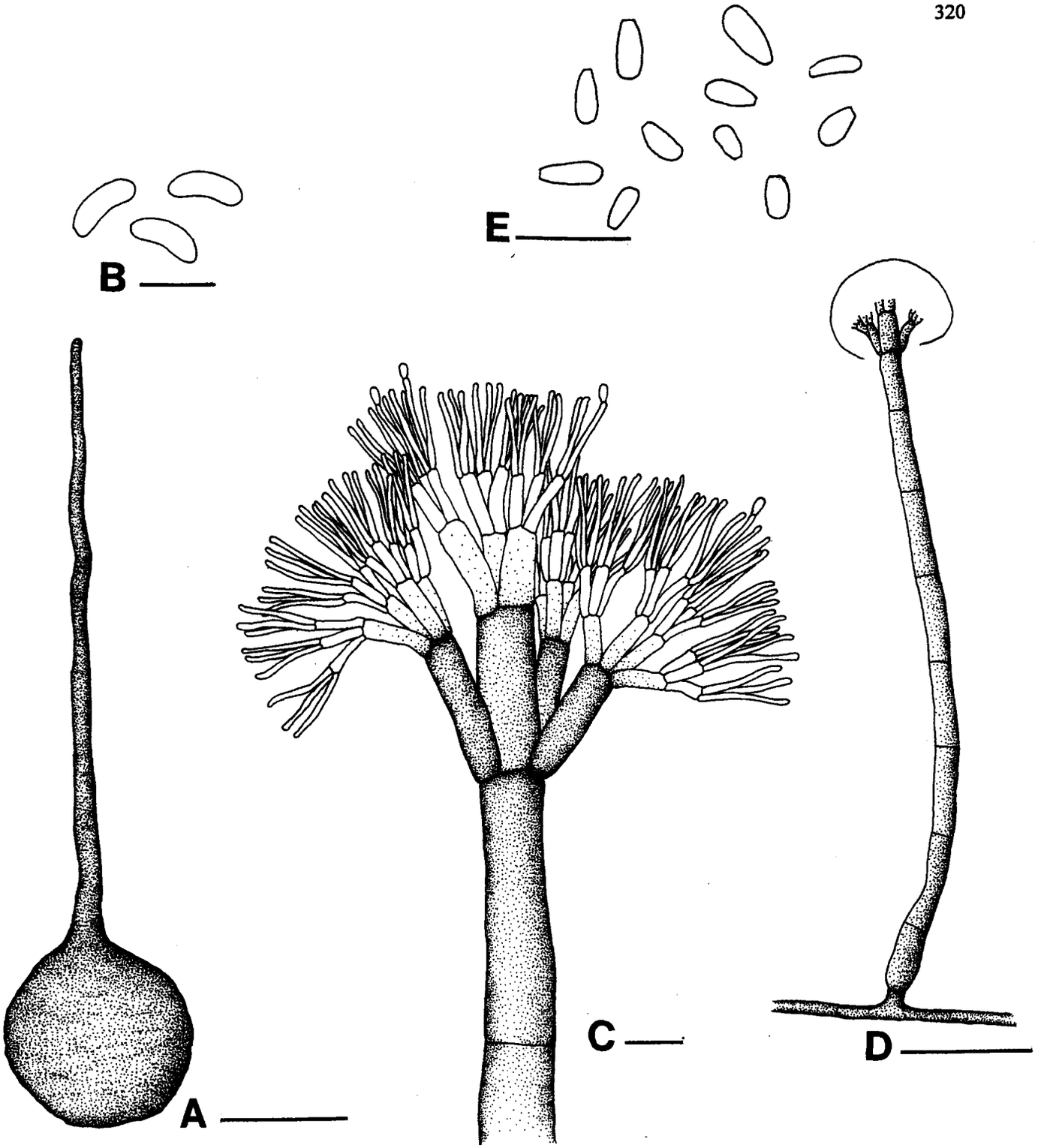

Fig. 141. Teleomorph (herbarium type material) and anamorph structures of *O. wageneri* (CMW 2812). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 10 µm). **C.** Conidiophore (Bar = 100 µm). **D.** Conidiogenous apparatus (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

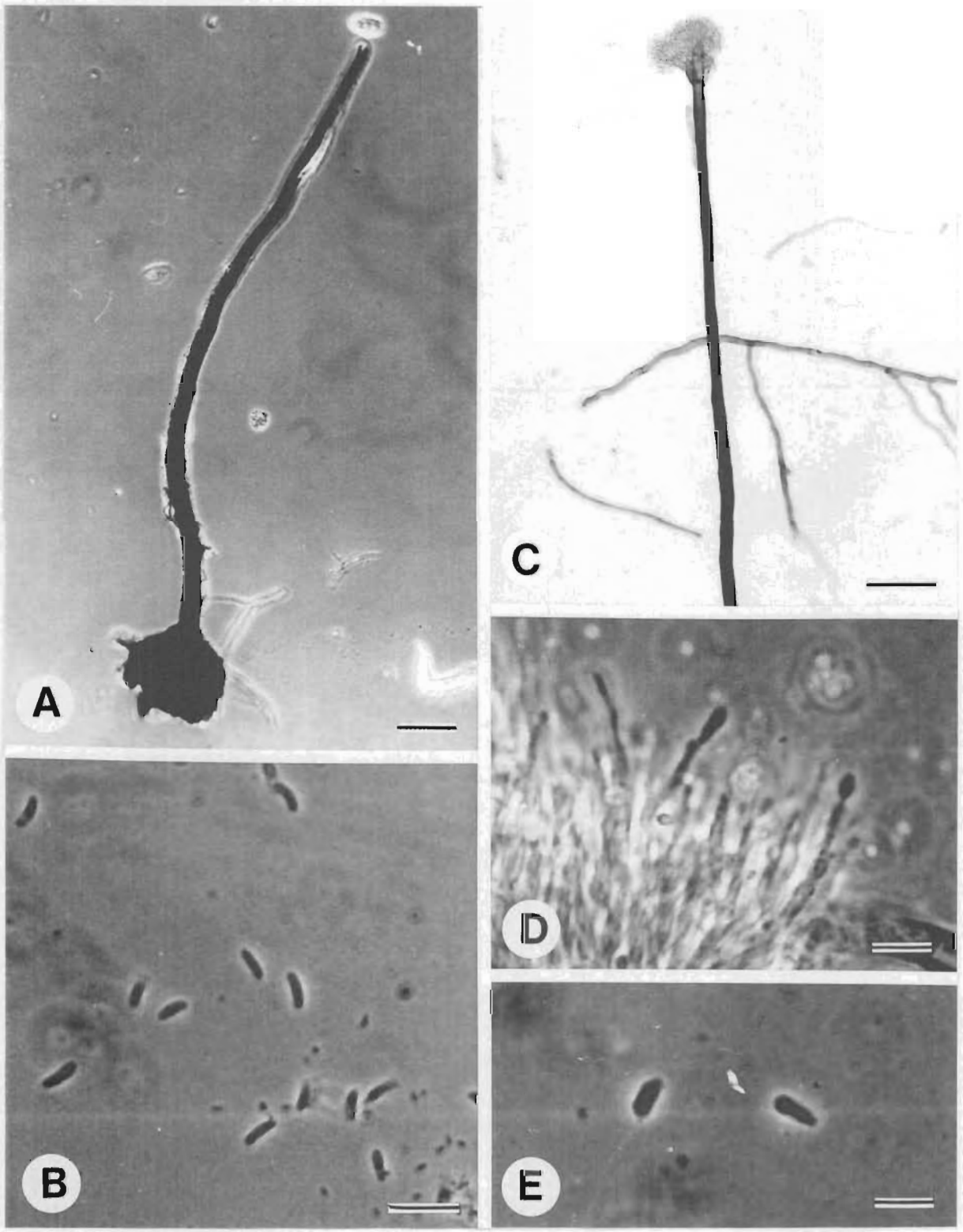

Fig 142. Light micrographs of the teleomorph (herbarium type material) and anamorph structures of *O. wageneri* (CMW 2812). **A.** Perithecium (Bar = 100 µm). **B.** Ascospores (Bar = 20 µm). **C.** Conidiophore (Bar = 100 µm). **D.** Conidiogenous cells (Bar = 10 µm). **E.** Conidia (Bar = 10 µm).

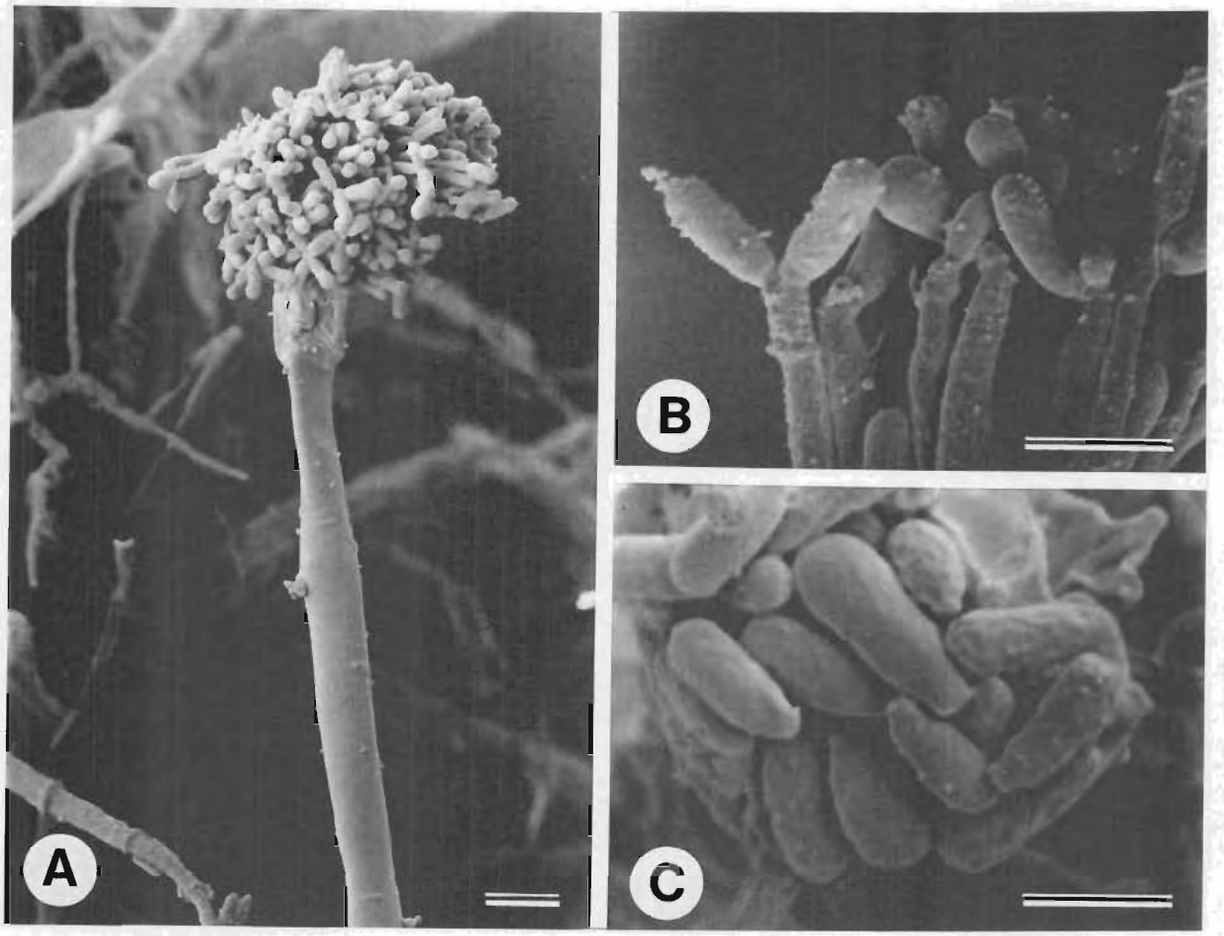

Fig. 143. Scanning electron micrographs of the conidiophores and conidia of *O. wageneri* (CMW 2812). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 5 µm). **C.** Conidia (Bar = 5 µm).

43. *Leptographium wageneri* (W.B. Kendr.) M.J. Wingf. **var. *pseudotsugae*** T.C. Harr. & F.W. Cobb, *Mycotaxon* **30**, 505. 1987. **(Figs. 144-146).**

Teleomorph: Not known.

Conidiophores occurring singly or in groups of up to six, arising directly from the mycelium, erect, macronematous, mononematous, (610-) 761.5 - 775.5 (-1030) µm in length, rhizoid-like structures absent. *Stipes* olivaceous, smooth, cylindrical, simple, 6-12 septate, (490-) 654 - 668 (-930) µm long, (5.0-) 9.0 - 12.5 (-15) µm wide below primary branches, apical cell not swollen, 12.5 - 20 µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* (60-) 97.5 - 111.5 (-200) µm long, excluding the conidial mass, with 2 to 3 series of cylindrical branches, 2-5 primary branches, olivaceous (21"m), smooth, cylindrical, 0-1 septate (14-) 30.5 - 41.5 (-59) µm long and 6.0 -12.0 (-15.5) µm wide, arrangement of the primary branches on the stipe - type C, secondary branches light olivaceous (21"k), aseptate, (6.0-) 9.5 - 22.5 (-26.5) µm long, (2.5-) 3.5 - 5.5 (-9.0) µm wide, tertiary branches hyaline, aseptate, (7.5-) 10.5 - 13.0 (-18.5) µm long, 2.0 - 6.0 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, cylindrical, tapering slightly at the apex, (7.5-) 11 - 13.5 (-15.5) µm long and 1.0 - 4.0 µm wide. *Conidia* hyaline, aseptate, oblong with truncate ends and rounded apices, 4.0 - 8.0 x 1.0 - 2.5 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, turning amber (21'b) when dry.

Colonies with optimal growth at 20°C on 2% MEA, reaching 23 mm in diam. in 7 days. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 37% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15""k) with olivaceous edges. *Colony margin* smooth. *Hyphae* submerged on agar with little aerial mycelium, hyaline to light olivaceous (21"k), smooth, straight, occasionally constricted at the septa, (3.0-) 3.5 - 5.5 (-12.5) µm diam.

Holotype: U.S.A., El Dorado County, California, *Pseudotsuga menziesii,* July, 1980, collected T.C. Harrington, UCB 1475052. **Cultures**: *Pseudotsuga menziesii*, 1982, collected: T.C. Harrington and Lowensburg, CAD 55 (same as CMW 2542), U.S.A., Jackson State Forest, *Pseudotsuga menziesii,* 1986, collected: T.C. Harrington, CMW 54.

Known distribution: Western North America.

Hosts/substrate: *Pseudotsuga menziesii* (Miller & Veirs, 1968; Cobb & Platt, 1967; Hansen, 1978; Mielke, 1979; Witcosky, 1981; Harrington, 1982; Harrington & Cobb, 1987), *Tsuga heterophylla* (Harrington & Cobb, 1987; Morrison & Hunt, 1988).

Notes: see notes provided with *Ophiostoma wageneri.*

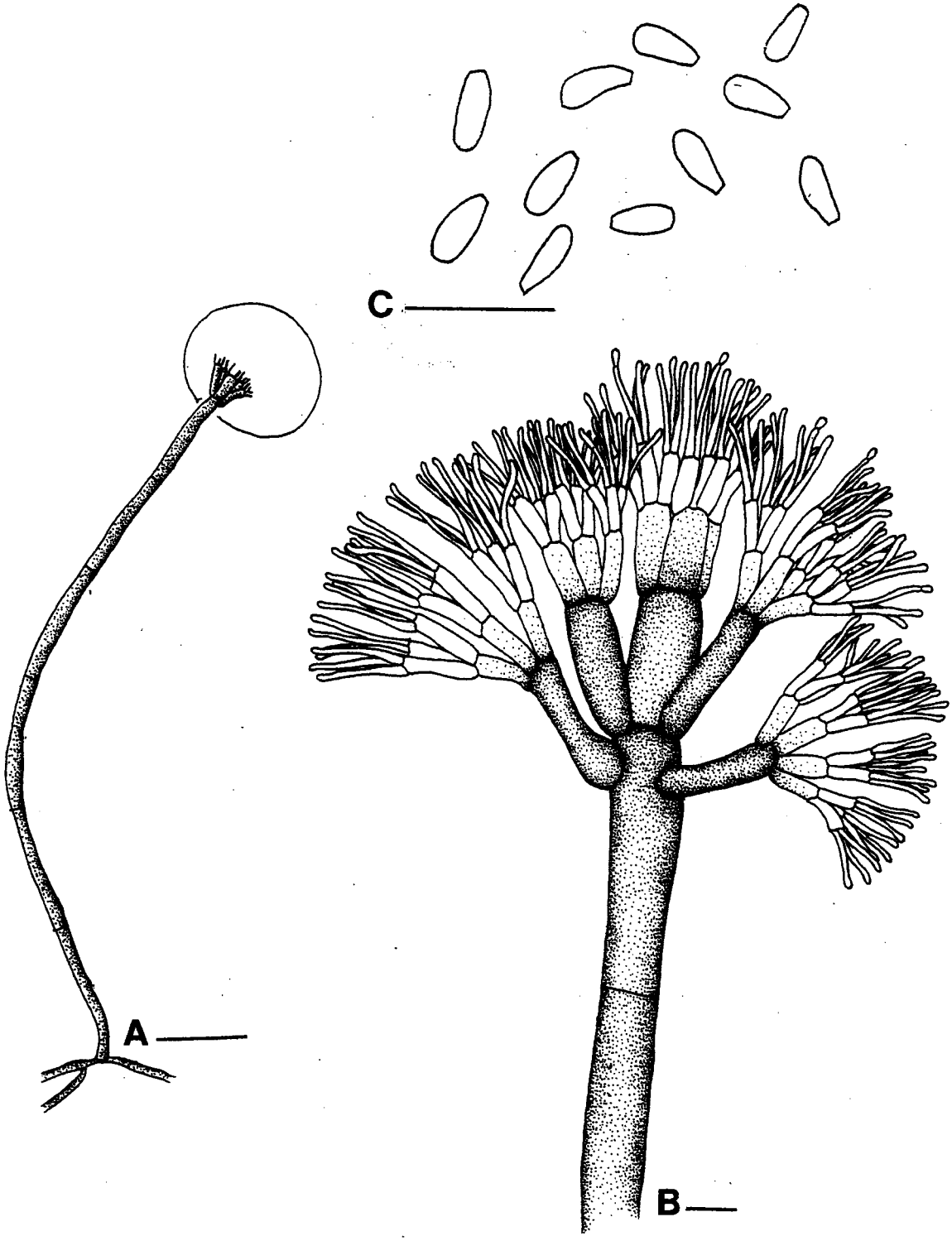

Fig. 144. Conidiophores and conidia of *L. wageneri* var. *pseudotsugae* (CMW 2542). **A.** Habit sketch (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

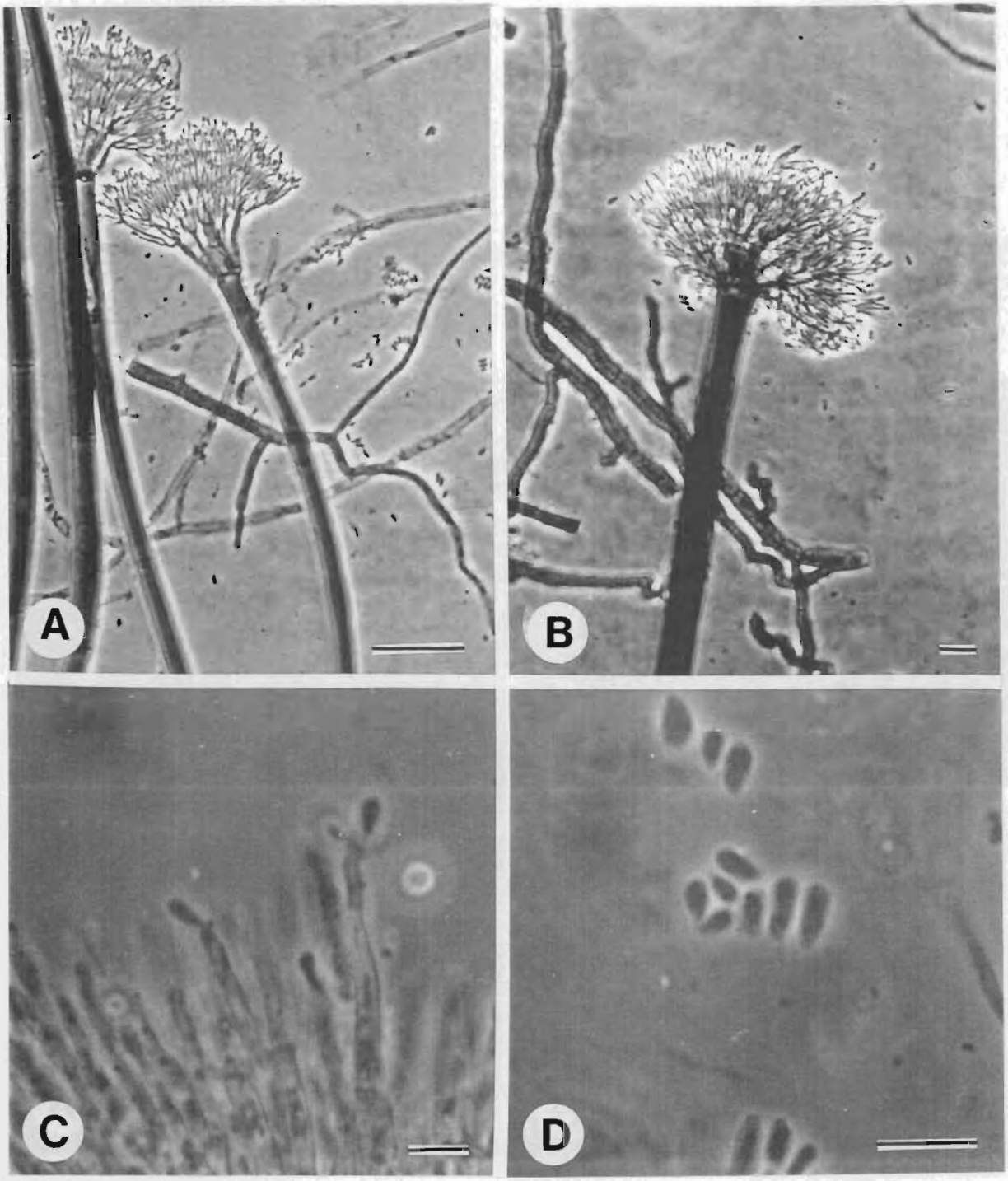

Fig 145. Light micrographs of the conidiophores and conidia of *L. wageneri* var. *pseudotsugae* (CMW 2542). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

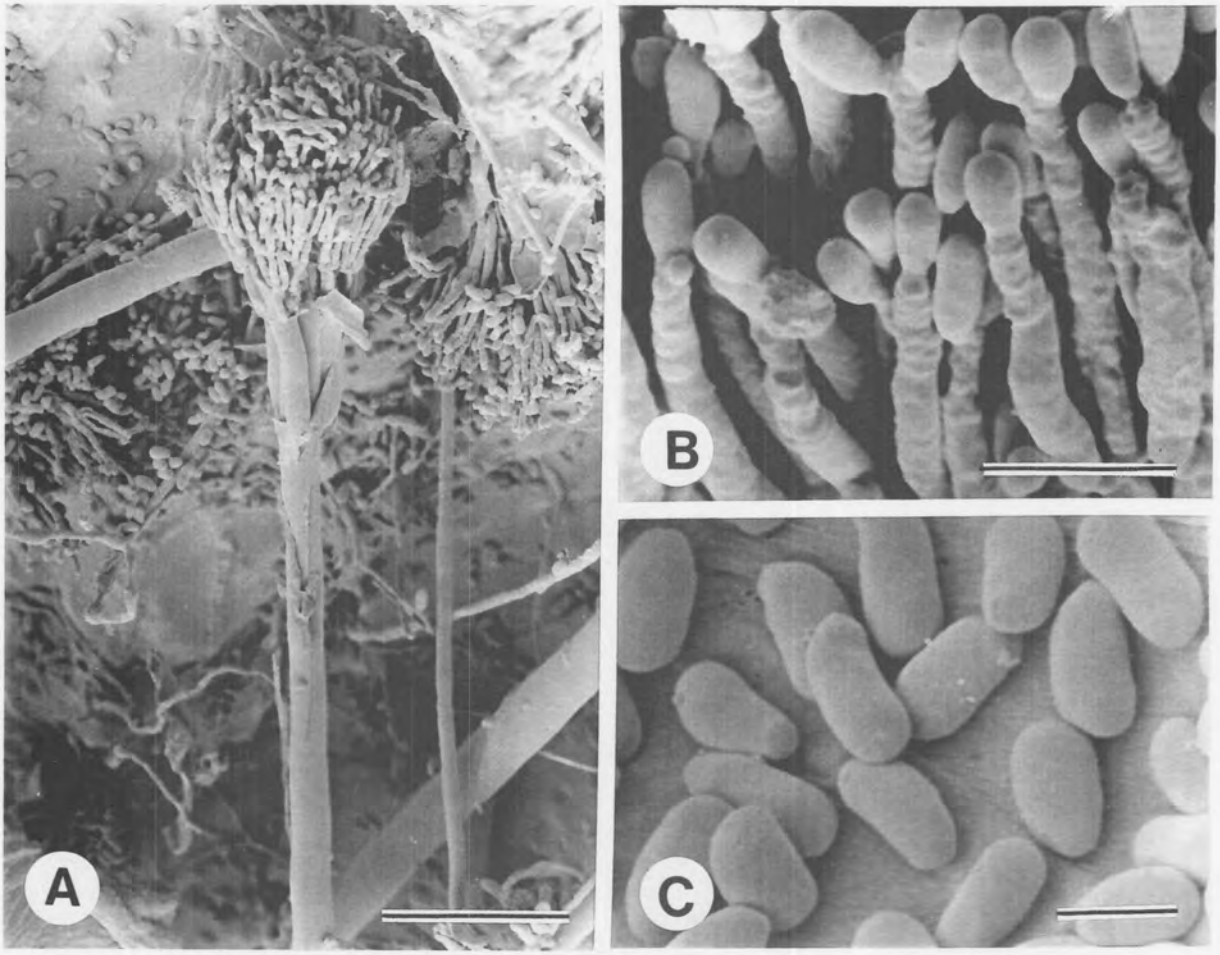

Fig. 146. Scanning electron micrographs of the conidiophores and conidia of *L. wageneri* var. *pseudotsugae* (CMW 2542). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 5 µm).

44. *Leptographium wageneri* (W.B. Kendr.) M.J. Wingf. **var. *wageneri*** *Transactions of the British Mycological Society* **85**, 92. 1985. **(Figs. 147-149).**

≡ *Verticicladiella wageneri* W.B. Kendr. *var. wageneri. Canadian Journal of Botany* **40**, 793. 1962.

Teleomorph: Not known.

Conidiophores occurring singly or in groups of up to four, arising directly from the mycelium, erect, macronematous, mononematous, (640-) 830 - 886.5 (-1040) µm in length, rhizoid-like structures absent. *Stipes* olivaceous (21"m), smooth, cylindrical, simple, 6 - 10 septate, (580-) 743.5 - 814.5 (-920) µm long, 5.0 - 12.5 µm wide below primary branches, apical cell not swollen, (7.5-) 10.5 - 14 (-15) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* (50-) 70.5 - 84.5 (-120) µm long, excluding the conidial mass, with 2 to 3 series of cylindrical branches, 2-5 primary branches, light olivaceous (21"k), smooth, cylindrical, aseptate (14-) 23 - 37 (-50) µm long and (2.5-) 6.0 - 8.5 (-18.5) µm wide, arrangement of the primary branches on the stipe - type C, secondary branches hyaline to light olivaceous, aseptate, (7.5-) 12 - 15 (-26.5) µm long, 2.0 - 3.0 (-7.0) µm wide, tertiary branches hyaline, aseptate, (8.0-) 12.5 - 13.5 (-20) µm long, 1.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, cylindrical, tapering slightly at the apex, (9.0-) 9.5 - 15.5 (-17) µm long and 1.0 - 2.5 µm wide. *Conidia* hyaline, aseptate, oblong with truncate ends and rounded apices, (4.0-) 5.0 - 6.0 (-9.0) x 1.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, turning amber (21'b) when dry.

Colonies with optimal growth at 20°C on 2% MEA, reaching 16 mm in diam. in 7 days. No growth below 5°C or above 30°C. Able to withstand high concentrations of cycloheximide with a 10% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15""k). *Colony margin* smooth. *Hyphae* submerged on agar with no aerial mycelium, light olivaceous (21"k), smooth, straight, not constricted at the septa, (2.5-) 3.5 - 7.0 (-7.5) µm diam.

Specimens examined: Holotype: U.S.A., Baldwin Lake, California, *Pinus monophylla*, Feb. 1961, collected: J.R. Parmeter, DAOM 87324. **Cultures:** collected: T.C. Harrington, COE 1 (same as CMW 2773), U.S.A., White Mountain, California, 1981, collected: T.C. Harrington, CMW 1827 (ATCC 64195), CMW 1828; U.S.A., White Mountain, California, *Pinus monophylla,* 1981, collected: T.C. Harrington, CMW 55.

Known distribution: Western North America.

Hosts/substrate: *Abies grandis* (Mielke, 1979), *Larix occidentalis* (Mielke, 1979), *Picea glauca* (Morrison & Hunt, 1988), *Picea engelmannii* (Morrison & Hunt, 1988), *Pinus aristata* (Mielke, 1979), *Pinus attenuata* (Smith & Graham, 1975), *Pinus contorta* (Cobb & Platt, 1967; Smith & Graham, 1975; Goheen & Hansen, 1978; Mielke, 1979; Hunt & Morrison, 1986), *Pinus edulis* (Wagener & Mielke, 1961; Kendrick, 1962; Cobb & Platt, 1967; Smith & Graham, 1975; Landis & Helburg, 1976; Walters & Walters, 1977), *Pinus jeffreyi* (Wagener & Mielke, 1961; Kendrick, 1962; Cobb & Platt, 1967; Smith & Graham, 1975), *Pinus lambertiana* (Smith & Graham, 1975), *Pinus monophylla* (Smith & Graham, 1975), *Pinus monticola* (Kulhavy *et al.,* 1978; Smith & Graham, 1975; Mielke, 1979), *Pinus ponderosa* (Wagener & Mielke, 1961; Kendrick, 1962; Cobb & Platt, 1967; Goheen, 1976; Goheen & Cobb, 1978; Goheen & Hansen, 1978; Mielke, 1979), *Pinus strobus* (Smith & Graham, 1975), *Pinus sylvestris* (Mielke, 1979), *Pseudotsuga menziesii* (Miller & Veirs, 1968; Mielke, 1979; Smith & Graham, 1975; Hansen, 1978), *Tsuga heterophylla* (Morrison & Hunt, 1988), *Tsuga mertensiana* (Leaphart, 1960; Byler *et al.,* 1983; Leaphart, 1960; Goheen & Hansen, 1978; Leaphart, 1960).

Associated insects: *Dendroctonus brevicomis* (Wagener & Mielke, 1961; Goheen, 1976; Goheen & Cobb, 1980), *Dendroctonus ponderosae* (Goheen, 1976; Goheen & Cobb, 1980; Hunt & Morrison, 1986; Morrison & Hunt, 1988), *Dendroctonus valens* (Goheen, 1976; Harrington & Cobb, 1983; Harrington, 1988; Perry, 1991), *Hylastes macer* (Goheen, 1976; Goheen & Cobb, 1978; Harrington, 1982; Harrington & Cobb, 1983; Harrington, 1988), *Hylastes nigrinus* (Witcosky, 1981, 1989; Harrington, 1982;

Harrington & Cobb, 1983; Witcosky *et al.,* 1986; Harrington, 1988; Jacobi, 1992), *Hylurgops porosus* (Wagner, 1977; Harrington, 1982), *Ips latidens* (Morrison & Hunt, 1988), *Ips mexicanus* (Morrison & Hunt, 1988), *Pissodes fasciatus* (Witcosky, 1981, 1989; Witcosky *et al.,* 1986; Jacobi, 1992), *Steremnius carinatus* (Witcosky, 1981, 1989; Witcosky *et al.,* 1986; Jacobi, 1992).

Notes: see notes provided with *Ophiostoma wageneri.*

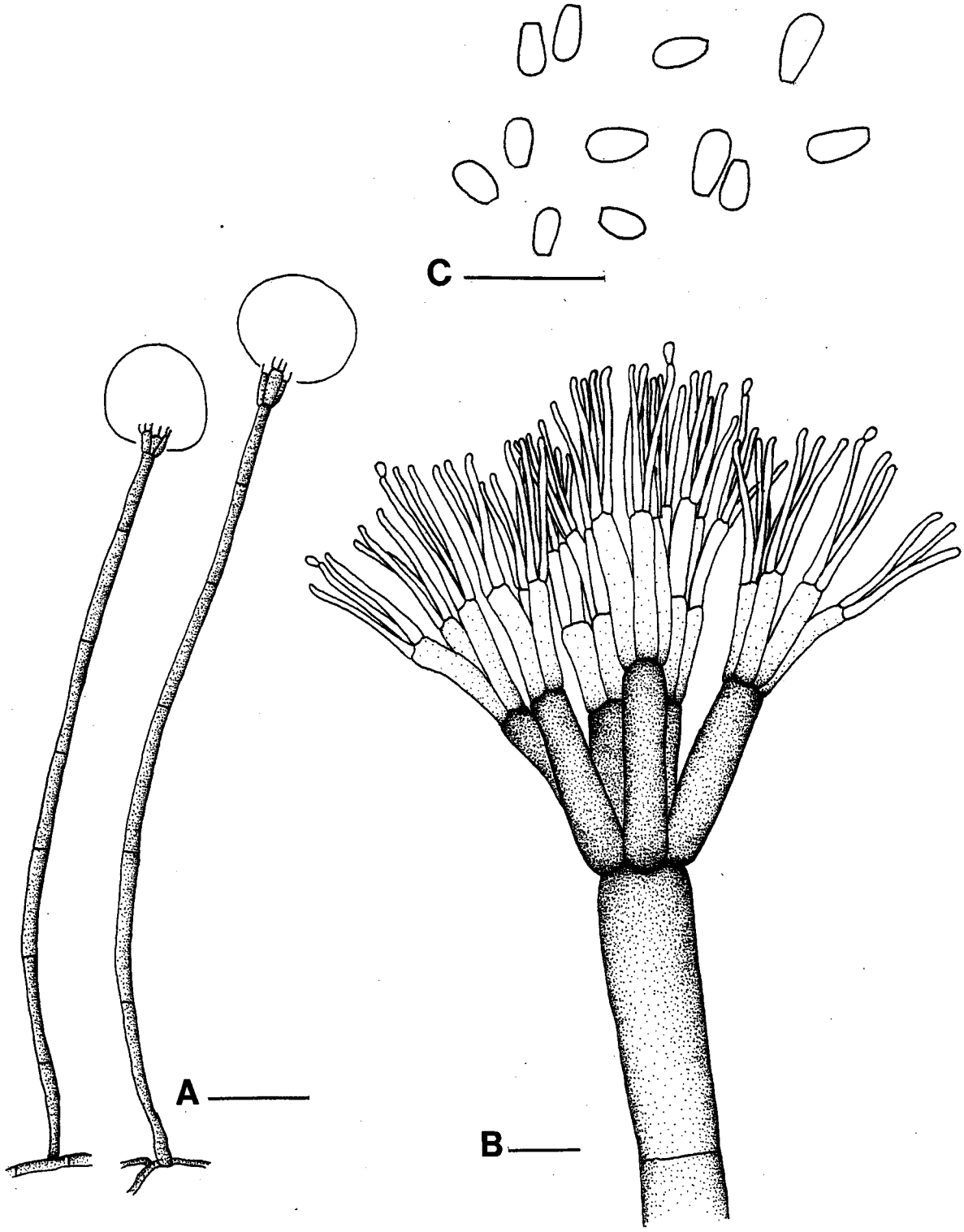

Fig. 147. Conidiophores and conidia of *L. wageneri* var. *wageneri* (CMW 2773). **A.** Habit sketch (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

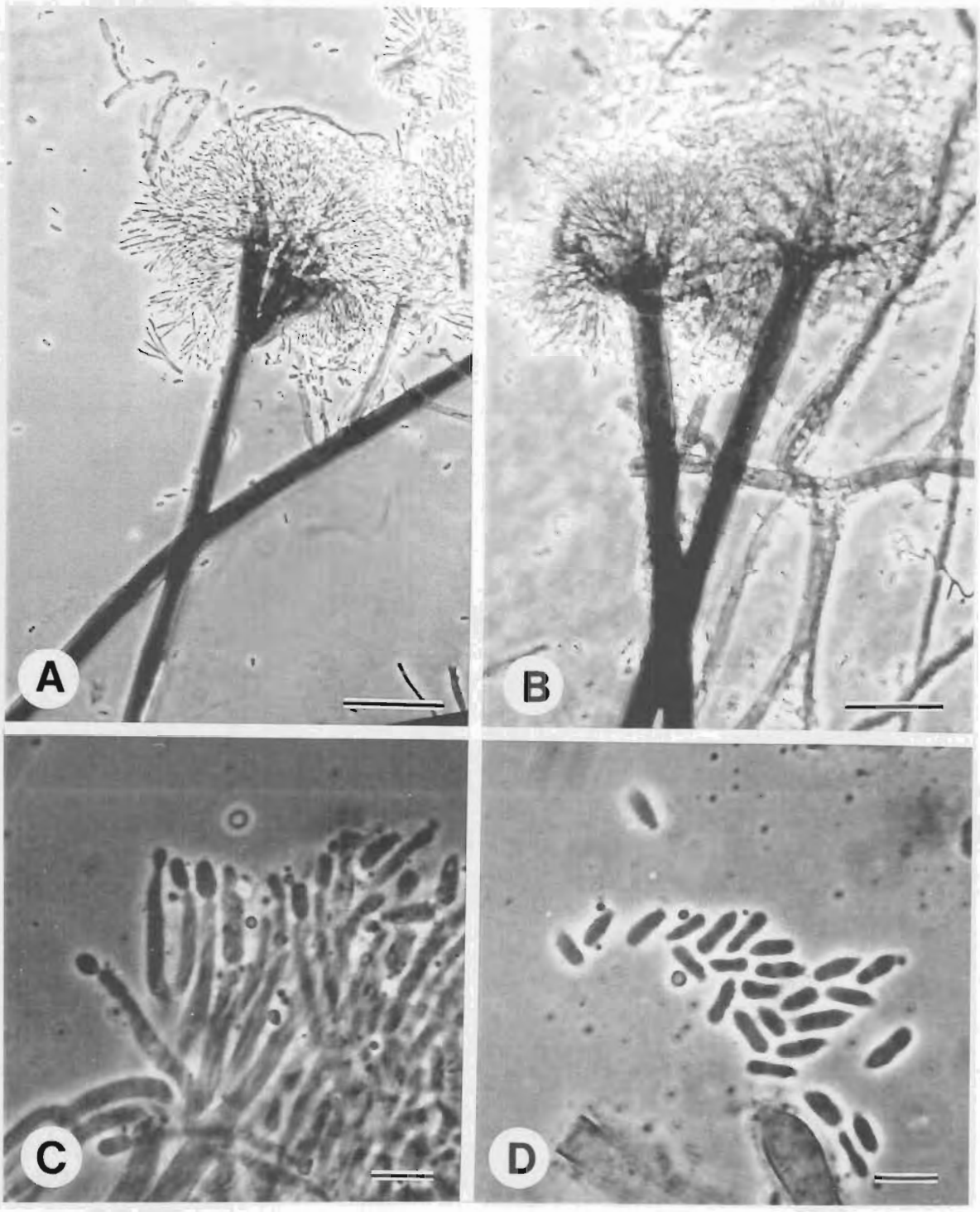

Fig 148. Light micrographs of the conidiophores and conidia of *L. wageneri* var. *wageneri* (CMW 2773). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 50 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

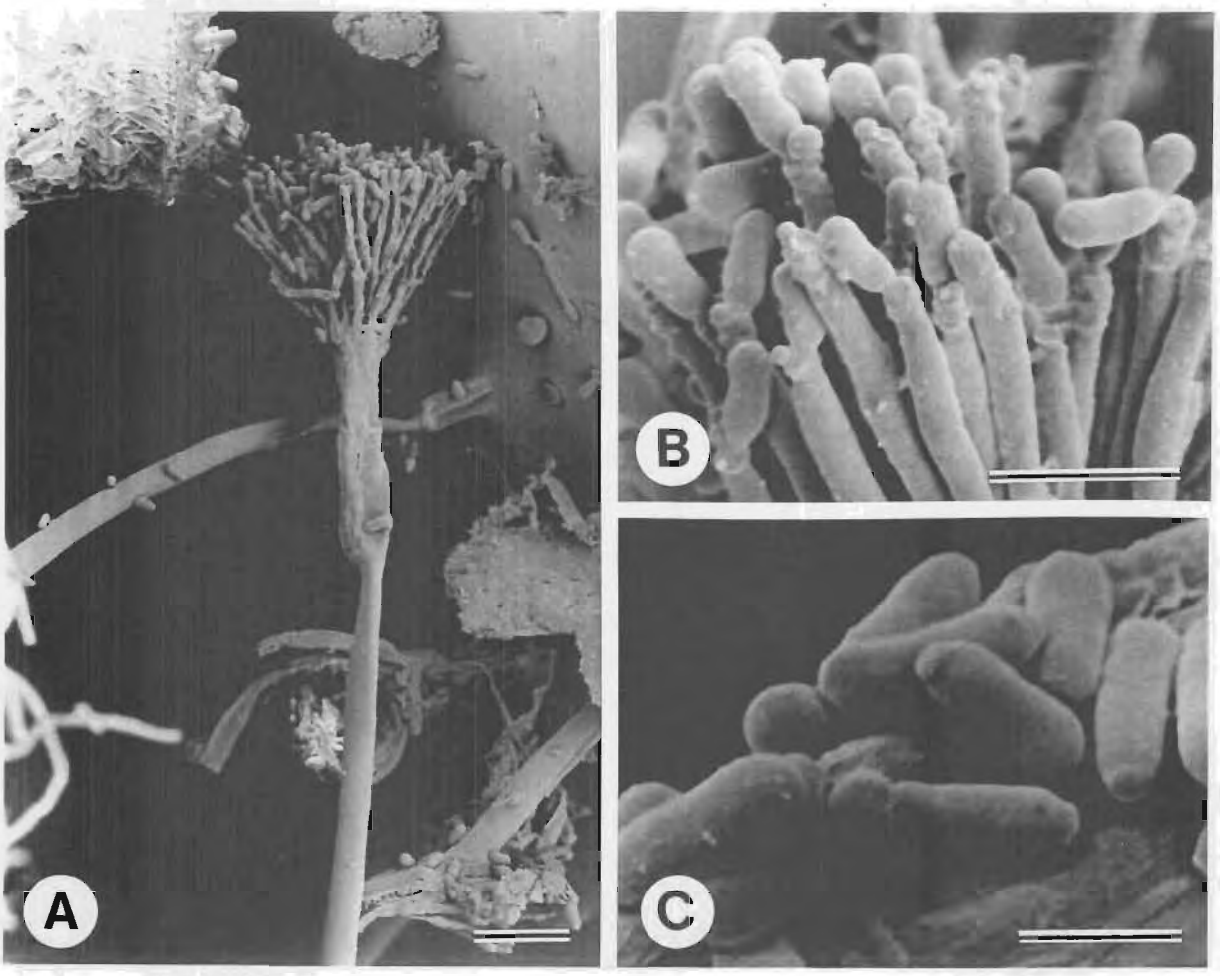

Fig. 149. Scanning electron micrographs of the conidiophores and conidia of *L. wageneri* var. *wageneri* (CMW 2773). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 5 µm).

45. *Leptographium wingfieldii* M. Morelet *Ann. d.l. S.S.N.A.T.V.* **40**, 43. 1988. **(Figs. 150-152).**

Teleomorph: Not known.

Etymology: wing-fiél-di-i: genitive of Wingfield. This specific epithet honors M.J. Wingfield.

Conidiophores occurring singly or in groups of up to six, mostly on aerial mycelium, erect, macronematous, mononematous, (180-) 161 - 521.5 (-735) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous (21"k), smooth, cylindrical, simple, 2 - 12 septate, 125 - 494.5 (-645) µm long, 5.0 - 10 µm wide below primary branches, apical cell not swollen, 5.0 - 7.0 (-10) µm wide at base, basal cell not swollen. *Conidiogenous apparatus* 50 - 97.5 (-115) µm long, excluding the conidial mass, with 2 to 4 series of cylindrical branches, 2-4 primary branches, light olivaceous (21"k), smooth, cylindrical, aseptate, 15.5 - 26 µm long and (4.5-) 6.0 - 8.0 (-11) µm wide, arrangement of the primary branches on the stipe - type B, secondary branches light olivaceous (21"k), aseptate, 7.0 - 23 (-25) µm long, 2.5 - 6.0 µm wide, tertiary branches light olivaceous (21"k), aseptate, (6.0-) 9.0 - 15 (-22) µm long, 2.0 - 5.0 µm wide, quaternary branches aseptate, 8.0 - 15 (-17) µm long, 2.0 - 4.0 µm wide. *Conidiogenous cells* discrete, 2-4 per branch, cylindrical, tapering slightly at the apex, (7.0-) 13 - 14 (-23.5) µm long and 1.0 - 3.0 µm wide. *Conidia* hyaline, aseptate, oblong with truncate ends and rounded apices, 4.0 - 6.0 x 2.0 - 3.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream colored (19'f) with age. Conidial mass cream colored when wet, turning amber (21') when dry.

Colonies with optimal growth at 25°C on 2% MEA, reaching 38 mm in diam. in 8 days. Little growth below 5°C and above 35°C. Able to withstand high concentrations of cycloheximide with a 13% reduction in growth on 0.5 g/l cycloheximide after 8 days at 20°C in the dark. Colonies dark mouse gray (15""'k). *Colony margin* smooth. *Hyphae* submerged on agar with abundant aerial mycelium, hyaline to light olivaceous, smooth, straight, occasionally constricted at the septa,

(3.0-) 4.5 - 7.0 (-9.0) µm diam.

Specimens examined: Holotype: France, Lionet, *Pinus sylvestris* infested by *Tomicus piniperda*, August 1984, collected: M. Morelet, PFN herbarium no. 1380. **Cultures**: *Pinus strobus*, collected: M. Morelet, CMW 2095 (PREM 56403), France, *Pinus brutia,* collected: M. Morelet, CMW 2093; *Pinus strobus*, collected: M. Morelet, CMW 2779 (PREM 56356), France, *Pinus sylvestris,* collected: M. Morelet, CMW 2096 (PREM 56357).

Known distribution: England, France, Sweden.

Hosts/substrate: *Pinus sylvestris* (Morelet, 1988; Wingfield & Gibbs, 1991), *Pinus brutia* (Morelet, 1988), *Pinus strobus* (Morelet, 1988); *Pinus densiflora* (Masuya *et al.,* 1998).

Associated insects: *Hylastes opacus* (Wingfield & Gibbs, 1991), *Hylurgops palliatus* (Wingfield & Gibbs, 1991), *Tomicus piniperda* (Morelet, 1988; Lieutier *et al.,* 1989; Wingfield & Gibbs, 1991; Gibbs & Inman, 1991; Solheim & Långström, 1991).

Notes: *Leptographium wingfieldii* is characterized by conidiophores with slightly elongated conidiogenous apparatuses. The conidiophores are in some cases slightly yellow, in contrast to the olivaceous conidiophores of other *Leptographium* spp. *Leptographium wingfieldii* is also characterized by slightly elongated oblong conidia. The colonies of this species are characterized by abundant aerial mycelium. The optimal growth temperature of 25 °C in this study confirm the results of Lieutier and Yart (1989).

Leptographium wingfieldii displays a low but uniform frequency of association with the bark beetle, *Tomicus piniperda* (Wingfield & Gibbs, 1991; Masuya *et al,* 1998) suggests that *L. wingfieldii* might play a role in the establishment of *Tomicus piniperda* in *Pinus sylvestris* (Lieutier *et al.,* 1989b). Wingfield & Gibbs (1991) attributed no definite pathological role to this fungus. *Leptographium wingfieldii* was shown to cause staining of the sapwood after the trees had been attacked by *T.*

piniperda. This species was also able to kill trees when mass inoculated into trees (Solheim & Långström, 1991). Similarly, in cases of high inoculum density, *L. wingfieldii* proved to be pathogenic to Scots pine (*Pinus sylvestris*) (Solheim *et al.*, 1993). Gibbs and Inman (1991) suggested that the beetle plays a direct role in the introduction of *L. wingfieldii* to pines.

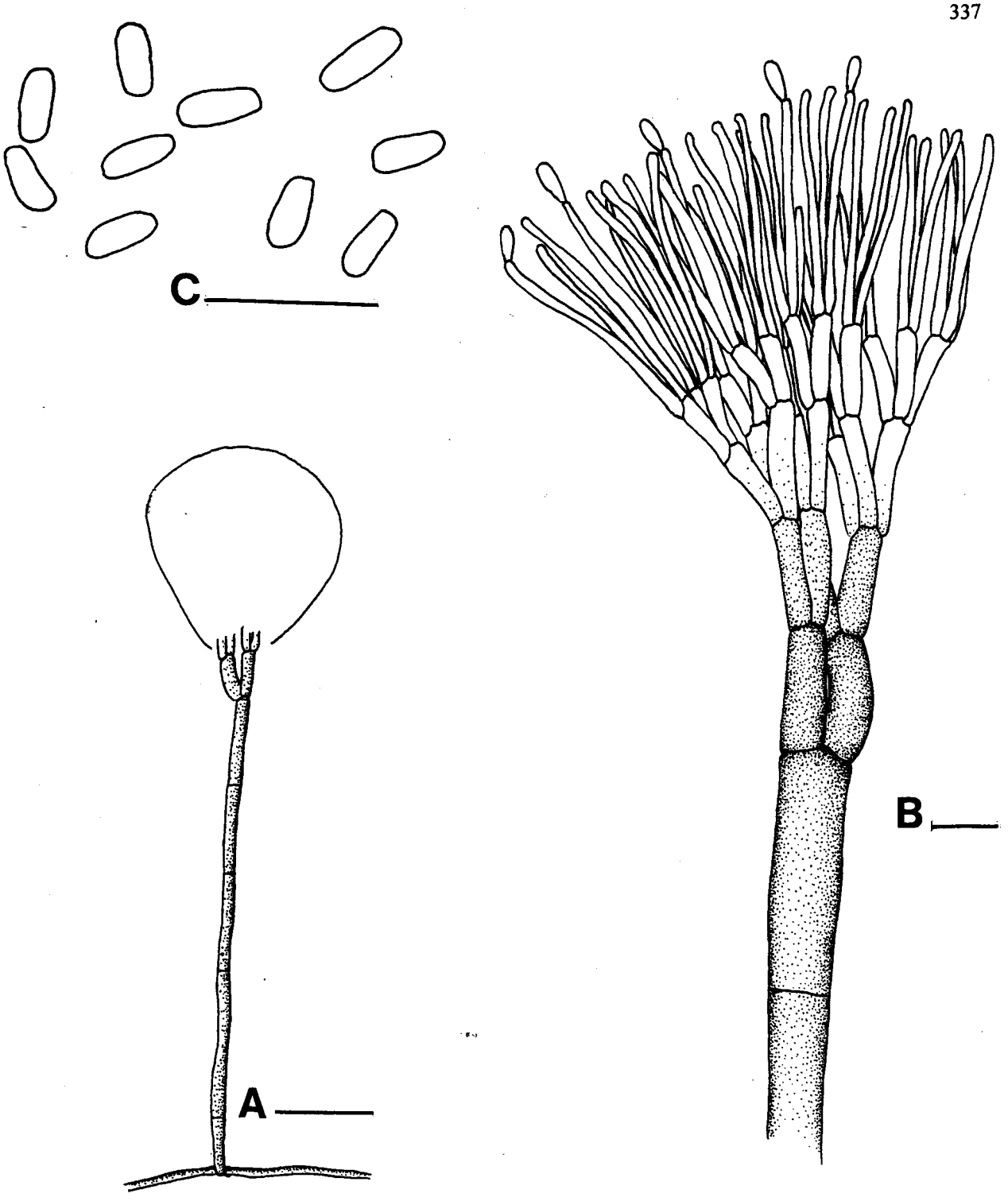

Fig. 150. Conidiophores and conidia of *L. wingfieldii* (PREM 56403). **A.** Habit sketch (Bar = 100 µm). **B.** Conidiogenous apparatus (Bar = 10 µm) **C.** Conidia (Bar = 10 µm).

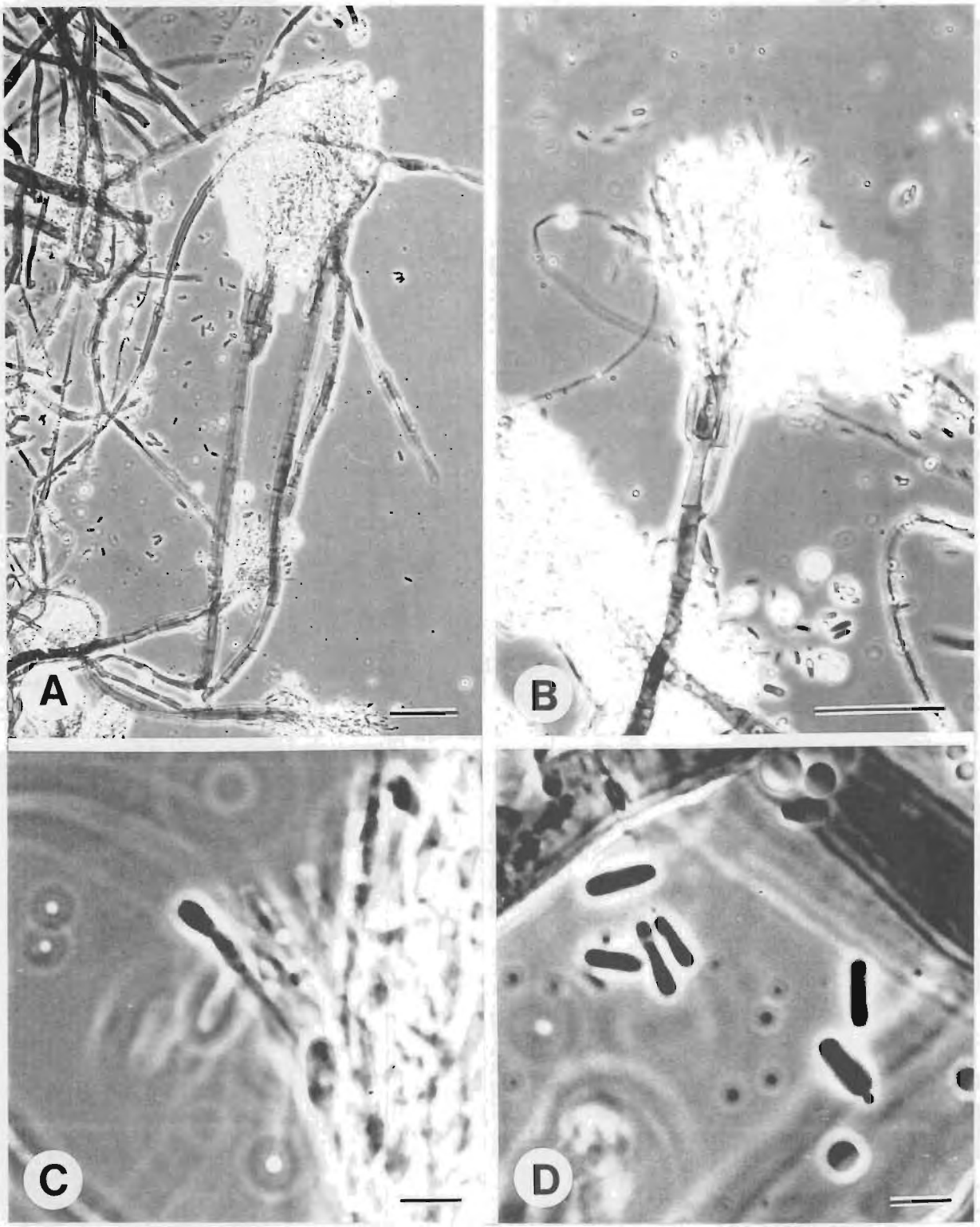

Fig 151. Light micrographs of the conidiophores and conidia of *L. wingfieldii* (PREM 56403). **A.** Conidiophore (Bar = 50 µm). **B.** Conidiogenous apparatus (Bar = 50 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 10 µm).

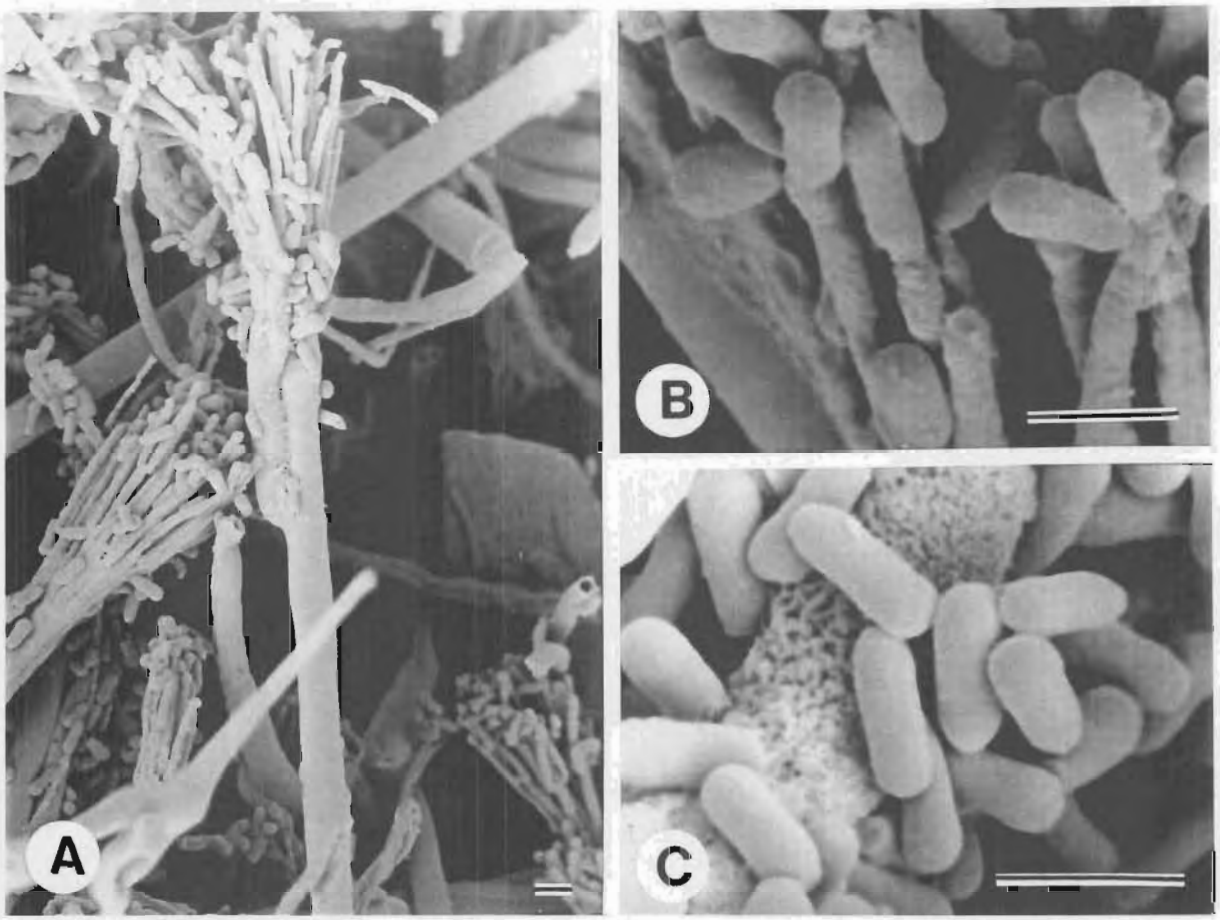

Fig. 152. Scanning electron micrographs of the conidiophores and conidia of *L. wingfieldii* (PREM 56403). **A.** Conidiophore (Bar = 10 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

46. *Leptographium yunnanensis* Zhou, Jacobs & Wingfield. *Mycoscience.* 1999. **(Figs. 153-155).**

Teleomorph: Not known.

Etymology: yun-na-nén-sis: growing in Yunnan. This specific epithet refers to Yunnan, South western China, from where the type is derived.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, 74 - 227 (-233) µm in length, rhizoid-like structures absent. Stipe light olivaceous, occasionally constricted, cylindrical, simple, 0-4 septate, 11 - 66 (-112) µm long (from first basal septum to below primary branches), 4.0 – 9.0 µm wide below primary branches, apical cells not swollen, (3.0-) 5.0 - 6.0 (-11.0) µm wide at base, basal cells not swollen. Conidiogenous apparatus (40-) 83 - 88 (-127) long, excluding the conidial mass, with 2 to 3 series of cylindrical branches; 2-3 primary branches, arrangement of primary branches - type B, light olivaceous to hyaline, smooth, cylindrical, 0-1 septate, (9.0-) 12 - 15 (-20) µm long and 3.0 - 6.0 (7.0) µm wide, secondary branches light olivaceous to hyaline, aseptate, (9.0-) 13 - 15 (-20) µm long, 3.0 - 5.0 (-6.0) µm wide, tertiary branches light olivaceous to hyaline, aseptate, (7.0-) 8.0 - 19 (-24) µm long, 2.0 - 5.0 µm wide, quaternary branches (11-) 14 - 17 (-20) µm long, 2.0 - 5.0 µm wide. Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (18-) 23 - 26 (-32) µm long and (2.0-) 3.0 - 4.0 (-6.0) µm wide. Conidia, oblong to obovoid, (4.0-) 7.0 - 8.0 (-11) x 2.0 – 6.0 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 13 mm in diameter in 7 days. No growth below 10 °C or above 30 °C. Able to withstand high concentrations of cycloheximide with a no reduction in growth on 0.5 g/l cycloheximide after 7 days at 25 °C in the dark. Colonies dark olivaceous (19"f). Colony margin smooth. Hyphae submerged or on top of agar with sparse aerial mycelia, dark olivaceous to hyaline, granular outer surface, not constricted at the septa, (2.0-) 3.0 - 7.0 (-9.0) µm diameter.

Specimens examined: Holotype: Cultures on 2 % malt extract agar, isolated from *Tomicus piniperda* infesting *Pinus yunnansensis*, Yunnan, South-western China, December 1996, collected: Xu Dong Zhou, Hui Ye & Hua Sun Ding, 5304. **Cultures:** isolated from *Tomicus piniperda* infesting *Pinus yunnansensis*, Yunnan, South-western China, December 1996, collected: Xu Dong Zhou, Hui Ye & Hua Sun Ding, CMW 5305, CMW 5152, CMW 5153.

Known distribution: South Western China

Hosts/substrate: *Pinus yunnanensis; Pinus gaoshanensis; Pinus shimaonensis* (Zhou *et al.*, 1999).

Associated insects: *Tomicus piniperda* (Zhou *et al.*, 1999).

Notes: *Leptographium yunnanensis* can easily be distinguished from other *Leptographium* spp. by its small conidiophores which are produced abundantly on the agar surface in culture. In older cultures, the spore masses at the apices of the conidiophores flow from the conidiophores, and cover the entire structure. *Leptographium yunnanensis* is morphologically similar to the *Leptographium* anamorph of *Ophiostoma crassivaginatum* and to *L. pyrinum*. These species are all characterized by short robust conidiophores without rhizoids and hyphae that appear to have a granular surface (Zhou *et al.*, 1999).

Leptographium yunnanensis can be distinguished from the anamorph of *O. crassivaginatum* based on its slightly longer conidiophores. *Ophiostoma crassivaginatum* is also characterized by an *Ophiostoma* teleomorph, whereas *L. yunnanensis* has not been associated with a teleomorph structure. *Leptographium yunnanensis* and *O. crassivaginatum* have conidia of similar length, while those of *L. yunnanensis* are twice as broad as those of *O. crassivaginatum*. *Leptographium yunnanensis* is distinguishable from *L. pyrinum* based on the considerably longer conidiophores in the latter species. *Leptographium yunnanensis* and *L. pyrinum* have conidia of similar dimension but can be distinguished based on the pear-shaped conidia of *L. pyrinum,* compared to the obovoid conidia of *L. yunnanensis.*

Leptographium pyrinum is also characterized by rhizoids (Davidson, 1978), while these structures are absent in *L. yunnanensis* (Zhou *et al.*, 1999).

Leptographium yunnanensis is one of several *Leptographium* species associated with *T. piniperda* in Europe and Asia. The other species are *L. wingfieldii, L. huntii, L. procerum, L. guttulatum* and *L. lundbergii* (Morelet, 1988; Gibbs & Inman, 1991; Solheim & Långström, 1991; Wingfield & Gibbs, 1991; Solheim *et al.*, 1993; Jacobs *et al.*, 1999). *Tomicus piniperda* is considered to be a secondary pest, which usually colonizes weakened, stressed and recently killed trees (Bevan, 1962; Klepzig, Raffa & Smalley, 1991; Långström & Hellqvist, 1993). However, in China, where this species occurs, it can also attack healthy, non-stressed trees (Ye & Dang, 1986; Ye, 1991).

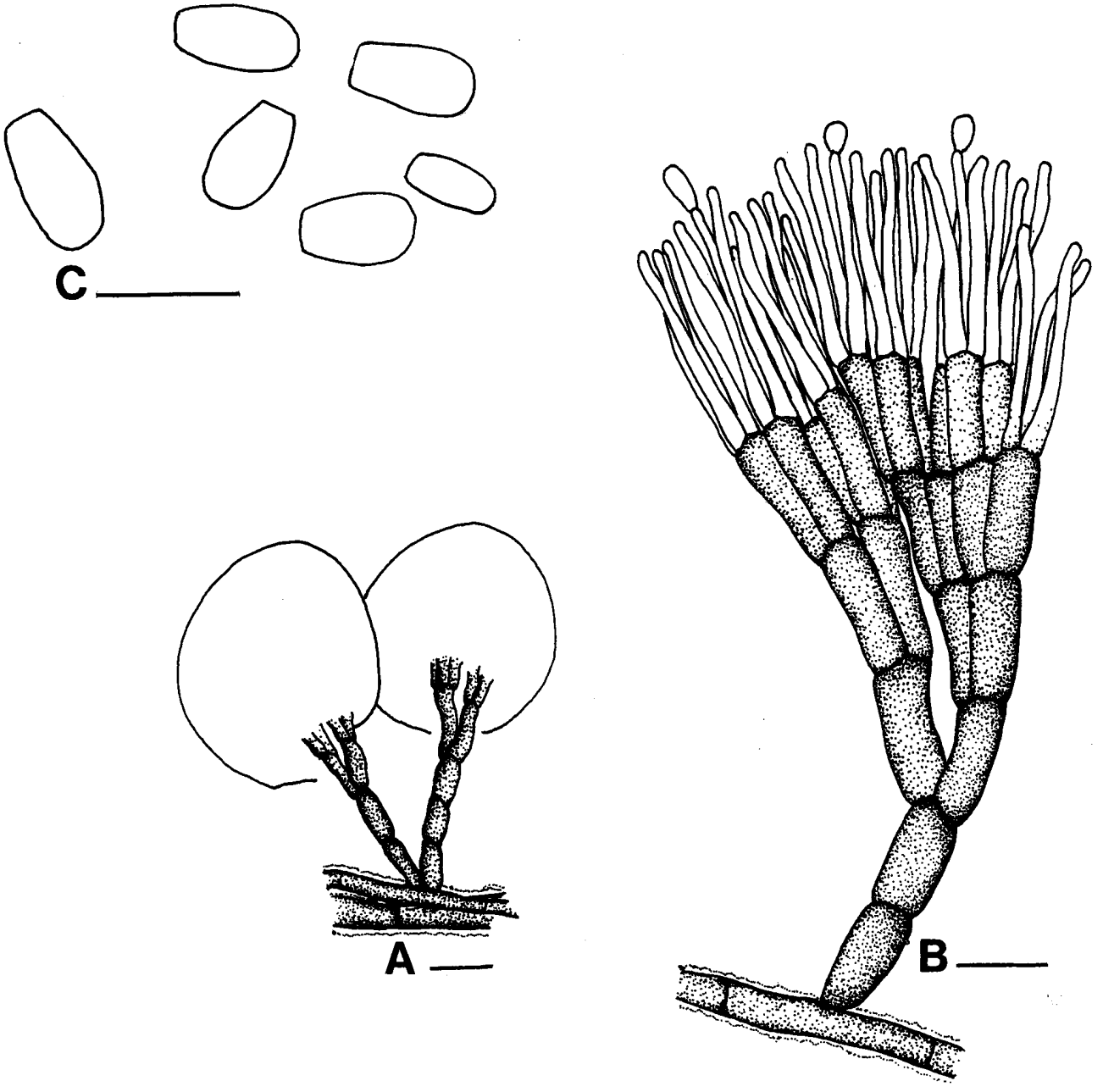

Fig. 153. Anamorph structures of *L. yunnanensis* (CMW 5204). **A.** Conidiophore. Note granular deposit on the surface of the hyphae (Bar = 10 µm). **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

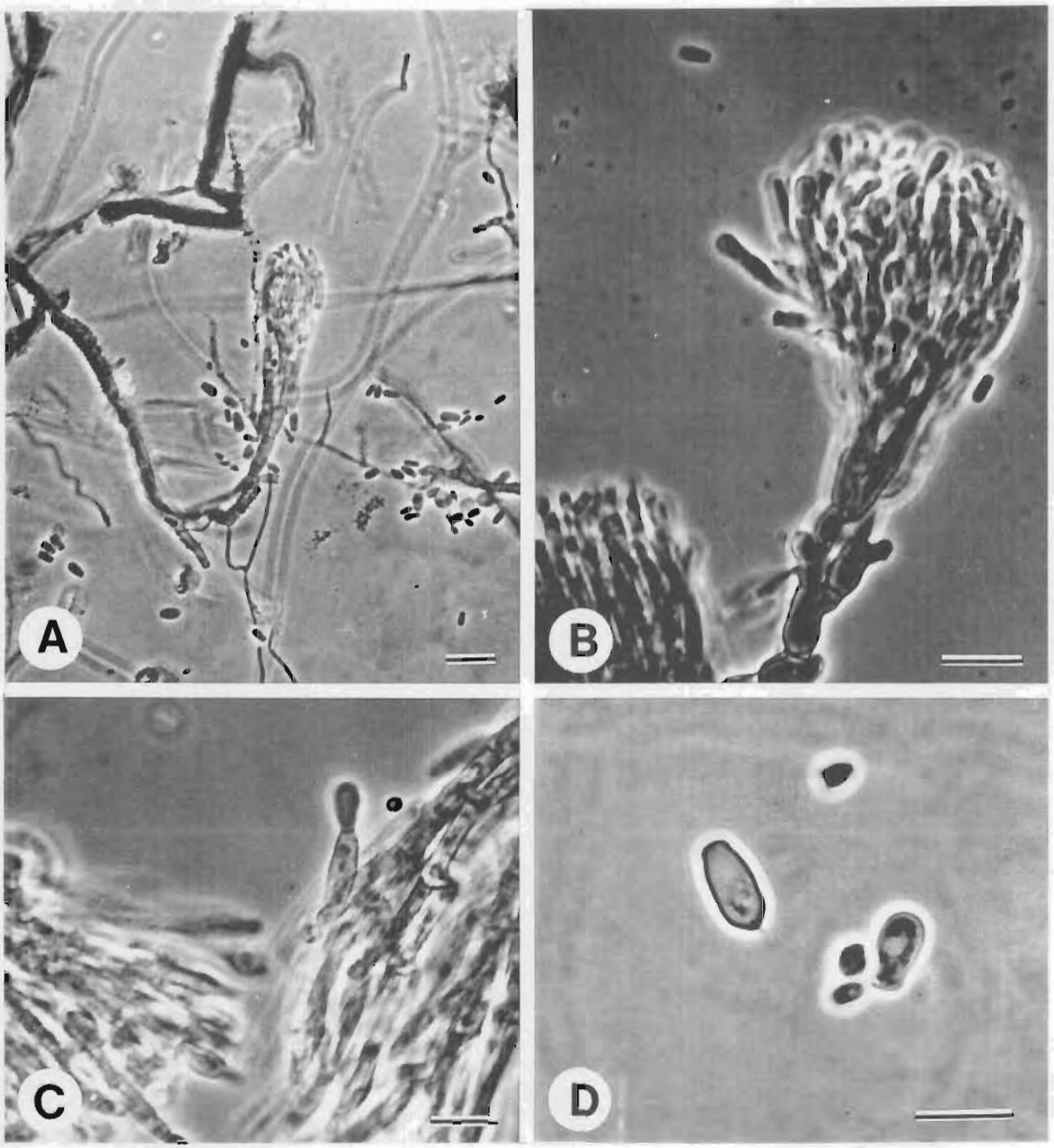

Fig 154. Light micrographs of the anamorph structures of *L. yunnanensis* (CMW 5204). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous apparatus (Bar = 20 µm). **C.** Conidiogenous cells (Bar = 10 µm). **D.** Conidia (Bar = 15 µm).

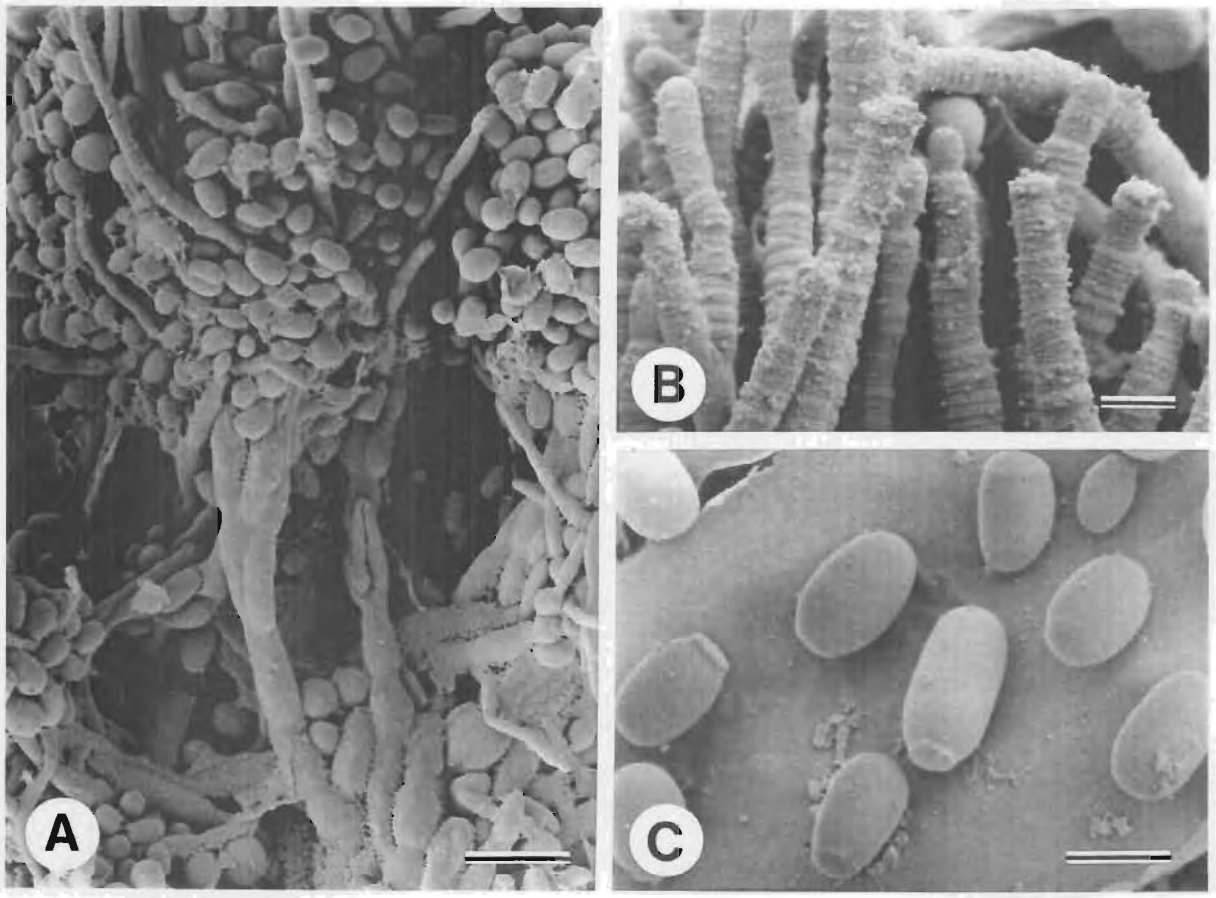

Fig. 155. Scanning electron micrographs of the conidiophores and conidia of *L. yunnanensis* (CMW 5204). **A.** Conidiophore (Bar = 20 µm). **B.** Conidiogenous cells (Bar = 10 µm). **C.** Conidia (Bar = 5 µm).

SPECIES NOT INCLUDED OR OF DUBIOUS VALIDITY

Ophiostoma abiocarpum (R.W. Davidson) T.C. Harr. *Mycotaxon* **28**, 41, 1987

≡ *Ceratocystis abiocarpa* R.W. Davidson *Mycopathologia et Mycologia Applicata*. **28**, 273, 1966.

Davidson (1966) described the teleomorph of this fungus and compared it to *O. huntii* that has a *Leptographium* anamorph. Anamorph structures were, however, never observed in *O. abiocarpum*. Upadhyay (1981) reported and described a *Leptographium* state for this fungus. The authors has, however, never observed anamorph structures associated with living cultures of this species. The location of the type material of this species is also unknown and it could not be located for examination in the present study. It is possible that the relationship of this species to *Leptographium* will only be resolved through molecular analysis.

Ophiostoma rostrocylindricum (R.W. Davidson) Arx, *Antonie van Leeuwenhoek* **18**, 212. 1952.

≡ *Ceratocystis rostrocylindrica* (R.W. Davidson) Hunt, *Lloydia* **19**, 26. 1956.

= *Ceratostomella (Grosmannia) rostrocylindricum* R.W. Davidson, *Mycologia* **34**, 658. 1942.

Anamorph: *Verticicladiella rostrocylindrica* H.P. Upadhyay, 1981. A Monograph of *Ceratocystis* and *Ceratocystiopsis*.

This species was described in the genus *Ceratostomella*, and a *Leptographium* anamorph was reported (Davidson, 1942). We have not been able to locate a type specimen and no record of living cultures exists. Hunt (1956) reported that no type was designated for this species, which would thus makes the name invalid.

Ophiostoma tetropii, Mathiesen, *Svensk. Bot. Tidskr.* **54**: 228. 1951.

= *Ceratocystis tetropii* (Mathiesen) J. Hunt, *Lloydia* **19**, 45. 1956.

Anamorph state: *Verticicladiella/Sporothrix.*

This fungus was described by Mathiesen (1950) as having a *Scopularia*-like anamorph. The original description of this fungus was accompanied by a poor line-drawing and no photographs. Solheim (1986) showed this anamorph of this fungus as having a *Sporothrix* state. As in the case of *O. rostrocylindricum,* no record of any herbarium material has been found and the species remains of uncertain validity.

Ceratocystis valdaviana Butin, *Phytopathologische Zeitschrift* **109**, 86. 1984.

Anamorph: *Verticicladiella/Sporothrix.*

This species was described from a *Nothofagus* sp. which is native to Chile (Butin, 1984). This species has only been reported once and has been reported from a host not characteristic of other species in *Leptographium.* No live cultures or herbarium material could be located and we were thus unable to verify the existence of a *Leptographium* anamorph.

Leptographium microsporum R.W. Davidson *J. Agric. Res.* **50**, 805. 1935.

Teleomorph: *Scopularia microspora* Goid. *Boll. Stm. Patol. Veg. Roma* **16**, 38. 1936.

This species was described briefly by Davidson (1935) and is the only record of this fungus (Harrington, 1988). No record of live cultures or herbarium specimens could be located and the existence of a *Leptographium* state could not be confirmed.

Leptographium hymenaeae A. Ram & C. Ram, *Broteria* **41**, 94. 1972.

This species was described from an unusual host (*Hymenaeae altissima*) in Brazil. *Leptographium hymenaeae* produces chlamydospores and seta-like structures that are unusual in species of *Leptographium* (Ram & Ram, 1972; Harrington, 1988). The conidiogenous cells were also reported to be phialidic, which make the placement of this species in *Leptographium* highly unlikely (Ram & Ram, 1972). No type material is available and we doubt the placement of the fungus in *Leptographium.*

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Part 2

Chapter 1

Jacobs, K., Wingfield, M.J. and Wingfield, B.D. (1999). Phylogenetic relationships in *Leptographium* based on morphological and molecular characters. Submitted to Canadian Journal of Botany.

Phylogenetic relationships in *Leptographium* based on morphological and molecular characters

Species in *Leptographium* are characterized by mononematous conidiophores with dark stipes and conidiogenous apparatuses with complex series of branches. These fungi generally inhabit woody substrates, are associated with bark beetles (Coleoptera: Scolytidae) and are known to cause blue-stain on conifers. Few phylogenetic studies have been conducted on *Leptographium* spp. and those that have been undertaken, have been focused on a small number of species. The aim of this study was to investigate the phylogenetic relationships between species in *Leptographium* based on partial DNA operon sequences and to ascertain whether morphological characters are congruent with DNA based phylogeny. Morphological characters were, analyzed and compared with results from DNA sequence analysis. Results indicate that there are three groups within *Leptographium* based on DNA sequence analysis. There was, however, no congruence between these groups and those emerging from morphological characters. Data from this study strongly support the connection between *Leptographium* and *Ophiostoma.* They have also provided us with a non-subjective means to confirm the identity of many *Leptographium* spp. that are difficult to distinguish based on morphological characters.

Keywords: *Leptographium,* phylogeny, morphology, *Ophiostoma,* rRNA

INTRODUCTION

Species of *Leptographium* Lagerberg & Melin can be recognized by their dark mononematous conidiophores and complex conidiogenous apparatuses. Conidia are produced through annelidic conidium development and are single-celled, hyaline spores (Kendrick, 1962). Some *Leptographium* spp. are known as anamorphs of *Ophiostoma* Sydow & Sydow spp. (Harrington, 1988; Wingfield, 1993). These fungi are able to tolerate high levels of cycloheximide (Harrington, 1981) and have rhamnose and cellulose in their cell walls (Marais, 1996; Horner, Alexander & Julian, 1986).

Ophiostoma and *Leptographium* spp. mostly occur on conifers in association with insects, and particularly bark beetles (Harrington, 1988; Solheim, 1986; 1993). Most *Leptographium* spp. are known to cause blue-stain in the sapwood of lumber. Only one species is well recognized as a pathogen. This includes the three varieties of *L. wageneri* (Kendrick) Wingfield that cause black stain root disease in the western USA (Wagener & Mielke, 1961; Kendrick, 1962; Cobb, 1988). *Leptographium procerum* (Kendrick) Wingfield has been associated with a root decline disease, primarily in Eastern North America (Harrington & Cobb, 1983; Alexander, Horner & Lewis, 1988) and *L. serpens* with a root disease in pines in South Africa and Italy, but the role of these fungi in disease remains unclear (Lorenzini & Gambogi, 1976; Wingfield & Knox-Davies, 1980; Wingfield, Capretti & Mackenzie, 1988).

Several new species have been described in *Leptographium* in recent years (Wingfield, Crous & Tzean, 1994; Van der Westhuizen *et al.*, 1995; Jacobs, Wingfield & Bergdahl, 1997; Jacobs *et al.*, 1998, 1999; Webber, Jacobs & Wingfield, 1999). Some of these are unusual in that they are associated with niches such as soil and non-coniferous hosts. Several have also been described from tropical areas, which is an unusual niche for *Leptographium* spp. (Wingfield *et al.*, 1994; Webber *et*

al.,1999). This has posed some questions regarding the relatedness of species described in *Leptographium* and the phylogenetic placement of the atypical species.

Zambino and Harrington (1992) used isozymes to determine phylogenetic relationships within some *Leptographium* spp. They concluded that isozyme variation can be useful in determining relationships within the genus. In general, their data also supported morphological species groupings. In a recent study of a selected group of *Leptographium* spp., Coetsee (1999) showed an apparent correlation between conidium length and phylogenetic groupings. Although the correlation was not equivocal, it did suggest that conidium length might be used to infer phylogeny in *Leptographium*.

Sequences of the ribosomal DNA genes have proved useful in determining phylogenetic relationships within groups of Ascomycetes (Gaudet *et al.,* 1989; Okada, Takematsu & Takamura, 1997; Ward & Adams, 1998; Myburg, Wingfield & Wingfield, 1999; Witthuhn *et al.,* 1999). This is especially true for morphologically similar taxa (Glenn *et al.,* 1996; Wingfield *et al.,* 1996; Dupont, Laloui & Roquebert, 1998; O'Donnell, Cigelnik & Nirenberg, 1998, Chen, Shrearer & Crane, 1999; Myburg *et al.,* 1999; Witthuhn *et al.* 1999). In the ophiostomatoid fungi, comparison of ribosomal gene sequences has been valuable in resolving various taxonomic questions, at least at generic and ordinal levels (Hausner, Reid & Klassen, 1993a,b; Wingfield, Viljoen & Wingfield, 1999). The majority of these studies have made use of the ITS and 5.8 S ribosomal RNA operon sequences. However, the large subunit of the ribosomal gene (28S) is sufficiently conserved to allow determination of relationships between genera, but is also sufficiently sensitive to distinguish relationships between species, in many cases (Gaudet *et al.,* 1989; Yamada & Kawasaki, 1989; Yamada *et al.,* 1989; Guého, Kurtzman & Peterson, 1989; Kurtzman & Robnett, 1991; Peterson & Kurtzman, 1991; Hausner, Reid & Klassen, 1993b; Vilgalys & Sun, 1994; Wingfield *et al.,* 1994).

Various authors have attempted to correlate morphological characters and DNA sequence phylogeny, with various levels of success. Berbee and Taylor (1992) concluded that morphological characters can be misleading and are not a reflection of true relationships in ascomycetes. This was also the case with Hausner *et al.* (1992) and Wingfield *et al.* (1994) who found that relationships based on ascospore shape were not congruent with phylogenies based on DNA sequence data. They thus cautioned against the use of certain morphological characters in taxonomy. In other studies, a strong correlation has been found between relationships based on morphology and phylogeny (Kurtzman, 1993; Strydom, Wingfield & Wingfield, 1997; Jacobs and Rehner, 1998; Witthuhn *et al.,* 1998; Myburg *et al.,* 1999).

The combination and comparison of morphological with molecular characters is difficult. Hibbett and Vilgalys (1993) coded the morphological data for *Lentinus* spp. in a manner similar to that of sequence data and analyzed these data as they would sequence data. From the results they determined whether relationships based on morphology could be correlated with those determined through phylogeny. A similar approach was followed by Viljoen (1996) who studied the phylogeny of *Ceratocystis,* s.l. based on morphological characters. Patterson, Williams and Humphries (1993) concluded that comparison of phylogenies based on morphology and molecular data can only be made if large data sets of morphological characters for the organisms in question, exist.

The aim of this study was to determine the phylogenetic relatedness of species within *Leptographium* through comparison of the partial sequence of the ITS2 and 28S ribosomal RNA operon. A selected set of isolates were also used to determine the placement of *Leptographium* in a larger group of Ascomycetes. Secondly, morphological characters from a large data set were coded and analyzed. Derived trees were compared to those generated from the molecular data to determine whether relationships based on morphological characters are congruent with those generated based on DNA sequence data.

MATERIAL AND METHODS

Molecular comparisons

Representative isolates were selected for known *Leptographium* spp. (Table 1). These isolates represent the majority of the described *Leptographium* spp. Species not included in the study are *L. aenigmaticum* Jacobs, Wingfield & Yamaoka, *L. neomexicanus* Wingfield, Harrington, & Crous and *L. serpens* (Goidanich) Arx. These species were omitted because we failed to amplify the DNA of the desired region despite repeated attempts. Where possible, and within the limitations of our budget, more than one isolate per species was included.

DNA extractions were performed using a modification of the technique described by Raeder & Broda (1985). Genomic DNA was extracted from two-week-old cultures grown in liquid ME (malt extract). This was done by grinding a small amount of mycelium in liquid nitrogen to a fine powder and adding 1.0 µl Extraction buffer (1 % CTAB). This was then incubated in a 60 °C waterbath for 1 hour. Proteins were removed with phenol and chloroform (1:1), followed by a series of chloroform steps, until the interface was clean. The DNA was precipitated with cold 100 % ethanol and left for 2 hours at -20 °C. This solution was then centrifuged at 13000 rpm for 30 min., the resulting pellet was washed with cold 70 % ethanol and dissolved in 100 µl sterile water. The presence of DNA was confirmed by agarose gel electrophoresis and visualized through ethidium bromide staining under the UV light.

The ITS2 (internal transcribed spacer region) and part of the large subunit (28S) of the ribosomal DNA gene were amplified using the Polymerase Chain Reaction (PCR) (Saiki *et al.*, 1988) on a Hybaid™ Touchdown thermo cycling system (Life Sciences International, UK). The primers ITS3 (5'-GCATAGATGAAGAAGCAGC-3') (White *et al.*, 1990) and LR3 (5'-CCGTGTTTCAAGACGGG-3') (White *et al.*, 1990) were used

to amplify the required DNA fragment. Reaction volumes were 100 µl and contained 10 µl 10X PCR buffer (Boehringer Mannheim, Germany), 5 mM $MgCl_2$ (Boehringer Mannheim, Germany), 10 mM dNTP's, 20 pmol of each primer, 0.5 µl DNA and 1.75 U Expand *Taq* polymerase (Boehringer Mannheim, Germany). The PCR reaction conditions were as follows: 2 min. at 94 °C, annealing at 48 °C for 1 min., 10 s at 62 °C, 2 min. at 72 °C with an increase of 5 °C/s. This was repeated for 40 cycles and a final elongation reaction was done at 72 °C for 8 min. The resulting products were purified with the High Pure PCR product Purification Kit (Boehringer Mannheim Kit) and used in DNA sequence reactions.

Sequencing was done using the primers ITS3, LR3, LR1R (5'-AGGAAAAGAAACCAACC-3') (White *et al.*, 1990) and 404X (5'-CCCTTTCAACAATTTCAC-3') (designed based on consensus sequences of selected *Leptographium* and *Saccharomyces cereviseae* LSU sequences). Sequencing was performed on a ABI 377 automated sequencer using the Thermo Sequenase dye terminator cycle sequencing pre-mix kit (Perkin Elmer Applied Biosystems). Sequence data were edited using Sequence Navigator (Perkin Elmer Applied Biosystems) and aligned using the alignment algorithm, CLUSTAL. Analyses were performed in PAUP* (version 4.0) (Phylogenetic Analysis Using Parsimony *and other methods) (Swofford, 1999). Gaps were treated as missing data. Analyses were performed using parsimony and heuristic search option (TBR-tribisection reconnection). Bootstrap values were determined by 100 Bootstrap replicates. Selected *Leptographium* rRNA operon DNA sequences were also compared with other genera of the Ascomycetes. Sequences for the taxa of for comparisons were obtained from genbank (Table 1).

A partition homogeneity test was performed on the different data sets (Huelsenbeck, Bull & Cunningham, 1996). This test gives an indication whether data sets can be combined, or analysed separately. Partition homogeneity tests were performed to determine whether ITS2 sequence data and LSU sequence data can be combined.

This test was also used to determine whether the morphological data set could be combined with that representing molecular data.

Morphological comparison

The data used for comparison were obtained from a morphological study of all described species in *Leptographium* (Jacobs, unpublished). Representative isolates of each species were grown on 2 % Malt extract agar (MEA) (Table1). These were compared with the original description of each species as well as with available herbarium specimens. 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.

Nineteen different characters were used in the comparison. Approximately half of all described *Leptographium* spp. have been connected to a teleomorph (Harrington, 1988; Wingfield, 1993) and for this reason only anamorph characters were used. Morphological characters included conidiophore length, stipe length and conidiogenous apparatus length, morphology of the hyphae and stipe, the presence or absence of rhizoids, primary branch pattern and number of primary branches. They also included primary, secondary, tertiary and quaternary branch length, length of the conidiogenous cells, conidium shape and length, ratio of conidium length to width, optimal growth temperature as well as host and insect associations (Table 2). Species of *Leptographium* can be divided into three groups according to the length of the conidiophore. However, the ranges of the groups overlap considerably. Therefore, the character was reduced to a multi-state character and species were coded according to their range. Similarly, there are three forms of primary branch arrangement in *Leptographium,* which we refer to as type A (only two branches), type B (two or more branches) and type C (two or more branches with a single branch that are twice to three times as broad as the others). All species of *Leptographium* can be defined in terms of one of these types. The last character

considered was the length to width ratio of conidia. All species in *Leptographium* can broadly be defined in terms of small, medium or large conidia. As in the case of the conidiophores, this character was reduced to multiple characters to incorporate the overlapping ranges for the different species.

Morphological characters were coded according to the binary coding system proposed by Viljoen (1996). Characters were defined as multi-state and coded as present (1) or absent (0) (Table 2). A matrix was compiled for the data set and data analyzed using parsimony analysis, as well as distance analysis (UPGMA) using the PAUP* v.4 program (Swofford, 1999). Weighting of taxonomically important characters was done as proposed by Viljoen (1996). These characters include conidiophore length, number of primary branches and the type of arrangement. The weight assigned to a specific character was calculated as the largest number of character states in a character divided by the number of character states in the given character, times a 100. Parsimony analysis, as well as distance analysis (UPGMA) was used for the data set. A bootstrap analysis (100 replicates) was done to calculate the bootstrap values. No outgroup taxa were used and the trees were rooted to midpoint.

RESULTS

Molecular comparison

A single band of 1500 bp was obtained for all the isolates using the primers ITS3 and LR3. Of these 645 bp (base pairs) were used for comparison. This region included the last ten base pairs of the 5.8S gene, the whole ITS2 region and the first part of the 28S gene. Results from the partition homogeneity test indicated that the ITS2 and 28S regions should be analyzed separately (P=0.01). Heuristic analysis of data for the ITS2 region resulted in 452 trees with identical topologies. For the ITS2

region 221 characters were used. Of these 187 were parsimony informative and 24 parsimony uninformative. For the 28S region 423 characters were used. Of these 101 were parsimony uninformative and 69 parsimony informative. The shortest tree length was 1085 with a RI of 0.749 and CI of 0.489 (Fig. 1). Heuristic analysis of the 28S region resulted in 10068 trees with similar topologies. The shortest tree length was 344 with a RI of 0.755 and CI of 0.692. Comparison of the trees from the different analyses, revealed that in both cases, clades will consist of the same set of species. However, due to the conserved nature of the 28S gene, relationships within the genus could only be resolved into larger clades. Analysis of the ITS2 region was necessary to determine relationships within the *Leptographium* group.

Three distinct clades are obvious from the analysis (fig. 1). The first of these includes *Ophiostoma trinacriforme* (Parker) Harrington, *Leptographium brachiatum* (Kendrick) Wingfield, *L. antibioticum* (Kendrick) Wingfield, *O. brevicolla* Davidson, *L. costaricense* Weber, Spaaij & Wingfield, *L. pineti* Jacobs & Wingfield, *L. pityophilum* Jacobs, Wingfield & Uzunovic, *O. leptographioides* (Davidson) Arx and *L. elegans* Wingfield, Crous & Tzean. Apart from *L. brachiatum* and *L. antibioticum* that are morphologically similar, there is no obvious correlation between morphology and phylogeny within this group.

The second clade included *L. abietinum* (Peck) Wingfield, *L. engelmannii* Davidson, *O. abiocarpum* (Davidson) Harrington, *O. huntii* (Robinson De Hoog & Scheffer, *L. guttulatum* Jacobs & Wingfield, *L. euphyes* Jacobs & Wingfield, *L. alethinum* Jacobs & Wingfield, *L. wingfieldii* Morelet, *O. americanum* Jacobs, Wingfield & Bergdahl, *L. pyrinum* Davidson, *L. hughesii* Jacobs, Wingfield & Harrington, *L. eucalyptophilum* Jacobs, Wingfield & Roux, *L. peucophilum* Jacobs & Wingfield, *O. franke-grosmanniae* (Davidson) De Hoog & Scheffer, *O. penicillatum* (Grosmann) Siemaszko, *O. dryocoetidis* (Kendrick & Molnar) Harrington, *O. robustum* (Robinson-Jeffrey & Davidson) Harrington, *O. crassivaginatum* (Griffin) Harrington and *O. grandifoliae* (Davidson) Harrington.

The third clade has two subgroups. The first of these is comprised of *L. reconditum* Jooste, *O. laricis* Van der Westhuizen *et al.*, *O. europhioides* (Wright & Cain) Solheim and *O. piceaperdum* (Rumbold) Arx and the three varieties of *Leptographium wageneri.* The second group consists of *L. albopini* Wingfield, Harrington & Crous, *O. aureum* (Robinson-Jeffrey) Harrington and *L. lundbergii* Lagerberg & Melin. *Leptographium procerum* and *L. terebrantis* Barras & Perry grouped together separate from the other species (Fig. 1).

Comparison of a sub-set of *Leptographium* spp. with other Ascomycetes using only the 28S region and the heuristic search option in PAUP, produced 288 trees with identical topologies. The group belonging to the Pezizales was used as an outgroup. The shortest tree was 596 with a RI of 0.802 and CI of 0.542 (Fig. 2). The dendogram consisted of three distinct clades. The first clade consisted of species belonging to the Eurotiales and Dothideales. The second clade included species residing in the Microascales, Xylariales, Phyllachorales, Ophiostomatales, Hypocreales and Laboulbeniales and the third clade included the Pezizales. All *Leptographium* spp. grouped closely together within the Ophiostomatales.

Morphological comparison

Analysis of the unweighted morphological characters using the heuristic search option in PAUP, produced 1228 trees with similar topologies with species differing only within clusters. The shortest tree length was 403 with a CI of 0.216 and RI of 0.556 (Fig. 3). The partition homogeneity test on the molecular and morphological data, resulted in a P-value of 0.01, suggesting that the data sets cannot be combined. No correlation could be found between trees produced based on molecular characters (Fig. 1) and those from the analysis of morphological characters (Fig. 3). It was, however, of interest that *O. laricis, O. piceaperdum* and *O. europhioides* clustered together in both the molecular and morphological analysis. The same was true for the three *L. wageneri* varieties.

Weighting of morphological characters produced trees that were not congruent with those produced by the analyses of the unweighted characters (data not shown). Three morphologically important characters, conidiophore length, primary branch type and conidium length to width ration were weighted preferentially. No correlation could be found between any of the weighted trees based on morphology and those based on molecular characters. It is interesting, however, that *Ophiostoma laricis, O. europhioides* and *O. piceaperdum* clustered together as before. The same was true for the three varieties of *L. wageneri.*

The morphological data were also subjected to distance analysis using UPGMA. The resulting cladogram, as in the case of the parsimony analysis, was not congruent with the molecular data. The cladogram consists of four clades and each of these included species of all three groups in the molecular analysis.

DISCUSSION

DNA based analysis of species in *Leptographium* has confirmed some of the taxonomic questions regarding species complexes in this genus. The species that group together in the different clusters based on ribosomal DNA sequence data are morphologically diverse and inhabit different niches. In most cases, no obvious relationships emerged.

Based on analysis of the ribsomal DNA data, *Leptographium abietinum* and *L. engelmannii* were found to be closely related. This confirms the synonymy of *L. engelmannii* with *L. abietinum* as proposed by Zambino and Harrington (1992) and implemented by Jacobs *et al.* (1999). The anamorph of *O. abiocarpum* was not described when the teleomorph of this fungus was described (Davidson, 1966). However, Upadhyay (1981) reported a *Leptographium* state for this species. We have not observed this state in material available to us but, its close relationship with

Leptographium in this study confirms that it fits appropriately with other *Leptographium* spp.

Furthermore, *O. europhioides* appears to be closely related to *O. piceaperdum.* This close relationship confirms the recent synonymy of *O. europhioides* with *O. piceaperdum* proposed by Jacobs, Wingfield & Crous (1999). These species were also found to be closely related to *O. laricis* based on DNA analysis and they are morphologically similar (Van der Westhuizen *et al.,* 1995). Cluster III of the DNA based analysis also includes *L. procerum* and *L. terebrantis.* Although these species are morphologically distinct, they have both been considered similar in terms of disease, they occur on the same hosts and are associated with the same insects (Kendrick, 1962; Barras & Perry, 1971; Wingfield, 1983; Highley & Tattar, 1985; Alexander *et al.,* 1988; Harrington, 1988; Wingfield & Gibbs, 1991). In addition, this cluster includes the three varieties of *L. wageneri.* These varieties are difficult to distinguish morphologically, but can be distinguished based on molecular characters and isozyme analyses (Zambino & Harrington, 1992).

The outcome from the DNA sequence based comparison in this study is similar to that of Zambino and Harrington (1992), which was based on isozyme analyses. Although these authors used a smaller number of species, a significant correlation can be seen between the results of the two studies. Species from the *L. serpens* and *L. lundbergii* cluster (Zambino & Harrington, 1992) corresponded well to cluster III of the ribosomal DNA analysis in this study. *Leptographium albopini* (*L.* sp I), *L. aureum, L. terebrantis, L. lundbergii* and *L. europhioides* formed part of the *L. lundbergii,* together in the isozyme analysis of Zambino and Harrington (1992). This corresponds with the grouping of these species in cluster III of our study. The three varieties of *L. wageneri* clustered together closely in the isozyme study (Zambino and Harrington, 1992) and this was also confirmed in the current study.

The synonymy of *Leptographium engelmannii* with *L. abietinum* as proposed by Zambino and Harrington (1992) and implemented by Jacobs *et al.,* (1999), was confirmed using DNA sequence analysis. These species, together with *O. penicillatum* and *O. abiocarpum* formed part of a separate clade based on isozyme analysis (Zambino & Harrington, 1992). This corresponds to clade II derived from the DNA analysis in the present study. From the DNA sequence data, it appears that *O. abiocarpum* is most closely related to *O. huntii.* However, isozyme analyses on these species placed them in two different groups (Zambino & Harrington, 1992).

Ophiostoma crassivaginatum (Griffin) Harrington clustered separately from the other species based on isozyme comparison (Zambino & Harrington, 1992). This was also reflected in the molecular analysis of this study where *O. crassivaginatum* clustered separately from other *Leptographium* spp in clade II. This species is unlike other species and is characterized by very short, robust conidiophores (Griffin, 1968). *Leptographium procerum* resides in the *L. serpens* group based on isozyme analysis (Zambino & Harrington, 1992). From the DNA sequences data in this study, however, it appears to be more closely related to *L. terebrantis* than to *L. wageneri.* Two species, *O. huntii* and *L. pyrinum,* that reside in cluster II of the DNA sequence study, grouped in the *L. lundbergii* cluster of Zambino & Harrington (1992).

Based on partial sequence of the ITS 1 and 2, as well as the 5.8 S gene, Coetsee (1999), speculated that conidium size might be an indication of phylogeny in some species of *Leptographium.* This was based on the fact that species with long spores clustered separately from those with shorter spores. This observation was also apparent in the current study with all the species having long spores namely, *L. penicillatum, O. americanum* and *O. dryocoetidis,* grouping together. However, this group also contained species with medium sized and small spores and natural relationships do not appear to be reflected by conidium size.

The close relationship between *O. europhioides, O. piceaperdum* and *O. laricis* found in a previous study (Van der Westhuizen *et al.,* 1995; Jacobs *et al.,* 1998), is confirmed here. *Leptographium procerum,* although part of one group in this study, grouped away from the cluster accommodating *O. laricis* and *O. europhioides*. This is consistent with the findings of Coetsee (1999). The only discrepancy between the two studies was with the placement of *Leptographium guttulatum.* Coetsee (1999) found that this species grouped separately from *O. penicillatum.* However, in the current study, *L. guttulatum* groups together with *O. penicillatum* in clade II.

This study has shown that there is no single morphological character that corresponds with phylogeny based on rDNA sequences. However, the rDNA sequence-based data might be reflected in a combination of morphological characters. To test this hypothesis, we coded the morphological characters using a binary code (Viljoen, 1996). Analyses of the unweighted characters produced a dendogram including three clades. Each clade included a group of species from the three main clusters emerging from the sequence analysis. The only groups within the morphological dendrograms that were similar to those emerging from the molecular comparison was the group with *O. piceaperdum, O. europhioides* and *O. laricis* and the cluster including the three varieties of *L. wageneri*. Weighting of different taxonomically important characters did not produce trees that were congruent with results based on rDNA based analysis. This was confirmed through the homogeneity test, which resulted in P values of lower than 0.05. Taxonomically important morphological characters, therefore, cannot be used to infer phylogeny, and only seem to be useful in the identification of these species.

From the molecular data presented in this study, it is apparent that there is no distinction between *Leptographium* spp. that are not known to be associated with a teleomorph, and those that are known to be associated with an *Ophiostoma* state. Earlier researchers have reported a close relationship of morphologically similar genera based on cycloheximide tolerance (Harrington, 1981; Marais, 1996) and the

presence of cellulose in the cell walls of both these genera (Horner *et al.*, 1986; Marais, 1996). This relationship is confirmed in our study. Comparison with other genera of Ascomycetes using partial sequence of the 28S gene, placed all *Leptographium* spp. within the Ophiostomatales, together with *Ophiostoma* spp. *Leptographium* spp. without teleomorphs grouped together with species with known *Ophiostoma* teleomorphs. This implies a strong association between *Leptographium* and *Ophiostoma* as previously suggested (Harrington, 1981; Wingfield, 1993).

Many *Leptographium* spp. are morphologically similar and they are generally difficult to identify (Wingfield, 1993). Thus, many misidentifications have emerged in the scientific literature and these have in some cases also led to misdiagnoses of disease problems. Results of this study have, for the first time provided DNA sequence data for a relatively large set of *Leptographium* spp. We are already using these data to routinely confirm the names of species that are sent to us for identification and we believe that this particular output of this study is especially valuable. Although availability of isolates and cost precluded us from including additional specimens, in future, it will be desirable to extend this initial database. We also hope that similar data sets for other genes will emerge and provide tools for the identification of *Leptographium* spp.

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Table 1. Isolates used in the study.

Species	Ascession number	Isolate number	Host	Origin	Collector
L. abietinum	Not available	CMW 2817	*Picea engelmannii*	USA	T.C. Harrington
L. abicolens	"	CMW 2865	*Abies balsamea*	USA	D.R. Bergdahl
L. albopini	"	CMW 26	*Pinus strobus*	USA	A Lackner
		CMW 2065	*Pinus strobus*	USA	A Lackner
L. antibioticum	"	CMW 2777	*Pinus taeda*	USA	S. Alexander
L. brachiatum	"	CMW 2855	*Picea rubens*	USA	S. Alexander
L. alethinum	"	CMW 3766	*Hylobius abietis* galleries	England	A. Uzunovic
L. costaricense	"	CMW 3041	soil	Costa Rica	P.W. Crous
L. douglasii	"	CMW2078	*Pseudotsuga menziesii*	USA	D. Goheen
L. elegans	"	CMW 2245	*Chaemacyparis* sp.	Japan	M.J. Wingfield
L. eucalyptophilum	"	2.1	*Eucalyptus* spp.	Democratic Republic of Congo	J.Roux
L. euphyes	"	CMW 301			

L. hughesii	"	CMW 4052	*Aquilana* spp.	Vietnam	B. Blanchette
		C930			
L. guttulatum	"	CMW 742	*Pinus sylvestris*	France	M. Morelet
L. lundbergii	"	CMW 30	*Pinus strobus*	New Zealand	M. Dick
L. pineti	"	CMW 3831	*Pinus* sp.	Indonesia	M.J. Wingfield
L. pityophilum	"	CMW 2840	*Pinus nigra*	Italy	S. Frisullo
L. procerum	"	FHM 93-36	*Pinus* sp	USA	M.J. Wingfield
L. pyrinum	"	CMW 169	*Pinus ponderosa*	USA	R.W. Davidson
L. reconditum	"	CMW 15	*Zea mays*	RSA	W. Joost
			rhizosphere		
L. terebrantis	"	CMW 9	*Pinus sylvestris*	Minnesota	M.J. Wingfield
L. wageneri var.	"	CMW 154	*Pseudotsuga*	USA	T.C. Harrington
pseudotsugae			*menziesii*		
L. wageneri var.	"	CMW 402	*Pinus* spp.	USA	T.C. Harrington
wageneri					
L. wingfieldii	"	CMW 2096	*Pinus sylvestris*	France	M.Morelet
O. americanum	"	CMW 495	*Larix decidua*	USA	D.R. Bergdahl
		CMW 2963	*Larix decidua*	USA	D.R. Bergdahl
O. aureum	"	CMW 714	*Pinus contorta* var.	Canada	R.W. Davidson
			latifolia		& T.C.
					Harrington

O. brevicolla	"	CMW 447	*Populus* sp	USA	R.W. Davidson
O. crassivaginatum	"	CMW 90		USA	T. Hinds
O. dryocoetidis	"	CMW 442	*Abies lasiocarpa*	Canada	A.C. Molnar
O. francke-grosmanniae	"	CMW 445	*Quercus* sp.	Germany	H. Francke-Grosmann
O. grandifoliae	"	CMW 703	*Fagus grandifoliae*	USA	R.W. Davidson
O. huntii	"	CMW 2824	*Pinus* sp.	USA	T.C. Harrington
O. laricis	"	CMW 1980	*Larix* sp.	Japan	Y. Yamaoka
O. leptographioides	"	CMW 2803	*Quercus alba*	USA	B. Moss
O. penicillatum	"	CMW 435	*Picea abies*	Germany	
O. piceaperdum	"	CMW 660	*Picea abies*		M. Hallaksella
O. robustum	"	CMW 2805		USA	T. Hinds
O. trinacriforme	"	CMW 670	*Pinus monticola*	Canada	A.K. Parker
O. wageneri (*L. wageneri* var. *ponderosum*)	"	CMW 2821	*Pinus* sp.	USA	T.C. Harrington
Ceratocystis albofundus	AF043605				
Ceratocystis laricicola	AF043600				
Colletotrichum capsici	Z18982				
Colletotrichum	Z18983				

lindemuthianum	
Colletotrichum truncatum	Z18978
Diplodia tumefaciens	AF110816
Epichloe amarillans	U57680
Epichloe baconii	L07138
Epichloe festucae	X62987
Epichloe glyceriae	L07137
Epichloe typhina	L07132
Eupenicillium hirayamae	AF033418
Eupenicillium katangense	AF033458
Eupenicillium reticulosporum	AF033437
Fusarium oxysporum f.sp. *melonis*	M38153
Fusarium solani	L36634
Glomerella cingulata	Z18993
Glomerella graminicola	Z18984
Melanospora fallax	U47834

Melanospora fallax	U17404
Peziza cerea	AF133164
Peziza violacea	AF133171
Sarcosphaera coronaria	AF133172
Scabropezia scabrosa	AF133173
Sphaeropsis sapinea	AF110815
Verticillium lecanii	U17421
Verticillium lecanii	AF049176
Xylaria curta	U47840
Xylaria hypoxylon	U47841

CMW refers to the culture collection of M. J. Wingfield - Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute (FABI), Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, Republic of South Africa, C refers to the culture collection of T. C. Harrington - Department of Plant Pathology, 351 Bessey Hall, Iowa State University, Ames, Iowa 50011.

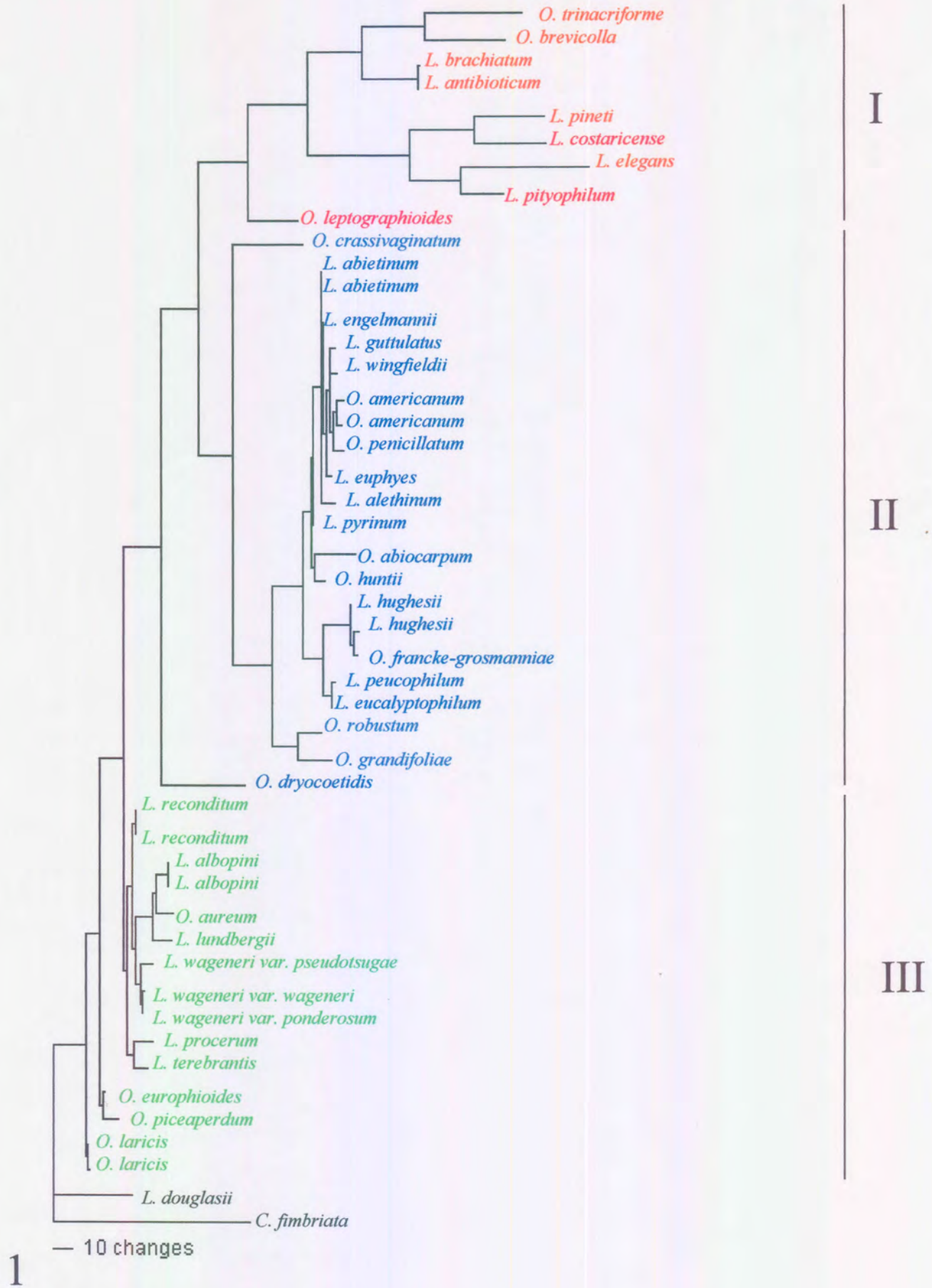
Table 2. Characters used in morphological comparisons

Charater states	1	2	3	4	5	6	7
1. Morphology of hyphae	Constricted at the septa	Not constricted at the septa	-	-	-	-	-
2. Conidiophore length	Less than 100 µm	100 – 200 µm	200 – 400 µm	400 – 600 µm	600 – 800 µm	800 – 1000 µm	1000 – 1500 µm
3. Stipe length	Less than 100 µm	100 – 200 µm	200 – 400 µm	400 – 600 µm	600 – 800 µm	800 – 1000 µm	1000 – 1500 µm
4. Stipe morphology	Not constricted at the septa	Constricted at the septa	-	-	-	-	-
5. Conidiogenous apparatus length	10 – 30 µm	30 – 50 µm	50 – 80 µm	80 – 100 µm	more than a 100 µm	-	-
6. Rhizoids	Present	Absent	-	-	-	-	-
7. Primary branch type	Type A	Type B	Type C	-	-	-	-
8. Number of primary branches	2	2 - 3	3 - 4	4 - 5	more than 5	-	-
9. Primary branch length	Less than 10 µm	10 – 15 µm	15 – 20 µm	more than 20 µm	-	-	-
10. Secondary branch length	Less than 10 µm	10 – 15 µm	15 – 20 µm	more than 20 µm	-	-	-
11. Tertiary branch length	Less than 10 µm	10 – 15 µm	15 – 20 µm	more than 20 µm	To complex to measure	Absent	-

Table 2. (cont.)

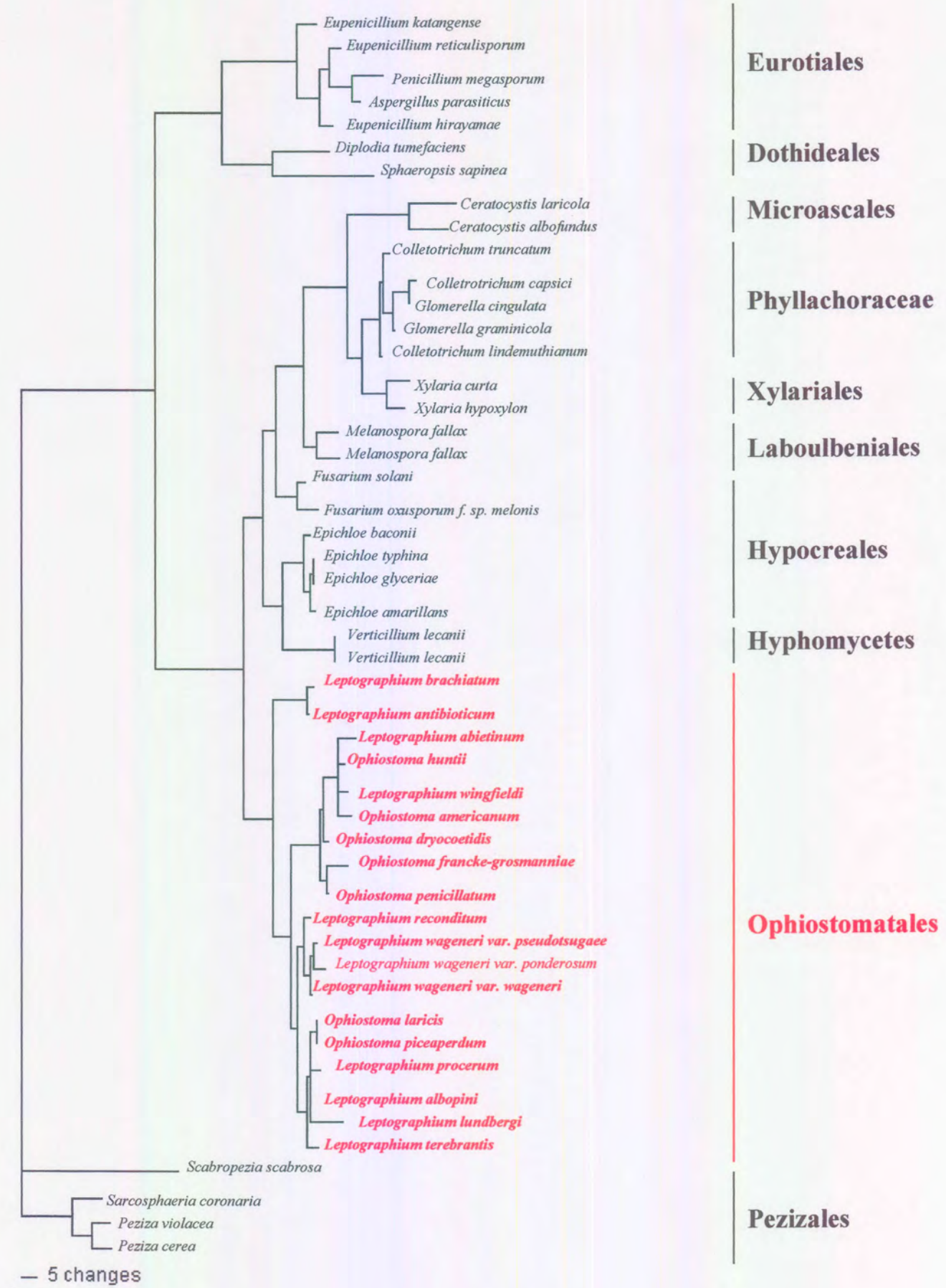
Charater states	1	2	3	4	5	6	7
12. Quaternary branch length	Less than 10 µm	10 – 15 µm	15 – 20 µm	more than 20 µm	Absent	To complex	-
13. Conidiogenous cell length	Less than 10 µm	10 – 15 µm	15 – 20 µm	more than 20 µm	-	-	-
14. Conidium shape	Oblong to obovoid	Obovoid	Distinctly curved	-	-	-	-
15. Conidium length	3 – 5 µm	5 – 7 µm	7 – 10 µm	10 – 12 µm	more than 12 µm	-	-
16. Associated hosts	*Pinus* spp	*Picea* spp.	*Larix* spp.	*Pseudotsuga* spp.	*Abies* spp	Other conifers	Non-conifers
17. Insect association	Associated with insects	Not associated with insects	-	-	-	-	-
18. Optimum growth temperature	15 °C	20 °C	25 °C	30 °C	-	-	-
18. Ratio of conidium length: width	1.5:1	2:1	2.5:1	3:1	4:1	5:1	4:3

Fig. 1. Dendrogram of the DNA based analysis of species in *Leptographium.* 100 trees with identical topologies were obtained through the PAUP analysis using a heuristic search. Trees are rooted to midpoint. The shortest tree length was 1085 with a RI of 0.749 and CI of 0.489.



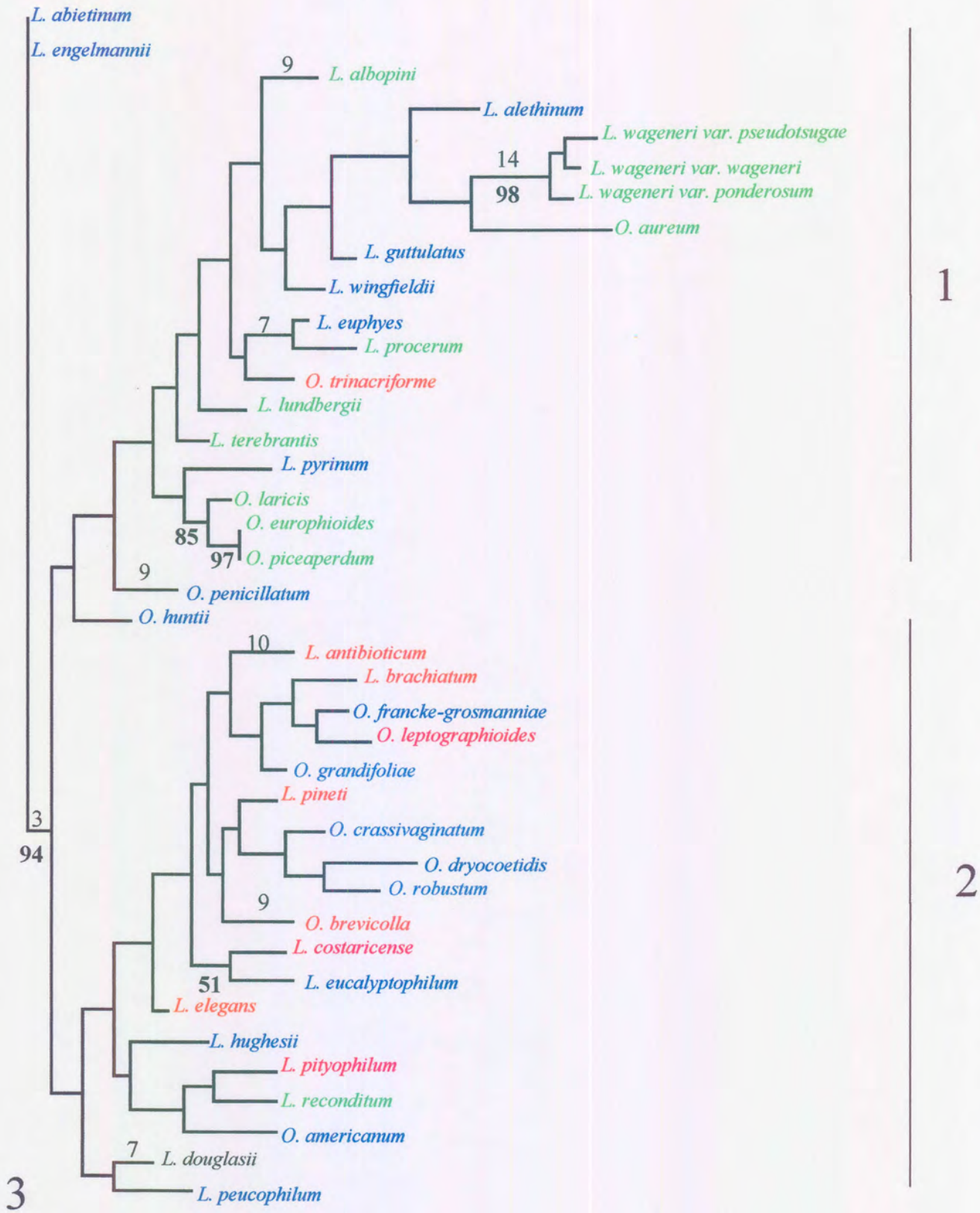
1

Figure 2. Comparison of a sub-set of *Leptographium* spp. to other Ascomycetes using the heuristic search option in PAUP, produced 288 trees with identical topologies . The group belonging to the Pezizales was used as an outgroup. The shortest tree was 596 with a RI of 0.802 and CI of 0.542.



2

Figure 3. Analysis of the unweighted morphological characters using the heuristic search option in PAUP, produced 1228 trees with similar topologies. The shortest tree length was 403 with a CI of 0.216 and RI of 0.556



Chapter 2

Jacobs, K., Wingfield, M.J., Crous, P.W. and Harrington, T.C. (1999). *Leptographium engelmannii,* a synonym of *L. abietinum*, and description of *L. hughesii* sp. nov. Canadian Journal of Botany 76, 1660-1667.

Leptographium engelmannii, a synonym of *L. abietinum*, and description of *L. hughesii* sp. nov.

Leptographium abietinum occurs in North America on various members of the Pinaceae, especially spruce (*Picea* spp.), always in association with bark beetles (Coleoptera: Scolytidae). It is characterized by noticeably curved, clavate conidia. All the isolates were from species of Pinaceae in North America except two isolates examined by Kendrick, originating from *Parashorea plicata* imported to England from Borneo and from *Melia* sp. imported into New Orleans, USA. After examination of the isolate from Borneo and a similar isolate from Vietnam, we have concluded that these do not represent *L. abietinum.* They are described as a new species, *L. hughesii. Leptographium engelmannii,* described from Engelmann spruce in Colorado, USA, is indistinguishable form *L. abietinum* and is considered a synonym of the latter species.

INTRODUCTION

The genus *Leptographium* Lagerb. & Melin includes a number of economically important species associated with root disease and sapstain of timber (Wagener & Mielke, 1961; Harrington, 1988; 1993; Wingfield, Capretti & Mackenzie, 1988; Wingfield, 1993). These fungi are mainly known from conifers, where they are generally associated with bark beetle (Coleoptera: Scolytidae) infestation (Harrington, 1988; 1993; Wingfield, 1993). Some species have also been isolated from non-coniferous hosts, roots and soil (Jooste, 1978; Weber, Spaaij & Wingfield, 1996). Many *Leptographium* spp. are anamorphs of *Ophiostoma*, although some species currently included in the genus lack teleomorphs and are, therefore, of unknown affinity (Jooste, 1978; Harrington, 1987; 1988; Wingfield, 1993; Wingfield, Harrington & Crous, 1994; Wingfield, Crous & Tzean, 1994; Weber *et al.,* 1996).

Leptographium abietinum (Peck) Wingfield occurs on members of the Pinaceae, especially *Picea*, and is associated with species of *Dendroctonus, Hylastes* and *Hylurgops* that infest these trees (Kendrick, 1962; Harrington & Cobb, 1983; Harrington, 1988; Zambino & Harrington, 1992). This species was first described by Peck (1879) as *Sporocybe abietina* Peck and was later transferred to *Periconia* Tode ex Schweinitz by Saccardo (1886). Hughes (1953) recognized the importance of conidium ontogeny as a taxonomic character in anamorphic fungi and established the genus *Verticicladiella* Hughes based on S. *abietina,* which then became known as *Verticicladiella abietina* (Peck) Hughes.

Verticicladiella was thought to be related to *Leptographium* but could be distinguished by differences in the proliferation of the conidiogenous cells. In species ascribed to *Verticicladiella,* proliferation is sympodial, whereas in *Leptographium* species, proliferation is percurrent (Hughes, 1953; Kendrick, 1962). Wingfield (1985) showed that some species in both of these genera displayed apparent sympodial proliferation, which in fact is annelidic with delayed secession

of the conidium, giving it a false sympodial appearance (Van Wyk, Wingfield & Marasas, 1988). He thus reduced *Verticicladiella* to synonymy with *Leptographium.* This included *V. abietina,* which became known as *L. abietinum* (Wingfield, 1985).

The first complete description of *L. abietinum* was provided by Kendrick (1962). Two of the isolates he examined were isolated from hosts other than spruce. One of these hardwood isolates, DAOM 62102 that was used to illustrate the protologue of *L. abietinum* (Kendrick, 1962; p.774), originated from *Parashorea plicata* imported to England from Borneo. The recent availability of an isolate of *Leptographium* from Vietnam that resembles *L. abietinum,* prompted us to re-examine the type material of *L. abietinum,* and the specimen from Borneo that was illustrated by Kendrick (1962).

Leptographium engelmannii, which is known from spruce and associated with the bark beetle *Dendroctonus rufipennis* Kirby (= *D. engelmannii* Hopkins), is also characterized by curved, clavate conidia (Davidson, 1955). Harrington (1988) suggested that *L. engelmannii* and *L. abietinum* might be synonymous, and isozyme analysis (Zambino and Harrington, 1992) supported this synonymy. In the present study, we re-examined *L. abietinum* and compare it to *L. engelmannii* in order to determine whether they could justifiably be maintained as separate species.

MATERIAL AND METHODS

Numerous isolates of *L. abietinum* as well as herbarium specimens of this and other similar species were included in the study. Herbarium isolates examined were *L. abietinum*: slide DAOM 33942, on the bark of spruce, Albany; DAOM 37980, *Picea engelmannii*, A. Molnar, 20 March 1953, Victoria, Canada; DAOM 64328 (DAVFP 11869), *Pseudotsuga menziesii*, C. Cottrell, 20 June 1958,

McGillivray Lake, British Colombia; *L. engelmannii:* US0 422466; *Picea engelmanii,* collected: R. W. Davidson; from Borneo: DAOM 62102, *Parashorea plicata*, Savary, Dec. 1957, Princess Risborough, England, on a ship from Borneo. The herbaria where these isolates are maintained, are as follows: DAOM represents the National Mycological Herbarium, Eastern Cereal and Oilseed Research Centre, William Saunders Building, Agriculture and Agri-food Canada, CEF, Ottawa, K1A 0C6, Canada and USO indicates the National Fungus Collections, Beltsville, Maryland, USA.

Cultures examined included for *L. abietinum*: CMW 2817 (=C699), *Picea engelmannii*, T. C. Harrington, 1993, Dixie Nature Forest, Utah; CMW 276, *Picea engelmannii,* A. Molnar, 1987, Victoria, BC; CMW 3083, *Picea* sp. M.J. Wingfield, August 1994, British Columbia; *L. engelmannii:* CMW 759 (=C29, C713, CO456, RWD971), collected by R. W. Davidson; Vietnam: CMW 4052 (= C930), isolated from the wounds of live *Aquilana* sp., R. A. Blanchette, June, 1996, Phu Quoc Island, southern part of Vietnam. The culture collections where these isolates are maintained, are as follows: CMW represents the culture collection of the Tree Pathology Co-operative Program (Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein, 9301, Republic of South Africa) and C represent the culture collection of T.C. Harrington, Department of Plant Pathology, Iowa State University, Ames, Iowa, 50011, USA

All measurements were made from fungal structures produced in culture on 2 % malt extract agar, (MEA, 20g Biolab malt extract, 20g Biolab agar and 1000 ml distilled water) in 90mm diameter plastic petridishes, containing 20 ml medium . Fungal structures for microscopic examination were mounted on slides in lactophenol. Fifty measurements of each relevant morphological structure were made and ranges and means computed. Herbarium specimens were examined by placing a drop of 1% KOH on the dried material. After five minutes, small portions of fungal material were removed and mounted in lactophenol on glass slides.

Isolates were also examined using 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 JSM 6400 scanning electron microscope.

The cardinal temperatures for growth of the isolates representing *L. abietinum* (CMW 2817), *L. engelmannii* (CMW 759) and the isolate from Vietnam (CMW 4052) were determined by inoculating eight MEA plates for each isolate at each temperature with a 6 mm diameter colonized agar plug taken from the actively growing margin of fresh colonies. The plates were incubated at temperatures ranging from 5 to 35 °C at 5 °C intervals. Colony diameters were measured after four and eight days, and the size of colonies was computed as an average of eight readings at each respective temperature.

Cycloheximide tolerance of *L. abietinum* (CMW 2817) and *L. engelmannii* (CMW 759) was determined after eight days of growth on 2 % MEA amended with 0.5 mg/ml cycloheximide. The plates were incubated at 25 °C and colony diameters were measured. Cycloheximide tolerance of the Vietnamese isolate (CMW 4052) was determined after eight days of growth on 2 % MEA amended with cycloheximide at 0, 0.05, 0.1, 0.5, 1.0, 2.5 and 5.0 mg/ml after eight days of growth.

RESULTS

The *Leptographium* sp. from Vietnam occurring on *Aquilana* sp. was morphologically identical to the fungus isolated from *Parashorea plicata* from Borneo (DAOM 62102) and illustrated by Kendrick (1962). Another isolate that we examined from hardwood material collected in Malaysia was also morphologically identical to the Borneo material, but this isolate is no longer available. These Southeast Asian isolates have slightly curved conidia and, thus, resemble the type material and other collections of *L. abietinum* from Pinaceae in North America. However, these fungi have very different hosts and geographic distributions, and on close examination they can be distinguished morphologically (Table 1).

Leptographium abietinum is characterized by dark olivaceous colonies on malt extract agar, with conidiophores arising directly from the agar with little aerial mycelium. In contrast, isolates of *Leptographium* sp. from Vietnam, Malaysia and Borneo are characterized by having a dense mat of aerial mycelium covering the colony, with conidiophores occuring in groups on the aerial mycelium and agar surface. The Asian isolates produce rhizoids at the bases of the conidiophore stipes, whereas, these structures are absent or very rarely found in isolates of *L. abietinum* (Fig. 1,2). The conidiophores of the Asian taxon and *L. abietinum* are similar (Fig. 3,4) but those of the Asian taxon are nearly twice as long as those of *L. abietinum* (Table 1). These two taxa can also be differentiated based on conidial morphology. Although the unnamed *Leptographium* sp. has similar curved conidia to *L. abietinum,* most of the conidia are ellipsoidal to obovoid (Fig. 5,6). The Vietnamese isolate also showed an increase in growth rate on 0.1 mg/ml cycloheximide compared to no cycloheximide, with growth inhibition only at higher concentrations of the antibiotic. In contrast, *L. abietinum* had a decreased growth rate when grown on 0.1 mg/ml cycloheximide.

From these observations we conclude that the isolates of the *Leptographium* sp. from Vietnam and Borneo represent an undescribed taxon which is described below.

The type specimen of *L. engelmannii* (USO 422466) was in a poor condition, making comparison with the holotype of *L. abietinum* (DAOM 33942) difficult. A culture of *L. engelmannii* from Davidson's collection, perhaps derived from the holotype, was available for comparison, and the two species appeared morphologically identical. Both species are morphologically similar. In culture, *L. abietinum* and *L. engelmannii* are virtually identical. Both have optimum growth temperatures at 25 °C and both produce cartridge buff to olivaceous (Rayner, 1970) colonies. *Leptographium abietinum* and *L. engelmannii* both tolerate high concentrations of cycloheximide, an indication that they are anamorphs of *Ophiostoma* (de Hoog & Scheffer, 1984; Harrington, 1981). Furthermore, *L. engelmannii* was described from spruce infested with *Dendroctonus rufipennis,* a common bark beetle associate of *L. abietinum* (Harrington, 1988). They also have similar isozyme electromorphs (Zambino & Harrington, 1992). From these data we conclude that *L. engelmannii* is conspecific with *L. abietinum,* and thus their synonymy is proposed below.

Taxonomy

***Leptographium abietinum* (Peck) Wingfield Trans Br. myco. Soc. 85, 92. 1985. Figs. 2,4,6.**

=*Sporocybe abietina* Peck, New York State Museum Report 31, 45. 1879.
=*Periconia abietina* (Peck) Sacc., Sylloge Fungorum, 4, 273. 1886.
=*Verticicladiella abietina* (Peck) Hughes, Can. J. Bot. 31, 653. 1953.

= *Leptographium engelmannii* Davidson, Mycologia 47, 59. 1955.

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 hughesii* Jacobs, M.J. Wingfield & Harrington sp. nov.**

Conidiophora evenientia singulatim vel usque ad octona aggregata, exorientia directe ex agaro vel ex mycelio aerio, erecta, macronematosa, mononematosa, 110 - 1120 (medius = 650) µm longitudine, rhizoidaceis structuris praesentibus. Stipites olivaceo-lutei, lenes, cylindracei, simplices, 4-18 septati, 80 - 1130 (medius = 598) µm. Apparatus conidiogenus 27.0 - 92.5 (medius = 60.5) µm longus, massa conidica exclusa, 2 vel 3 (aliquando 4) seriebus ramorum cylindricorum; 2-3 metulae primariae, olivaceo-luteae, leves, cylindricae, aseptatae, 11.0 - 35.5 (medius = 19.0) µm longae et 3.0 µ 6.0 (medius = 4.0) µm latae. Auctus conidii eveniens pariete reponendi causa constructo, holoblastica ontogenie et percurrenti proliferatione et retardata secessione efficiente impressionem falsam proliferationis sympodicae. Conidia hyalina, aseptata, ellipsoidea vel obovoidea, aliquando exique curvata 1.0 - 2.5 x3.0 µ 5.0 (medius = 1.5x4.0) µm.

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 8 mm in diameter after 8 days, with little growth at 5 °C and no growth at 35 °C . Colony olivaceous (21"m) (Rayner, 1970), with lacinate margins. Able to withstand high concentrations of cycloheximide with a 60 % increase in linear growth on 0.1 mg/ml cycloheximide, with a 63 % reduction in growth on 5 mg/ml cycloheximide after 8 days at 20 °C in the dark.

Colony covered in a dense mat of aerial mycelium, hyphae mostly submerged, hyaline, smooth, straight, not constricted at the septa, 1.5 - 6.0 (mean = 3.0) µm

diameter. Conidiophores occurring singly or in groups of up to eight, arising directly from the agar or aerial mycelium, erect, macronematous, mononematous, 110 - 1200 (mean = 650) µm in length, rhizoid-like structures present at the base (fig. 13a). Stipe olive-buff (21'"b), smooth, cylindrical, simple, 4 - 18 septate, 80 - 1130 (mean = 598) µm long (from first basal septum to below primary branches) 3.5 - 7.5 (mean = 5.5) µm wide below primary branches, apical cell not swollen; 5.0 - 12.0 (mean = 8.0) µm wide at base, basal cell slightly swollen (fig. 3, 7, 13b). Conidiogenous apparatus 27.0 - 92.5 (mean = 60.5) long, excluding the conidial mass, with 2 to 3 (occasionally 4) series of cylindrical branches; 2 to 3 primary branches, olive-buff (21'"b), smooth, cylindrical, aseptate, 7.5 - 35.5 (mean = 19.0) µm long and 2.0 - 6.0 (mean = 4.0) µm wide, secondary branches hyaline to olive-buff (21"b), aseptate, 6.0 - 16.0 (mean = 12.0) µm long, 2.0 - 4.0 (mean = 3.0) µm wide; tertiary branches hyaline, aseptate, 4.0 - 13.5 (mean = 8.0) µm long, 1.0 - 3.0 (mean = 2.0) µm wide, quaternary branches aseptate, 6.0 - 8.5 (mean = 8.0) µm long, 1.0 - 2.0 (mean = 1.7) µm wide (fig 8, 13c). Conidiogenous cells discrete, 2 to 4 per branch, tapering slightly from the base to the apex, 8.0 - 18.5 (mean = 12.0) µm long and 1.0 - 2.0 (mean = 1.2) µm wide. Conidium development occurring through replacement wall building with holoblastic ontogeny, percurrent proliferation and delayed secession, giving the false impression of sympodial proliferation (fig. 9-11). Conidia hyaline, aseptate, ellipsoid to obovoid, occasionally slightly curved, 1.0 - 2.5 x 3.0 - 5.0 (mean = 1.5 x 4.0) µm. Basal conidium frill absent (fig. 5, 12, 13d). Conidia accumulating in white, slimy droplets at the apex of conidiogenous apparatus.

SPECIMENS EXAMINED: Herbarium isolates: Holotype: CMW 4052, isolated from the wounds of live *Aquilana* sp., R.A. Blanchette, June, 1996, Phu Quoc Island, southern part of Vietnam; Paratype: DAOM 62102, *Parashorea plicata*, Savary, Dec. 1957, Princess Risborough, England, on a ship from Borneo. Dried specimens and cultures deposited at DAOM and CBS, respectively.

DISCUSSION

Leptographium abietinum is one of the most common fungi occurring on *Picea* spp. infested with *Dendroctonus rufipennis* in North America (Kendrick, 1962; Harrington, 1988; Solheim, 1995). The fungus is characterized by olivaceous colonies and conidiophores ranging in length from 90 to 570 µm and its distinctive narrow, prominently curved conidia. The latter feature was also recognized as taxonomically significant by Kendrick (1962), who unfortunately chose a culture from *Parashorea plicata* in Borneo to represent his revised description and illustration of *Verticicladiella abietinum*.

At present we regard *L. abietinum* as specific to hosts in the Pinaceae, and the species has been isolated from *Picea, Abies, Pinus* and *Pseudotsuga* in North America (Kendrick, 1962; Harrington & Cobb, 1983; Harrington, 1988; Zambino & Harrington, 1992). The fungus has been associated with bark beetles, *Dendroctonus rufipennis, D. pseudotsugae, Hylastes longicollis,* and *Hylurgops planirostris* (Harrington, 1988) and appears to be avirulent or weakly virulent to pine and spruce (Harrington & Cobb, 1983; Reynolds, 1992). *Leptographium engelmannii* from Engelmann spruce in North America is clearly the same fungus, as has been shown in morphological and isozyme comparisons (Zambino & Harrington, 1992). In our opinion, the importance of host, geographical distribution and vectors has been underestimated in the taxonomy of the Ophiostomatoid fungi, including *Ophiostoma* spp., *Ceratocystis* spp. and their anamorphs, including *Leptographium* (Wingfield, 1993).

Leptographium abietinum can easily be distinguished from other species of *Leptographium* based on morphology, particular by its distinct curved conidia. The slightly curved conidia of *L. hughesii* are similar to those of *L. abietinum,* but *L. hughesii* has longer conidiophores, with basal rhizoids and abundant aerial mycelia. The difference in the geographic distribution and host range of these two taxa is noteworthy. All identified *L. hughesii* isolates have been from Southeast

Asia. It appears that *L. abietinum* is restricted to North America. An isolate (C172) from spruce in Scotland is morphologically similar to *L. abietinum,* and it has similar isozyme electromorphs, but can be separated by conidiophore morphology and growth rate (Zambino & Harrington, 1992).

Leptographium hughesii peripherally resembles *L. procerum.* Both these fungi are characterized by long conidiophores (up to 1250 µm) and rhizoids at the bases of the conidiophores. However, these species can easily be distinguished based on the presence of abundant aerial mycelium in colonies of *L. hughesii.* Colonies of *L. procerum* are characterized by submerged mycelia that display concentric zones when grown in culture (Kendrick, 1962). *Leptographium hughesii* is characterized by ellipsoid to obovoid conidia that can be slightly curved in certain cases. In contrast, *L. procerum* are characterized by small (2.5 - 5 µm) obovoid conidia that are never curved. A further difference between these fungi is their host preference. *Leptographium hughesii* is known from non-coniferous hosts, whereas *L. procerum* occurs predominantly on *Pinus* spp. and exclusively on conifers (Kendrick, 1962; Wingfield, 1983; Wingfield *et al.,* 1988). It is best known on white pine (*Pinus strobus* L.), where it has been associated with a disease, known as white pine root decline (Wingfield, 1983; Wingfield *et al.,* 1988; Alexander *et al.*, 1988). No evidence is available to suggest that *L. hughesii* is a pathogen.

Many *Leptographium* spp. have been described from conifers infested with bark beetles (Harrington, 1988; 1993), and *L. hughesii* is unusual in its association with tropical hardwoods. Its vectors have yet to be identified. The cycloheximide tolerance of *L. hughesii* suggests a relationship to *Ophiostoma* (Harrington, 1981), but no perithecia have been associated with this fungus. Recognition of this species confirms the suggestion of Wingfield (1993) that many *Leptographium* spp. remain to be discovered, particularly in poorly studied regions such as Southeast Asia. Future collections in these areas would, therefore, most probably reveal a number of new species in the genus.

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Table 1. Comparison between *Leptographium abietinum*, *L. engelmannii* and *L. hughesii*.

	Species examined		
Characters	***L. abietinum***	***L. engelmannii***	***L. hughesii***
Host	*Abies grandis; Picea engelmannii; P. mariana; P. rubrens; P. ponderosa; Pseudotsuga mensiezii; Pinus contorta; Pinus* spp.	*Picea engelmannii, Pinus contorta*	*Parashorea plicata, Aquilana* sp.
Associated insect	*Dendroctonus Pseudotsuga; D. rufipennis; Hylastes longicollis; Hylurgops planirostris*	*D. rufipennis*	none reported
Distribution	British Columbia, Canada; Eastern and Western USA	Colorado	Borneo, Vietnam
Rhizoids	absent	absent	present
Conidiophore length	96 - 570 µm	100 - 450 µm	240 - 1200 µm
Conidium shape	clavate with curved ends	clavate with curved ends	ellipsoid to obovoid, occasionally curved
Conidium size	4.0 - 7.0 x 1.0 - 2.5 µm	4.0 - 6.5 x 1.0 - 2.5 µm	3.0 - 6.0 x 1.0 - 2.5 µm

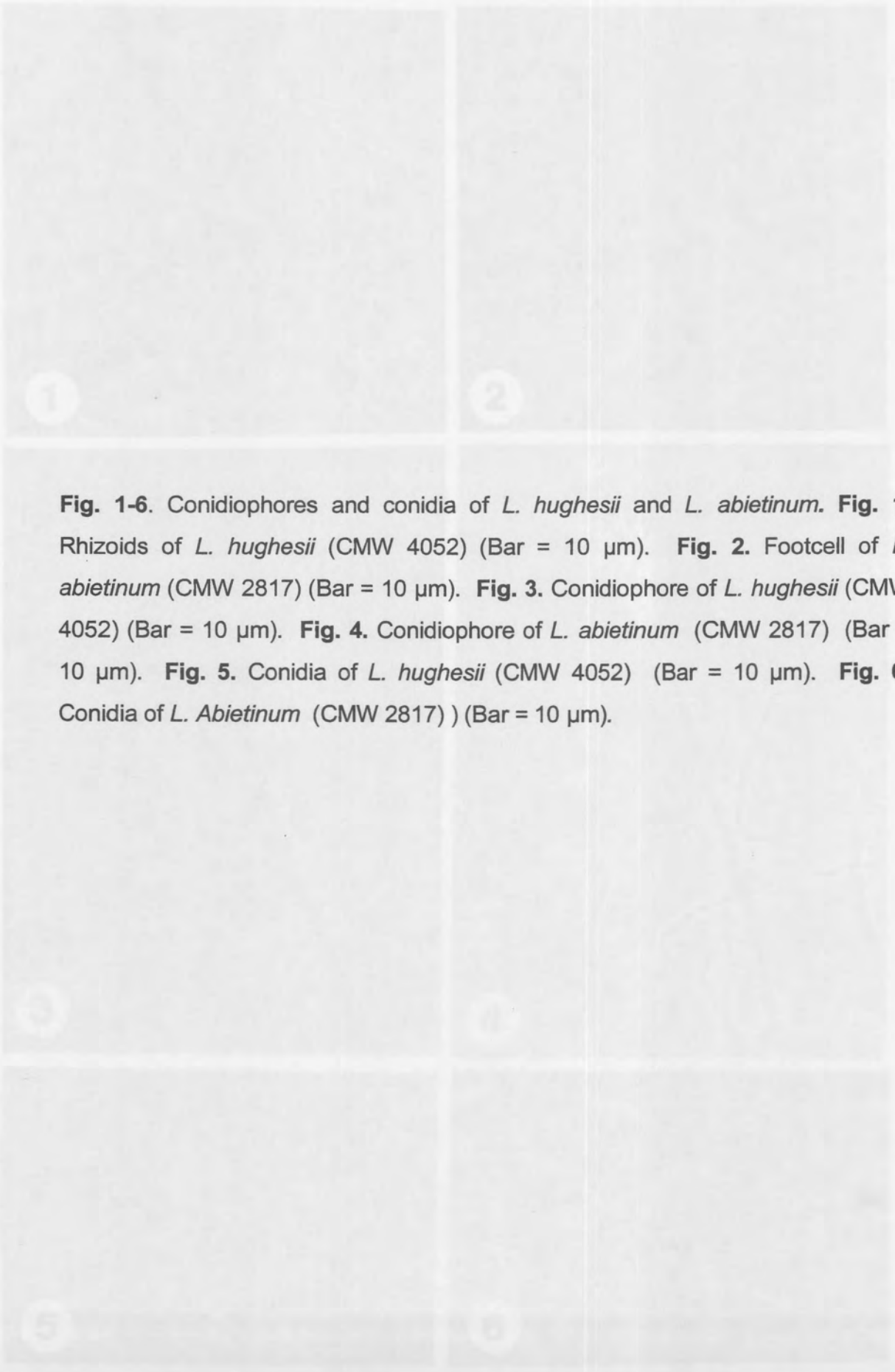
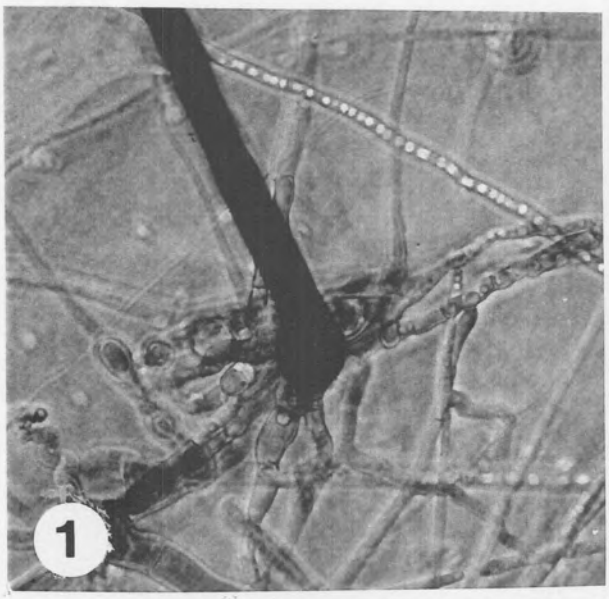
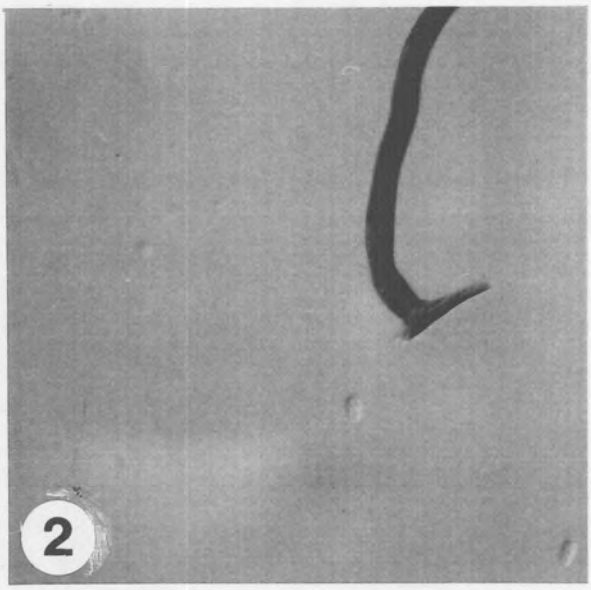
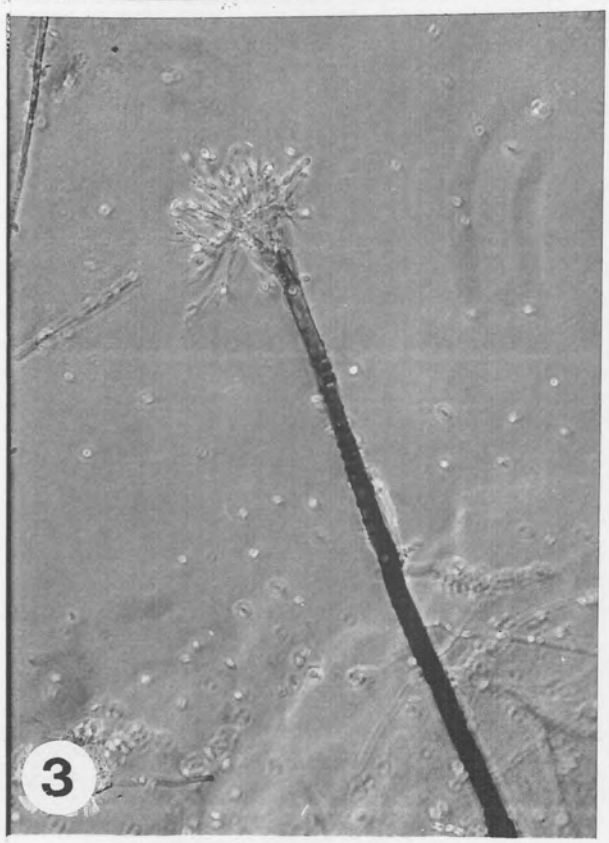
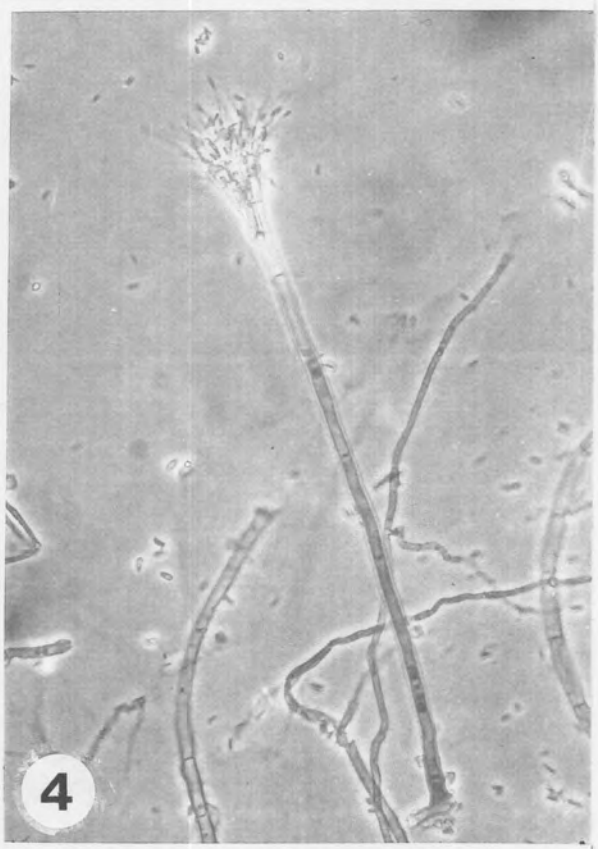


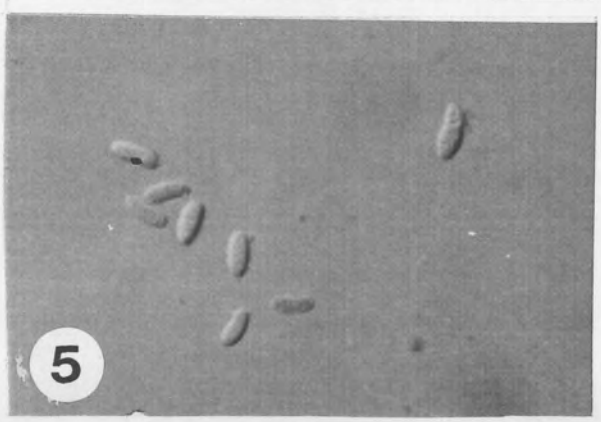
Fig. 1-6. Conidiophores and conidia of *L. hughesii* and *L. abietinum*. **Fig. 1**. Rhizoids of *L. hughesii* (CMW 4052) (Bar = 10 µm). **Fig. 2.** Footcell of *L. abietinum* (CMW 2817) (Bar = 10 µm). **Fig. 3.** Conidiophore of *L. hughesii* (CMW 4052) (Bar = 10 µm). **Fig. 4.** Conidiophore of *L. abietinum* (CMW 2817) (Bar = 10 µm). **Fig. 5.** Conidia of *L. hughesii* (CMW 4052) (Bar = 10 µm). **Fig. 6.** Conidia of *L. Abietinum* (CMW 2817)) (Bar = 10 µm).











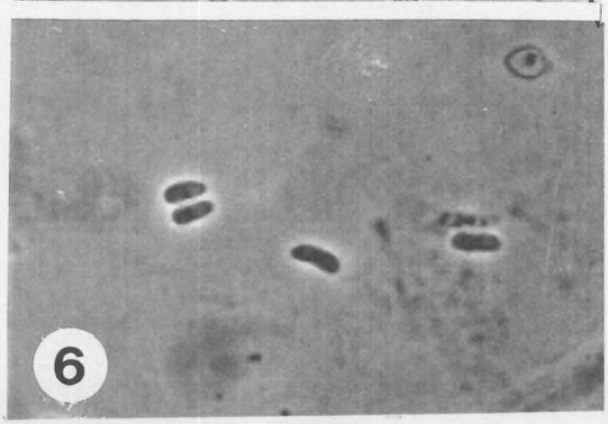
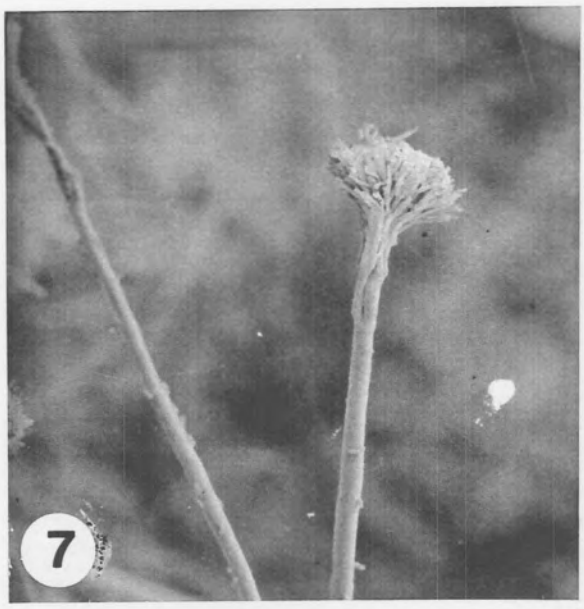
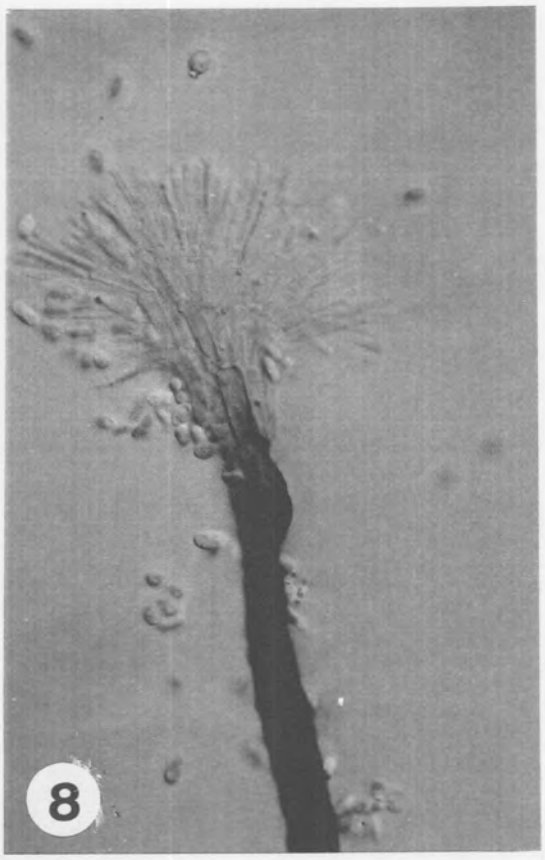
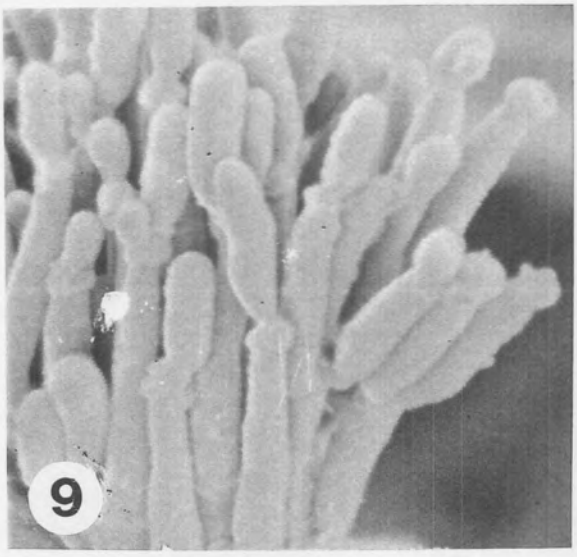
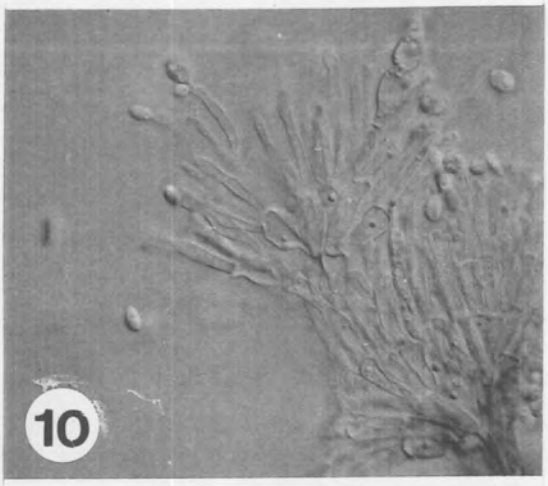


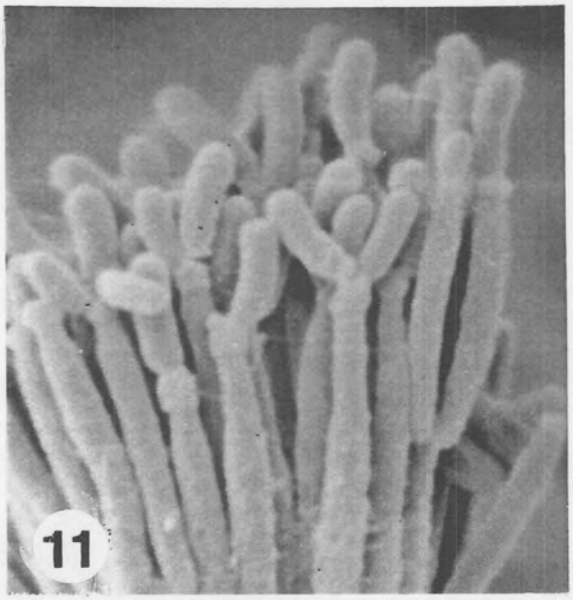
Fig.7-12. Conidiophores and conidia of *Leptographium hughesii* (CMW 4052). **Fig. 7**. Scanning electron micrograph of a conidiophore (Bar = 10 µm). **Fig. 8**. Light micrograph of the conidiogenous apparatus (Bar = 10 µm). **Fig. 9-11**. Light and scanning electron micrographs showing the conidiogenous cells with percurrent proliferation and annelidic conidiogenesis (Bar = 10 µm). **Fig. 12.** Scanning electron micrograph of the conidia (Bar = 10 µm).











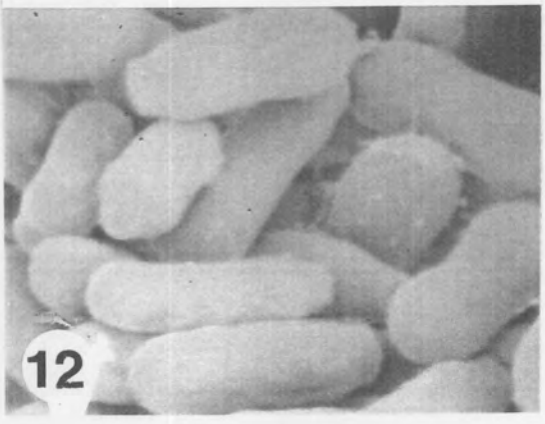
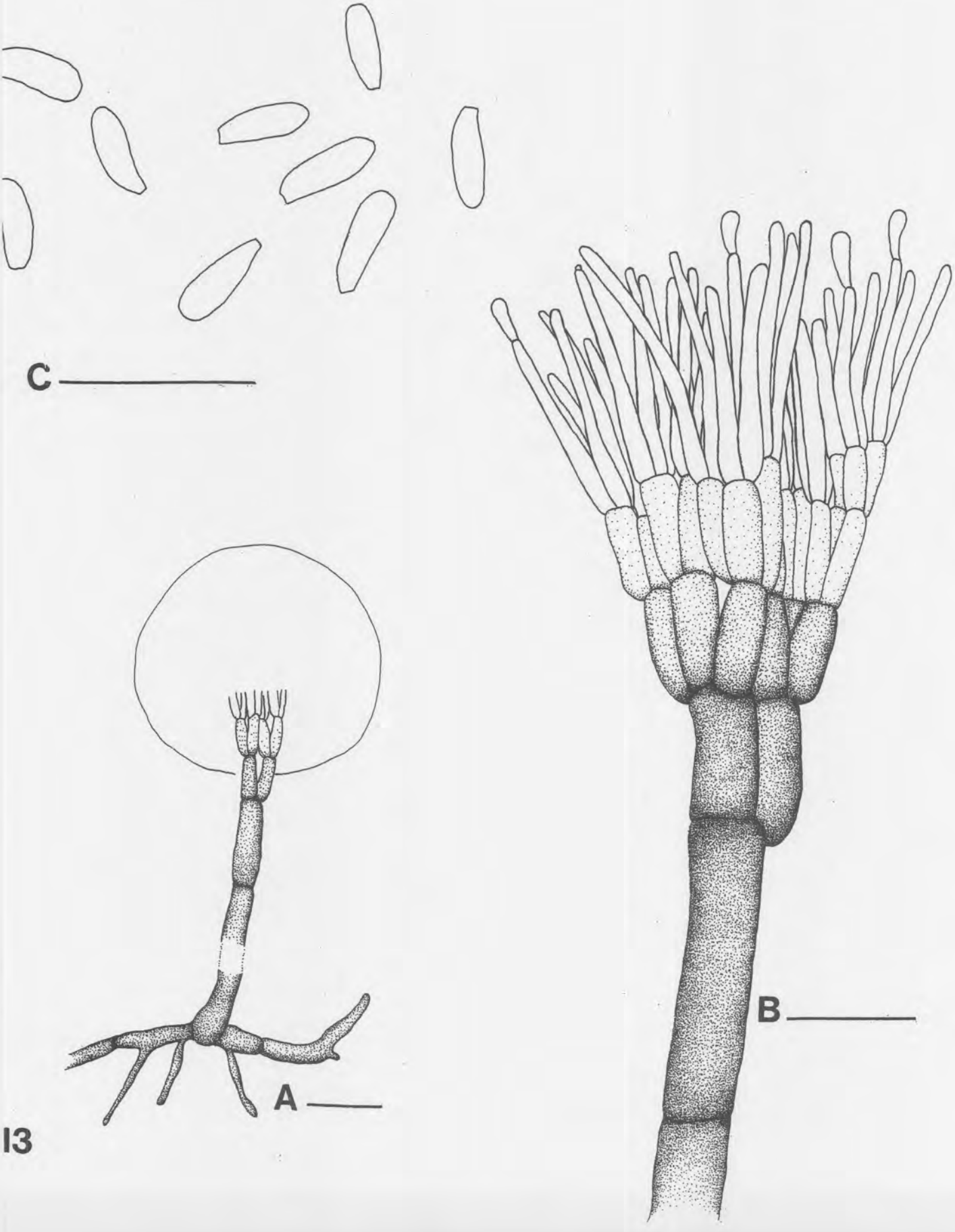


Fig. 13. Conidiophores and conidia of *L. hughesii* (CMW 4052). **Fig. 13a**. Conidiophore with rhizoids present. **Fig 13b**. Conidiophores occurring in groups. **Fig. 13c**. Conidiogenous apparatus (Bar = 10 µm). **Fig. 13d**. Conidia (Bar = 10 µm).



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Chapter 3

Jacobs, K., Wingfield, M.J. & Crous, P.W. (1999) *Ophiostoma europhioides* and *Ceratocystis pseudoeurophioides*, synonyms of *O. piceaperdum*. Mycological Research (in press).

Ophiostoma europhioides and *Ceratocystis pseudoeurophioides*, synonyms of *O. piceaperdum*

Ophiostoma piceaperdum and *O. europhioides* are well-known members of *Ophiostoma* and were first described from conifers in Canada. In previous monographic studies, these species were treated as synonyms. This synonymy was, however, not supported in later studies. After studying the type material, as well as a collection of isolates of *O. piceaperdum* and *O. europhioides,* we have concluded that they cannot be distinguished from each other. We, therefore, support the synonymy of *O. europhioides* and *O. piceaperdum* and provide a description for the *Leptographium* anamorph of *O. piceaperdum*. *Ceratocystis pseudoeurophioides* has previously been distinguished from *O. europhioides* based on differences in anamorph morphology. This species has also been reduced to synonymy with *O. penicillatum*, which was reported to have cucullate ascospores. Later descriptions of *O. penicillatum,* however, reported allantoid ascospores for this species. Examination of *C. pseudoeurophioides,* has led us to conclude that it cannot be distinguished from *O. piceaperdum* and is, therefore, reduced to synonymy with that species.

Keywords: *Ophiostoma, Ceratocystis,* bark beetle associated fungi, systematics

INTRODUCTION

The genus *Ophiostoma* Syd. & P. Syd. is an economically important group of fungi, best known for an ability to cause diseases of trees (Gibbs, 1978; Brasier, 1991). Many species cause considerable economic losses due to sapstain of lumber (Gibbs, 1993). Most are effectively dispersed by insects, especially bark beetles (Coleoptera: Scolytidae) (Münch, 1907; Lagerberg, Lundberg & Melin, 1927; Leach, Orr & Christensen, 1934; Upadhyay, 1981). This accounts for the extensive damage they can cause to plantation and forest trees (Solheim, 1986, 1992a, b).

Ophiostoma spp. are characterized by dark, flask-shaped ascocarps with ascospores that accumulate in slimy masses at the tips of ascocarp necks (Upadhyay, 1993; Wingfield, Siefert & Webber, 1993). Their anamorphs are found in many different genera including *Leptographium* Lagerberg & Melin, *Graphium* Corda, *Sporothrix* Hektoen & Perkins and *Hyalorhinocladiella* Upadhyay & Kendrick (Mouton, Wingfield & Van Wyk, 1993, 1994; Wingfield, Seifert & Webber, 1993). Most of these are also characterized by conidia that accumulate in mucilaginous masses at the apices of conidiophores, facilitating insect dispersal.

Ophiostoma piceaperdum (Rumbold) Arx was first described from sapwood of *Picea glauca* (Moench) Voss., associated with blue-stain and infestation by the bark beetle, *Dendroctonus piceaperda* Hopkins (Rumbold, 1936). Rumbold (1936) found that this fungus resembled the recently described *Ceratostomella penicillata* (= *O. penicillatum*) Grosmann. She could, however, distinguish the two species by the smaller conidia and conidiophores in *O. piceaperdum.*

Ophiostoma europhioides (Wright & Cain) Solheim was described as *Ceratocystis europhioides* from Canada by Wright & Cain (1961) from *Picea* and *Pinus* spp. Wright & Cain (1961) noted the similarity with *O. penicillatum*, but

could separate them because the allantoid ascospores and conidia of *O. penicillatum* (Grosmann, 1931, 1932) were unlike the cucullate ascospores and ellipsoid to obovoid conidia of *O. europhioides* (Wright & Cain, 1961).

Upadhyay (1981) treated *Ophiostoma piceaperdum* and *O. europhioides* as synonyms. So did Hutchison & Reid (1988) in their survey of ophiostomatoid fungi from New Zealand. Solheim (1986), however, treated the two species as different in his study of ophiostomatoid fungi from Norway spruce. So did Harrington (1988) in a review and Yamaoka *et al.* (1997) in a survey of ophiostomatoid fungi associated with *Ips typographus* f. *japonicus* in Japan.

Original descriptions of ascospore shapes suggest that *O. piceaperdum* and *O. europhioides* are distinct (Rumbold, 1936; Wright & Cain, 1961). Isolates of *O. piceaperdum* have been described as having ellipsoidal ascospores with a thin mucilaginous sheath, while ascospores of *O. europhioides* are reniform ascospores with sheaths that give it a cucullate appearance. The importance of this fungus as an agent of blue-stain and its occurrence in various parts of the world has prompted us to reconsider its taxonomy.

Olchowecki & Reid (1974) described *O. pseudoeurophioides* from spruce (*Picea* spp.) in Canada. The fungus can be distinguished from *O. europhioides* by apparently different anamorphs. *Ophiostoma pseudoeurophioides* was described as having a *Verticicladiella* Kendrick anamorph and *O. europhioides* a *Leptographium* anamorph. In his monograph of *Ceratocystis* and *Ceratocystiopsis,* Upadhyay (1981) treated *O. pseudoeurophioides* as a synonym of *O. penicillatum,* and reported the anamorph to be a species of *Verticicladiella.* He also reported cucullate ascospores for *O. penicillatum,* in contrast to the allantoid ascospores reported for the neotype of this species by Solheim (1986). Later, Wingfield (1985) reduced *Verticicladiella* to synonymy with *Leptographium*, thus eliminating the only obvious difference between *O. europhioides* and *O. pseudoeurophioides.* Harrington (1988) also treated these

species as synonyms in his monographic study of species in *Leptographium.* In view of the confused taxonomy of *O. europhioides, O. piceaperdum* and *O. pseudoeurophioides,* this study was undertaken to review their status.

MATERIALS AND METHODS

Material for examination included all available herbarium specimens of *O. piceaperdum, O. europhioides* and *O. pseudoeurophioides.* In addition, isolates of *O. europhioides* and *O. piceaperdum,* were obtained from a variety of culture collections and various colleagues. Characterisation of isolates was done on fungal structures produced on 2 % Malt extract agar (MEA, 20g Biolab malt extract, 20g Biolab agar and 1000 ml distilled water). For microscopy, relevant structures were mounted in lactophenol on glass slides. Herbarium specimens were examined by placing a drop of 1 % KOH on the dried tissue. After five minutes, small pieces of fungal tissue were removed and mounted in lactophenol on glass slides. Fifty measurements of each relevant morphological structure were made and ranges and averages computed. Colours were determined using the charts of Rayner (1970).

Available isolates of the fungi under consideration were examined using 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 JSM 6400 Scanning Electron microscope.

The optimal growth temperatures for two representative isolates of *O. europhioides* and *O. piceaperdum* [CBS 366.75 (incorrectly identified as *L. procerum*) and CMW 2811] were determined by inoculating eight MEA plates with 6 mm diameter agar disks taken from the actively growing margins of fresh

isolates. The plates were incubated at temperatures ranging from 5 to 20 °C at 5 °C intervals and between 20 and 30 °C at 2.5 °C intervals. Colony diameters were measured after eight days and growth was computed as an average of eight readings. Cycloheximide tolerance of these two isolates was determined on malt extract agar (MEA) plates (8 per isolate) amended with 0.5 g/l cycloheximide. The plates were incubated at 25 °C and colony diameters were measured on the eighth day.

RESULTS

Type specimens of *O. europhioides* (TRTC 45762, TRTC 36263, WIN(M) 71-18) and *O. piceaperdum* (BPI 595980, BPI 595981, BPI 595982), include both anamorph and teleomorph structures. Both species produced dark, olivaceous colonies and optimal growth occurred at 25 °C. Conidiophores developed abundantly on the surface of the mycelium in groups of two to seven. *Ophiostoma piceaperdum* and *O. europhioides*, have average stipe lengths of between 50 and 300 µm, and the conidiophores are characterized by the absence of rhizoid-like structures at the base. Both species have conidiophores with two to three primary branches and three to four series of branches. The conidia of *O. piceaperdum* and *O. europhioides* are ellipsoidal to obovoid with truncate ends and rounded apices. Conidia were also found to be of similar length, ranging from 3.0 to 9.0 µm (Table 1).

No distinction could be made between the teleomorph structures of *O. piceaperdum* and *O. europhioides* in culture. Perithecia of both species had neck lengths ranging from 250-1130 µm, and the apices of the necks were characterized by the absence of ostiolar hyphae. Ascospores were distinctly cucullate in both species, and their sizes ranged from 4.0-6.0 µm in length (Table 1).

Ophiostoma piceaperdum and *O. europhioides* cannot be distinguish based on morphology, and are, therefore considered to be synonyms. No anamorph names were provided in the descriptions of either *O. piceaperdum* or *O. europhioides*, although it was noted that a *Leptographium* anamorph was present (Rumbold, 1936; Wright & Cain, 1961). Given the fact that this fungus occurs commonly in the absence of the teleomorph, we provide a name for its *Leptographium* anamorph. A short Latin diagnosis for the anamorph of *O. piceaperdum* was provided by Rumbold (1936) and an additional Latin description is thus not required. The following emended description is provided to avoid further confusion.

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 piceaperdum* Jacobs & Wingfield sp. nov.**

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 34 mm in diam. after 8 days. No growth below 5 °C or above 30 °C. Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.5 g/l cycloheximide after 4 days at 20 °C in the dark. Colonies dark olive (21'"m) with smooth margins. Hyphae submerged on solid medium with little aerial mycelia, hyaline to light olivaceous (21"k), smooth, occasionally roughened by granular deposits, straight, not constricted at the septa, 1.5-6.0 (mean = 4.0) µm diameter. Conidiophores occurring singly or in groups of 2-7, arising directly from the mycelium with smaller conidiophores on aerial mycelia, erect, macronematous, mononematous, 140-300 (mean = 204) µm in length, rhizoid-like structures absent (Figs. 1, 5, 9a). Stipe light olivaceous (21"k), smooth,

cylindrical, simple, 3-8 septate, 70.0 - 195 (mean = 121) µm long (from first basal septum to below primary branches), 5.0-9.0 (mean = 6.0) µm wide below primary branches, apical cell not swollen; 6.0-12.5 (mean = 8.0) µm wide at base, basal cell not swollen. Conidiogenous apparatus 55.0-120 (mean = 84.0) long, excluding the conidial mass, with 2-5 series of cylindrical branches; 2-3 primary branches, light olivaceous (21"k), smooth, cylindrical, 0-1 septate 15.5-39.0 (mean = 21.0) µm long and 3.0-8.0 (mean = 5.0) µm wide, secondary branches light olivaceous (21"k), aseptate, 11.0-23.0 (mean = 12.0) µm long, 2.0-6.0 (mean = 4.0) µm wide; tertiary branches light olivaceous (21"k), aseptate, 9.0-22.0 (mean = 15.0) µm long, 2.0-5.0 (mean = 3.0) µm wide, quaternary branches, hyaline to light olivaceous (21"k), aseptate, 7.0-16.0 (mean = 11.5) µm long, 2.0-3.0 (mean = 2.5) µm wide (Figs 2, 9b). Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, 11.0-26.0 (mean = 17.5) µm long and 1.5-3.0 (mean = 2.0) µm wide. Conidium development occurring through replacement wall building with holoblastic ontogeny, percurrent proliferation and delayed secession giving the false impression of sympodial proliferation (Minter *et al.*, 1982; Minter *et. al.,* 1983; Van Wyk, Wingfield and Marasas, 1988) (Figs. 3, 6, 7). Conidia light gray olivaceous (19""), aseptate, obovoid to ellipsoid with truncated ends and rounded apices, 3.0-9.0 x 1.0-3.0 (mean =5.0 x 2.0) µm (Figs. 4, 8, 9c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream coloured (19'f) with age.

Specimens examined

Herbarium types: *Ophiostoma piceaperdum:* Canada, Nova Scotia, St. Peters, Cape Breton, *Picea glauca*, C.T. Rumbold, June 1930, BPI 595981; (holotype); Canada, Nova Scotia, St. Peters, Cape Breton, *Picea glauca*, R.E. Balch, June 1930, BPI 595980; Canada, Nova Scotia, St. Peters, Cape Breton, *Picea glauca*, R. E. Balch, June 1930, BPI 595982; *Ophiostoma europhioides:* Canada, Ontario, Shabotik River, Algoma district, *Picea mariana*, R.F. Cain, 20 June

1961, TRTC 45762; Canada, Ontario, Challenen Lake, Sudbury district, *Picea mariana*, R.F. Cain, J. Reid & W. Obust, 20 June 1961, TRTC 36263; Canada, Ontario, Shabotik River, Algoma district, *Picea mariana*, R.F. Cain, 20 June 1961, MFB 7439; Canada, Ontario, Shabotik River, Algoma district, *Picea mariana*, R.F. Cain, 20 June 1961, MFB 7439; Canada, Manitoba, Sandilands Forest Reserve, *Picea mariana*, A. Olchowecki, 20 June 1961, WIN(M) 71-18; *O. pseudoeurophioides:* Canada, Sandilands, Forest Reserve, *Picea mariana,* A. Olchowecki, Apr. 24, 1971 WIN(M) 71-13.

Cultures: *O. piceaperdum: Picea abies*, A.M. Hallakesela, CMW 660 (same as CBS 366.75); *O. europhioides:* USA, Nippletop Mountain, *Picea rubens*, T.C. Harrington, 1987, CMW 2811 (= C274); E.F. Wright, CMW 479 (= CBS 444-69); Austria, Nasswald, *Picea abies*, T. Kiristis, 1993, CMW 3314.

DISCUSSION

Ophiostoma piceaperdum and *O. europhioides* are indistinguishable based on morphological data. However, in the original description, the ascospores of *O. europhioides* were described as cucullate (Wright & Cain, 1961), whereas, those of *O. piceaperdum,* were noted as being allantoid (Rumbold, 1936). Examination of the herbarium type material, revealed that these species are both characterized by cucullate ascospores. The reason for this discrepancy is most probably because Rumbold (1936) did not described the sheaths surrounding the ascospores that give the spores their cucullate appearance. Both species displayed *Leptographium* anamorphs with obovoid conidia that could not be distinguished from each other. Thus, morphological comparisons of the type specimens, failed to distinguish between *O. europhioides* and *O. piceaperdum,* and from these observations we conclude that *O. piceaperdum* and *O. europhioides* are identical and thus support the synonymy proposed by Upadhyay (1981).

Ophiostoma piceaperdum and *O. europhioides* have both been considered to be similar to each other and also to *O. penicillatum* (Rumbold, 1936; Wright & Cain, 1961; Griffin, 1968). *Ophiostoma piceaperdum* can, however, easily be distinguished from *O. penicillatum* based on ascospore and conidial morphology. *Ophiostoma penicillatum* is characterized by the presence of curved ascospores and large, allantoid conidia. This is in contrast to the cucullate ascospores and obovoid conidia of *O. piceaperdum* (Grosmann, 1931, 1932; Rumbold, 1936; Wright & Cain, 1961).

The synonymy of *O. pseudoeurophioides* with *O. penicillatum,* proposed by Upadhyay (1981) is rejected. *Ophiostoma pseudoeurophioides* has been distinguished from *O. europhioides* based on the presence of a *Verticicladiella* state in the former species and a *Leptographium* state in the latter. When Wingfield (1985) synonomised *Verticicladiella* with *Leptographium,* this distinction became redundant. Examination of the type specimen [(WIN)M 71-13] and the original description of *O. pseudoeurophioides* (Olchowecki & Reid, 1974), revealed that this species cannot be distinguished from *O. piceaperdum* and we, therefore, propose the following synonymy:

Ophiostoma piceaperdum (Rumbold) Von Arx. Antonie van Leeuwenhoek 18, 211. 1952.

> *Ceratostomella piceaperda* Rumbold. J. Agric. Res. 52, 436. 1936.
>
> *Grosmannia piceaperda* (Rumbold) Goidanich. R. Staz. Pat. Veg. Bol. Rome, 16, 255, 1936.
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> *Ceratocystis piceaperda* (Rumbold) C. Moreau. Rev. Mycol Suppl. Col. 17, 22. 1952.

Ophiostoma europhioides (Wright & Cain) Solheim. Nord. J. Bot. 6, 203. 1986.

> *Ceratocystis europhioides* Wright & Cain. Can. J. Bot. 39, 1222. 1961.

Ceratocystis pseudoeurophioides Olchowecki & Reid. Can. J. Bot. 52, 1700. 1974.

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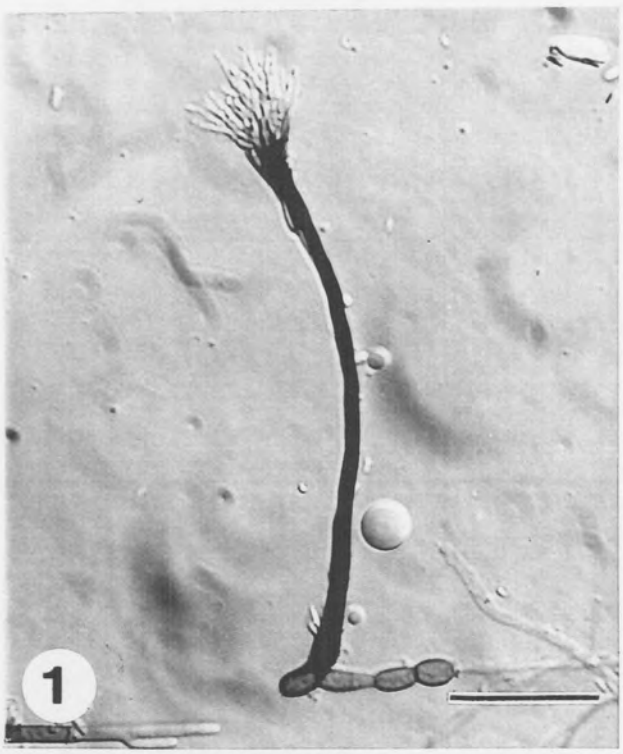
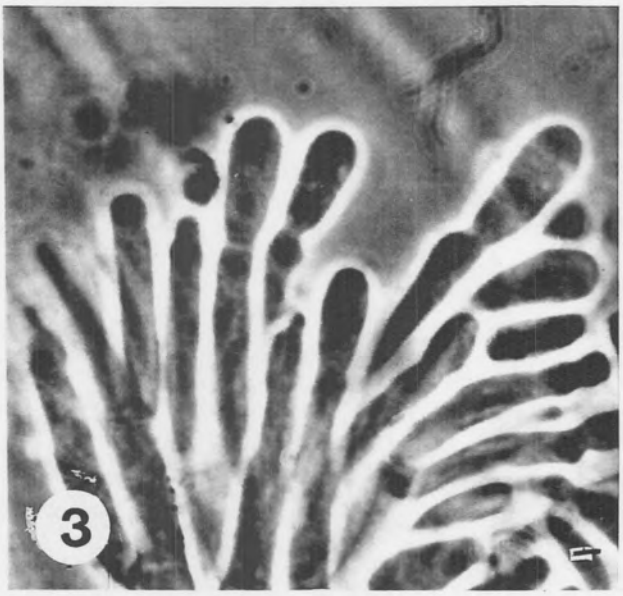
Table 1. Comparison of *Ophiostoma piceaperdum*, *O. europhioides* and *O. pseudoeurophioides*[1].

	O. piceaperdum	***O. europhioides***	***O. pseudoeurophioides***
Host	*Picea glauca, Picea abies*	*Picea abies ; Picea glauca; P. mariana; P. jezoensis; Pinus glauca; P. resinosa; P. strobus; P. sylvestris; P. banksiana; Pseudotsuga mensiezii*	*Picea mariana*
Associated insect	*Dendroctonus piceaperda*	*Dendroctonus rufipennis; D. valens; Dryocoetus* sp.; *Hylurgops palliatus; Ips typographus* f. *japonicus; Ips typographus; Pityogenes chalcographus*	*none reported*
Distribution	USA	Canada, USA	Canada
anamorph	*Leptographium*	*Leptographium*	*Leptographium*
Rhizoids	Absent	absent	Absent
Conidiophore length	170 – 250 µm	60 - 300 µm	150 – 500 µm
Conidium shape	Obovoid	Ellipsoid	Obovoid
Conidium size	3 - 11 µm	3.2 - 8.5 µm	2.5 - 5.0
Perithecium neck length	110 - 950 µm	Up to 950 µm	300 – 850 µm
Perithecium base size	90 - 350 µm	180 – 360 µm	150 – 300 µm
ascospore shape (including sheath)	Cucullate (Rumbold reported ellipsoid ascospores)	Cucullate	Cucullate
Ascospore size	3.6 - 4.7 µm	4.5 - 6.0 µm	4.5 - 5.0 µm

1: Data and information in this table are derived from publications of Rumbold (1936) (*O. piceaperdum*), Wright & Cain (1961) (*O. europhiodes*) and Olchowecki & Reid (1974) (*O. pseudoeurophioides*).

Fig 1-4. Light micrographs of the conidiophore and conidia of *L. piceaperdum* (CMW 660). **Fig. 1**. Conidiophore (Bar = 10 µm). **Fig. 2**. Conidiogenous apparatus (Bar = 10 µm). **Fig. 3.** Conidiogenous cells (Bar = 10 µm). **Fig. 4**. Conidia (Bar = 10 µm).





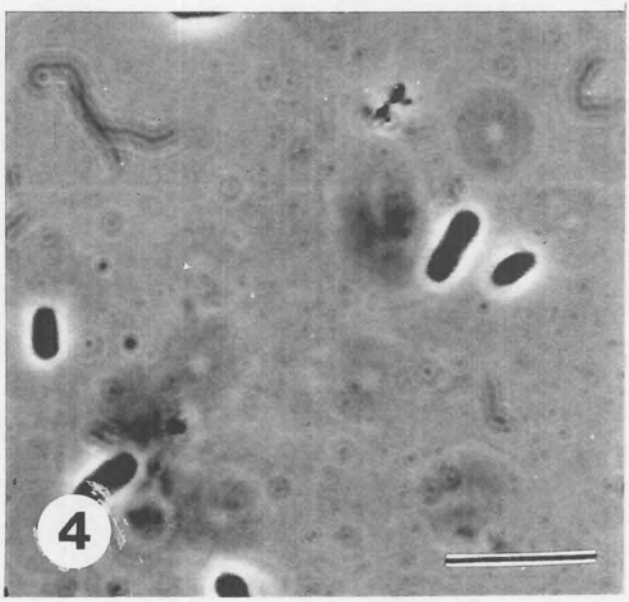
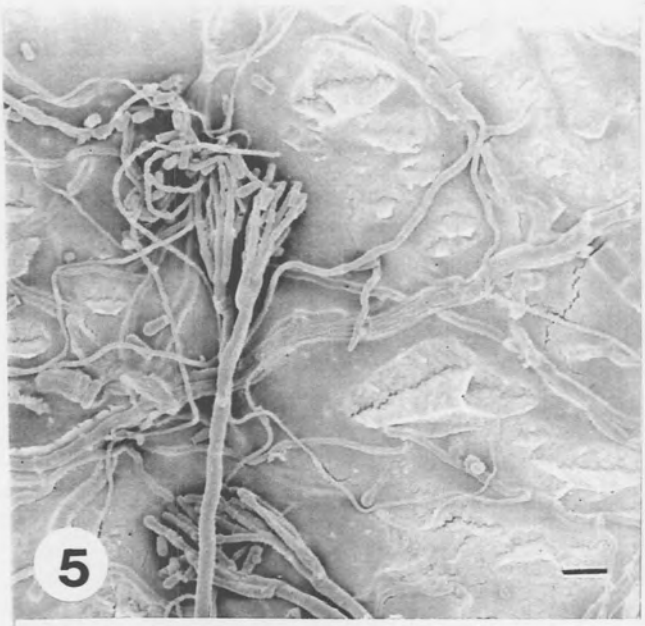
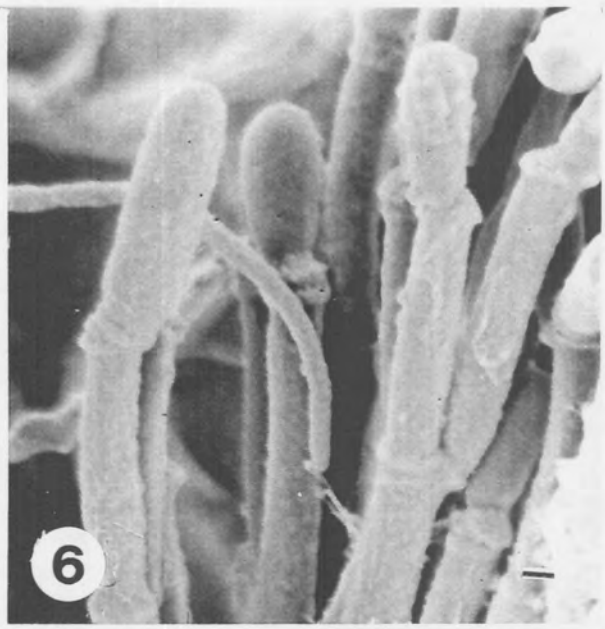
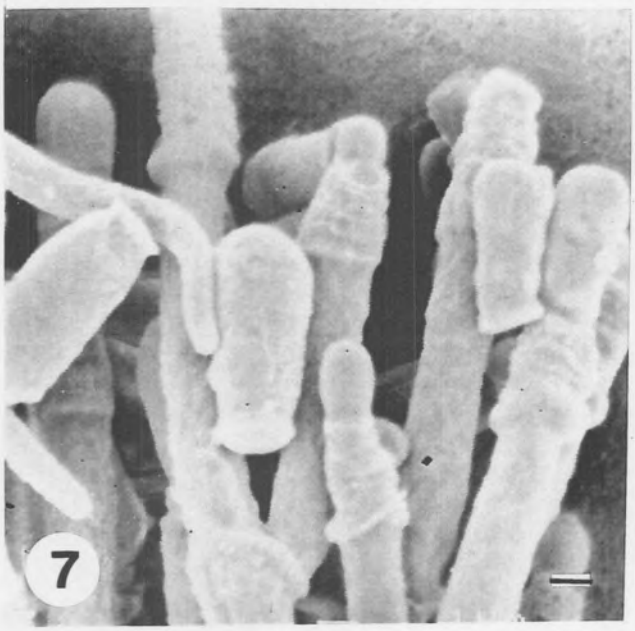


Fig.5-8. Scanning electron micrographs of a conidiophore and conidia of *L. piceaperdum* (CMW 660). **Fig. 5**. Conidiophore (Bar = 10 µm). **Fig. 6, 7**. Conidiogenous cells (Bar = 1 µm). **Fig. 8**. Conidia (Bar = 1 µm).







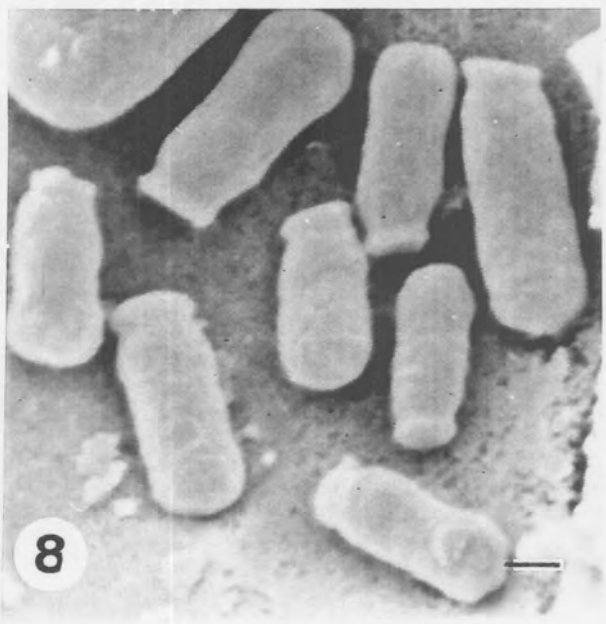
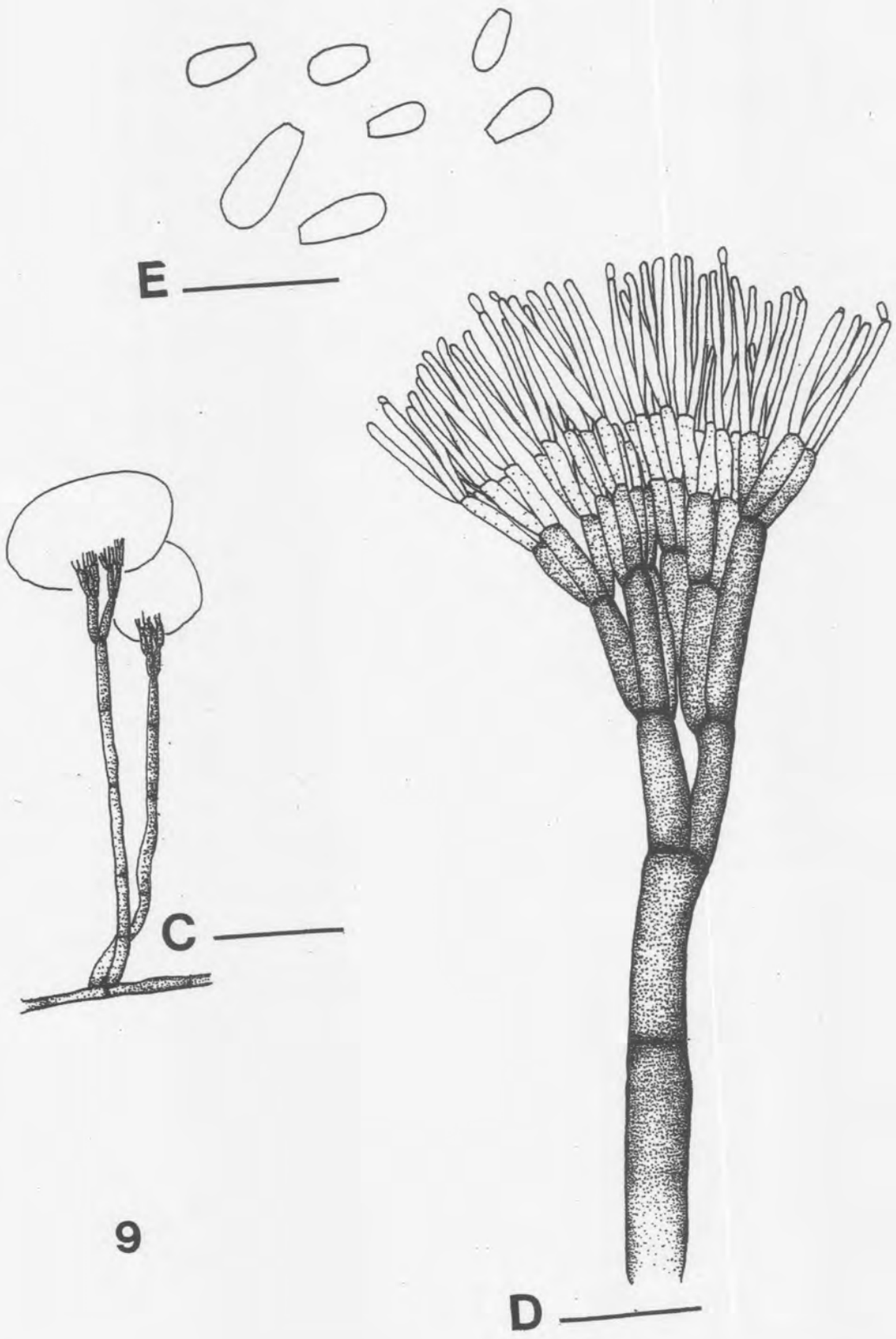


Fig. 9. Conidiophores and conidia of *L. piceaperdum* (CMW 660). **A.** Conidiophore without rhizoids. **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).



9

Chapter 4

Jacobs, K.; Wingfield, M.J., Jacobs, A. and Wingfield, B.D. (1999). A taxonomic re-evaluation of *Phialocephala phycomyces*. Canadian Journal of Botany (submitted).

A taxonomic re-evaluation of *Phialocephala phycomyces*

Phialocephala was established for species that produce their conidia phialidically at the apex of dark mononematous conidiophores. This is in contrast to *Leptographium* spp. that are characterized by annelidic conidium development. *Phialocephala phycomyces* was described more than a century ago as a single species in the genus *Scopularia.* It was later transferred to *Hantszchia,* then to *Leptographium,* and later to *Phialocephala.* Although characterized by phialidic conidium development, the phialides of *P. phycomyces* are not as deep-seated as those found in *P. dimorphospora,* the type of *Phialocephala. Phialocephala phycomyces* is, furthermore, characterized by reddish-brown conidiophores, unlike other species in *Phialocephala.* Based on morphological, as well as molecular comparisons, we concluded that *P. phycomyces* is a distinct taxon and it is described here as the type species of *Kendrickiella* gen.nov.

Keywords: *Leptographium* complex, morphology, phylogeny

INTRODUCTION

Phialocephala Kendrick together with *Leptographium* Lundberg & Melin, forms part of the *Leptographium*-complex, which originally included *Verticicladiella* Hughes. *Phialocephala* can be distinguished from *Leptographium* and *Verticicladiella* based on differences in conidium development (Kendrick, 1961, 1962, 1963, 1964a,b). *Phialocephala* is characterized by phialidic conidium development, in contrast to the annelidic and sympodial development of *Leptographium* and *Verticicladiella,* respectively (Kendrick, 1961, 1962). Wingfield (1985) reduced *Verticicladiella* to synonymy with *Leptographium* because species in these genera have indistinguishable conidium development when viewed at the electron microscope level.

Leptographium spp. are anamorphs of *Ophiostoma* (Harrington, 1987; 1988) and display the taxonomically useful character of being able to tolerate high concentrations of cycloheximide. In contrast, *Phialocephala* spp. are sensitive to low concentrations of cycloheximide (Harrington, 1988). *Phialocephala* and *Leptographium* spp. can also be distinguished based on differences in their host specificity. *Leptographium* spp. generally occur on conifers and living woody tissue (Lagerberg, Lundberg and Melin, 1927; Harrington, 1988), whereas *Phialocephala* spp. are generally associated with dead or decaying material and soil (Kendrick, 1961; 1963; Siegfried, Seifert and Bilmer, 1992).

Phialocephala phycomyces (Auersw.) Kendrick has been described as a single species of *Hantsczhia* by Auerswald in 1862 (Kendrick, 1964). This species was re-visited after Grosmann (1932) described several species of fungi causing blue-stain on spruce in Europe. After considering both *Scopularia* Preuss as well as *Hantsczhia* as possible genera for the new species, she found that the description and illustration of both *Scopularia* and *Hantszchia* were unclear. Grosmann, therefore, synonomised *Hantsczhia* with the newly described genus, *Leptographium* (Grosmann, 1932; Shaw and Hubert, 1952) and *H. phycomyces* became *L. phycomyces* (Auersw.) Grosm.

Kendrick (1961) established *Phialocephala* for species of the *Leptographium*-complex with phialidic conidium development. *Leptographium phycomyces* was

consequently transferred to *Phialocephala* as *P. phycomyces.* However, from the type material as well as the description (Kendrick, 1961) of *P. phycomyces,* it is clear that this fungus is characterized by inconspicuous collaretes on the phialides. Compared to the type of *Phialocephala,* namely *P. dimorphospora,* it is atypical of this genus. *Phialocephala dimorphospora* is characterized by deep-seated phialides. In addition, the habitat of *P. phycomyces* is also unlike other species in *Phialocephala.* It appears to be tropical, and has been described as a contaminant of a basidiomycete (Kendrick, 1964b). Recently, a single isolate of *P. phycomyces* has become available to us. This enabled us to restudy the species in detail. The aim of this study was, therefore, to reconsider the taxonomic placement of *P. phycomyces* based on morphology, physiology and molecular data.

MATERIALS AND METHODS

Molecular comparison

Isolates used for molecular comparisons included *L. abietinum* (Peck) Wingfield (CMW 2817), *L. penicillatum* Grosmann (CMW453), *O. piceaperdum* (Rumbold) Von Arx (CMW 2811), *O. francke-grosmanniae* (Davidson) De Hoog & Scheffer (CMW 445), *P. phycomyces* (CMW 2556), *P. dimorphospora* (CMW 168), *P. fortinii* Wang & Wilcox (CMW 815) and *P. xalapensis* Persiani & Maggi (CMW 807). Sequences for the *Penicillium* and *Aspergillus* species were obtained from genbank and the accession numbers are as follows: *Penicillium megasporum* (AF033494), *Penicillium implicatum* (AF033428), *Penicillium expansum* (AF003359), *and A. parasiticus (AB008418) and A. flavus* (AB008416).

Genomic DNA was extracted from two-week-old cultures grown in liquid ME (malt extract). This was done by grinding a small amount of mycelium in liquid nitrogen to a fine powder and adding 1.0 µl Extraction buffer (1 % CTAB). This was then incubated in a 60 °C waterbath for 1 hour. Proteins were removed with phenol and chloroform (1:1), followed by a series of chloroform steps, until the interface was clean. The DNA was precipitated with 2X cold 100 % ethanol and left ovemight at -20 °C. This was then centrifuged at 13000 rpm for 30 min., the resulting pellet was

washed with cold 70 % ethanol and dissolved in 100 -200 µl sterile water. The presence of DNA was checked on a 1 % agarose gel.

The ITS2 (internal transcribed spacer) region and part of the large subunit of the ribosomal DNA gene were amplified using the Polymerase Chain Reaction (PCR) (Saiki *et al.,* 1988) on a Hybaid™ Touchdown thermo cycling system (Life Sciences International, UK). The primers ITS3 (5'-GCATAGATGAAGAAGCAGC-3') and LR3 (5'-CCGTGTTTCAAGACGGG-3') were used in these reactions. Each reaction were done in 100 µl containing 10 µl 10X PCR buffer, 20 µl of 25 mM $MgCl_2$, 10 mM dNTP's, 20 pmol of each primer, 0.5 µl DNA and 1.75U Expand Taq polymerase (Boehringer Mannheim, Germany). The PCR conditions were as follows: 2 min. at 94 °C, annealing at 48 °C for 1 min., 10 s at 62 °C, 2 min. at 72 °C with an increase of 5 °C/s. This was repeated for 40 cycles and a final elongation reaction was done at 72 °C for 8 min. The resulting products were purified with the High Pure PCR product Purification Kit (Boehringer Mannheim Kit) and used in the sequence reactions.

Sequencing was done using the primers ITS3, LR3 and 404X (5'-CCCTTTCAACAATTTCAC-3'). Sequencing was performed on a ABI 377 automated sequencer using the Thermo Sequenase dye terminator cycle sequencing pre-mix kit (Perkin Elmer Applied Biosystems). Sequence data were edited in Sequence Navigator (Perkin Elmer Applied Biosystems) and manually aligned in PAUP (Phylogenetic Analysis Using Parsimony) (Swofford, 1993). Confidence intervals were determined by 1000 Bootstrap replicates.

Morphology

Material for examination included the herbarium type specimens (DAOM 34098; DAOM 64734; 63899) as well as a single live isolate (MUCL 38565) of *P. phycomyces*. These were compared with the herbarium type specimens of *Leptographium* (*L. lundbergii* Lagerberg & Melin, PREM 50548, CMW 30) and *Phialocephala* (*P. dimorphospora* Kendrick, DAOM 71465(c), ATCC 24087). Fungal structures produced on 2 % Malt extract agar (MEA, 20g Biolab malt extract, 20g Biolab agar and 1000 ml distilled water) were used for light as well as scanning and

electron microscopic study. For light microscopy, relevant structures from the cultures, as well as herbarium specimens, were mounted in lactophenol on glass slides. Fifty measurements of each relevant morphological structure were made and ranges and averages computed. Colours of structures and colonies were determined using the charts of Rayner (1970).

For scanning electron microscopy (SEM), small blocks of agar cut from sporulating colonies were fixed in 3 % glutaraldehyde and 0.5 % osmium tetroxide in a 0.1 M phosphate buffer, dehydrated in a graded acetone series and critical-point dried. Specimens were mounted and coated with gold palladium alloy and examined using a Joel JSM 6400 Scanning Electron microscope.

For ultrastructural examination, the isolate of *P. phycomyces* (MUCL 38565) was grown on 2 % malt extract agar in Petri dishes at 25 °C. Small blocks of agar were cut form the colony and fixed in the same manner as described for scanning electron microscopy. The material was then embedded in epoxy resin (Spurr, 1969) and ultrathin sections (60 nm) were cut with glass knives, using an LKB Ultratome III. Sections were stained for 20 min. with 6 % uranyl acetate and 10 min. in lead citrate and examined with a Philips CM100 Transmission Electron Microscope.

The optimal growth temperatures for *P. phycomyces* (CMW 2556), *Leptographium lundbergii* (CMW 30), *Phialocephala dimorphospora* (CMW 168) and *Ophiostoma piliferum* (Fries) H. & P. Sydow (CMW 2481) were determined by inoculating eight MEA plates with 6 mm diameter agar disks taken from the actively growing margins of a two week old isolate. The plates were incubated at temperatures ranging from 5 to 35 °C at 5 °C intervals. Cycloheximide tolerance was determined for *P. phycomyces* (CMW 2556), *L. lundbergii* (CMW 30), *P. dimorphospora* (CMW168) and *O. piliferum* (CMW2481) by inoculating 5 MEA plates amended with increasing concentrations (0, 0.05, 0.1, 0.5, 1.0 g/l) of cycloheximide and incubating them at 25 °C. Colony diameters were measured after eight days and growth was computed as an average of eight readings.

RESULTS

Molecular comparisons

Amplification of the ribosomal RNA operon, yielded products of more or less 1.3 kb (kilobasepairs). A region of 850 bp (basepairs) was successfully sequenced for all species. From the analysis, six most parsimonious trees were obtained displaying identical topologies. The shortest tree length was 269 with a consistency index (CI) of 0.777 a homoplasy index (HI) of 0.223 and a retention index of 0.814. Two distinct clades were observed (Fig. 1). One of these includes all *Leptographium* spp. with a 100% confidence interval. The second clade included species of *Phialocephala,* together with *Penicillium* spp. and *Aspergillus* spp. Although *P. phycomyces* falls within this clade, it appears to be more distantly related to other species of *Phialocephala,* than *Phialocephala* spp. are to *Penicillium.* This suggests that *P. phycomyces* probably does not belong in *Phialocephala.* However, it is more closely related to species of *Phialocephala* than it is to *Leptographium.*

Morphological and growth characteristics

Comparison of the isolate of *Phialocephala phycomyces* (CMW 2556) with the herbarium type specimens and the complete description provided by Kendrick (1964b), confirmed its identity. Both the isolate, as well as the herbarium material are characterized by reddish brown colonies as a result of the pigmentation of the conidiophore stipes. This was reported by Kendrick (1964b) and appears to be characteristic of the species. It is also unlike the colony colour typical of species in *Leptographium* or *Phialocephala.* Closer examination of the isolate of *P. phycomyces* using scanning as well as transmission electron microscopy indicated that the conidiogenous cells of *P. phycomyces* are distinctly phialidic with periclinal thickening (Fig. 5,6) although the collarettes are inconspicuous. These are unlike the pronounced collarettes of *P. dimorphospora* and most other *Phialocephala* spp. The isolate of *P. phycomyces* was found to be able to tolerate high concentrations of cycloheximide, which is atypical of most other species of *Phialocephala* that have been tested (Table 1).

These results lead us to conclude that *P. phycomyces* cannot be adequately accommodated in either *Leptographium* or *Phialocephala*. We, therefore, propose that this species be placed in a new genus, *Kendrickiella,* with *K. phycomycoides* comb. nov. as the type of this genus.

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

***Kendrickiella* K. Jacobs & M.J. Wingf. gen. nov.**

Conidiophora recta, solitaria. Stipites pigmentosi, rubrobrunnei. Apparatus conidiogenus complexus cum seriebus pluribus ramorum, in cellulis conidiogenis terminantium. Celluli conidiogeni phialidosi, collariculis inconspicuis, manifeste periclinaliter incrassatis. Ameroconidia non catenata, conidia in massa mucosa in apice conidiophori. Resistunt densissimo soluto corpori vulgo dictu *cycloheximide*.

Conidiophores straight, solitary. Stipes pigmented, reddish brown. Complex conidiogenous apparatus with several series of branches terminating in conidiogenous cells. Conidiogenous cells phialidic with inconspicuous collarettes, pronounced periclinal thickening. Ameroconidia not in chains, conidia in mucilaginous mass on the apex of the conidiophore. Able to tolerate high concentrations of cycloheximide.

Type species: *Kendrickiella phycomycoides* comb. nov.

***Kendrickiella phycomycoides* (Auerswald) K. Jacobs & M.J. Wingfield comb. nov.**

Teleomorph state: none known.

Colonia centro badia, cremo-fulvescentes. Conidiophora erecta, macronematosa, mononematosa, (150-) 316 (-520) µm longa, sine structuris rhizoideis similibus. Stipes rubrobrunneus, laevis, cylindricus, simplex. Apparatus conidiogenus (50-) 68 (-90) µm

longus, massa conidiali exclusa; ramis primariis 2-4, rubro-brunneis. Cellulae conidiogenae discretae, in ramo 2-3, cylindricae, apicem versus leviter angustatae, (8.0-) 13 (-22) µm longae et (1.0-) 1.5 (-2.0) µm latae. Progressus conidiorum phialidosus, collariculis inconspicuis. Conidia aseptata ellipsoidea (3.0-) 4.0 (-7.0) x (1.0-) 2.0 (-3.0) µm .

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 12 mm in diameter in 12 days. No growth below 20 °C or above 30 °C. Able to withstand high concentrations of cycloheximide with a 53 % reduction in growth on 0.1 g/l cycloheximide after days at 25 °C in the dark. Colony chestnut (9'm) in centre, becoming cream buff (19"f). Colony margin smooth. Hyphae submerged or on top of solid medium with no aerial mycelium, hyaline, smooth, not constricted at the septa, (2.0-) 2.5 (-4.0) µm diameter.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (150-) 316 (-520) µm in length, rhizoid-like structures absent (fig, 2, 8a). Stipe reddish brown, smooth, cylindrical, simple, 1 - 4 septate, (100-) 247 (-450) µm long (from first basal septum to below primary branches), (6.0-) 8.0 (-9.0) µm wide below primary branches, apical cell not swollen, (8.0-) 11 (-15.5) µm wide at base, basal cell not swollen. Conidiogenous apparatus (50-) 68 (-90) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches, 2-4 primary branches, reddish brown, smooth, cylindrical, aseptate, (12-) 15.5 (-20) µm long and (5.0-) 6.0 (-9.0) µm wide, secondary branches light reddish brown to hyaline, aseptate, (8.0-) 10 (-12) µm long, (3.0-) 4.5 (-6.0) µm wide, tertiary branches hyaline, aseptate, (4.0-) 8.5 (-12) µm long, (6.0-) 8.0 (-11) µm wide, quaternary branches aseptate, hyaline, (6.0-) 8.5 (-10) µm long, (1.0-) 2.0 (-3.0) µm wide (Fig. 3, 8b). Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (8.0-) 13 (-22) µm long and (1.0-) 1.5 (-2.0) µm wide. Conidium development phialidic with inconspicuous collarettes (Fig. 4, 5, 6). Conidia, aseptate, ellipsoid, (3.0-) 4.0 (-7.0) x (1.0-) 2.0 (-3.0) µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus (Fig. 7, 8c).

Holotype: DAOM 34098 (slides), oak barrel in Germany, collected C.A. Hantzsch, described: Auerswald in Rabenhorst, Fungi europaei nr 441. 1862.

Additional cultures: DAOM 64734 (herbarium material), Isolated as contaminant of culture of polypore, collected: R. Cailleux, St. Laurant du Maroni, French Guiana, August 1952; DAOM 63899 (herbarium material), isolated from soil, Belgian Congo, collected J. Meyer, December, 1954; MUCL 38565 (PREM 56321), (live isolate), isolated as a contaminant of a polypore (*Ganoderma* sp.), Reserva de Produccion Faunistica Cuyabeno, Prov. Sucumbios, Equador, collected C. Decock, July 1993.

DISCUSSION

Kendrickiella phycomycoides superficially resembles other species described in *Phialocephala.* However, *K. phycomycoides* can be distinguished form other *Phialocephala* and *Leptographium* spp. based on the characteristic reddish brown colour of the colony and conidiophore stipes. Species in *Leptographium* and *Phialocephala* are generally characterized by olivaceous colonies and olivaceous brown stipes (Lagerberg *et al.,* 1927; Van der Westhuizen *et al.,* 1995; Jacobs, Wingfield & Bergdahl., 1997; Jacobs *et al.,* 1999).

Leptographium and *Phialocephala* spp., have traditionally been distinguished based on the obvious differences in conidium development. In some cases, these differences are not as obvious. This was illustrated by Mouton, Wingfield and Van Wyk (1992) who studied the anamorph of *Ophiostoma francke-grosmanniae.* Based on morphology, this species was thought to best reside in *Phialocephala* (Upadhyay, 1981), and would thus have been the only *Ophiostoma* sp. with an anamorph in *Phialocephala.* However, closer examination revealed that conidium development in this species is annelidic, and it is thus a typical *Leptographium.* The closely packed annelations at the apex of the conidiogenous cells lead to the appearance of inconspicuous collarettes when the fungus is viewed using light microscopy. Thus, initial examination of the conidiogenous cells of *Kendrickiella phycomycoides,* prompted us to question the nature of its inconspicuous collarettes.

Electron microscope studies revealed that conidia in *K. phycomycoides* emerge from phialides that have pronounced periclinal thickening. This is despite the fact that the conidiogenous cells are tapered and the collarettes inconspicuous. In the broad sense, the species could be accommodated in *Phialocephala.* However, when

compared to the type of *Phialocephala,* namely *P. dimorphospora,* the morphology of the conidiogenous cells is entirely different. *Phialocephala dimorphospora* is characterized by deep-seated phialides with pronounced collarettes, whereas those of *K. phycomycoides* are inconspicuous and hardly visible under the light microscope.

Harrington (1988) concluded that species in *Phialocephala* are generally sensitive to the antibiotic cycloheximide, whereas *Leptographium* spp. are tolerant to high concentrations of the antibiotic. Marais (1996), however, found species in *Phialocephala* to be quite variable in their tolerance towards cycloheximide. *Kendrickiella phycomycoides* exhibits a distinct tolerance to cycloheximide and in this characteristic resembles *Leptographium,* but is generally unlike *Phialocephala.*

Kendrickiella phycomycoides, like other *Phialocephala* spp., has not been associated with a teleomorph genus. This is in contrast to many species of *Leptographium* that are known to be anamorphs of *Ophiostoma* (Grosmann, 1932; Kendrick and Molnar, 1965; Robinson-Jeffrey and Davidson, 1968). Comparison of the habitat of these taxa, revealed that *Leptographium* spp. occupy mostly woody substrates, in contrast to *Phialocephala* spp. that are found to inhabit leaves, soil and lignified tissues (Kendrick, 1961; 1963; Siegfried *et al.,* 1992). *Kendrickiella phycomycoides* has been reported from a variety of habitats including spruce and pine (Mathiesen, 1950), oak barrels, polypores and soil (Kendrick, 1964b) although the validity of these records on pine and spruce should be questioned. Two separate records of the fungus from polypores in the tropics (Kendrick, 1964b) suggest that it might be a mycoparasite.

Molecular data generated in this study show that *K. phycomycoides* cannot be accommodated in either *Leptographium* or *Phialocephala* and that it represents a distinct taxon. It appears to be more closely related to *Phialocephala* than to *Leptographium,* which is also consistent with its morphology. The relatedness of *Penicillium* and *Aspergillus* is also of interest and suggests that it might reside in the Eurotiales, although additional study and consideration of other parts of the genome would be needed to resolve this observation fully.

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Table 1. Tolerance of *Ophiostoma piliferum, Leptographium lundbergii, Phialocephala dimorphospora* and *Kendrickiella phycomyces* to various concentrations of cycloheximide after 6 days of growth at 25

Cycloheximide concentration (g/l)	Colony size (mm)			
	L. lundbergii	*O. piliferum*	*P. dimorphospora*	*K. phycomycoides*
0	40	14	9.0	9.0
0.05	29	11	7.0	6.0
0.1	30	11	6.0	4.0
0.5	24	9.0	4.0	0.0
1	24	10	4.0	0.0

Fig. 1. One of the six most parsimonious tree derived from analysis of a partial sequence of the large subunit of the ribosomal gene. Tree length = 269. The number of base substitutions are indicated above the branches and the bootstrap values are indicated below the branches.

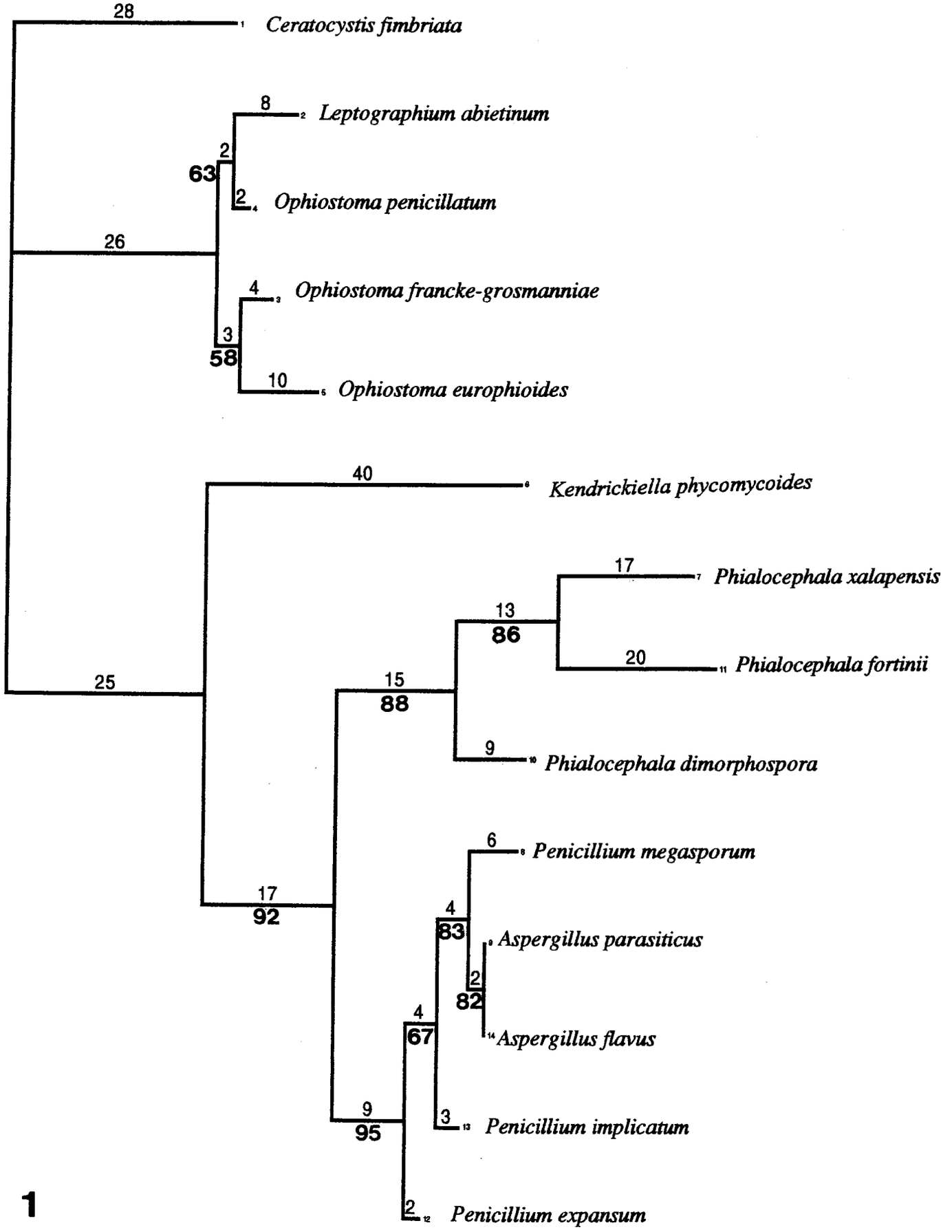

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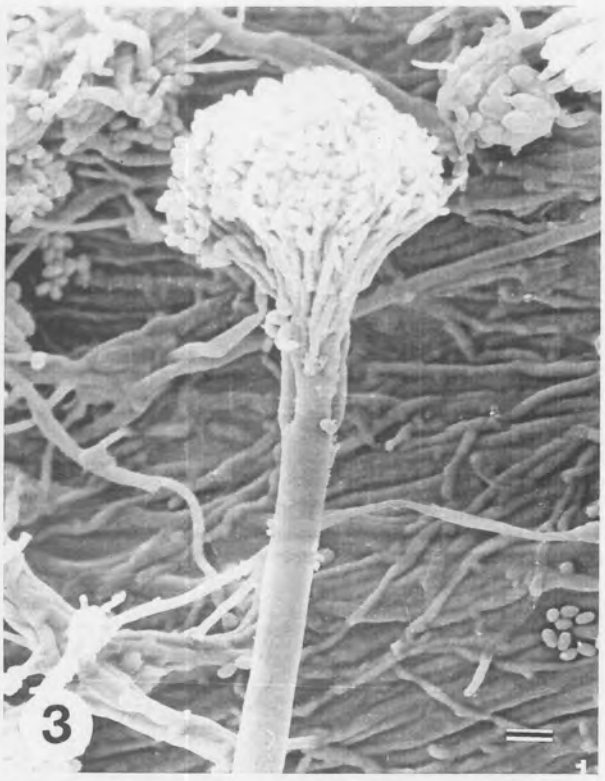
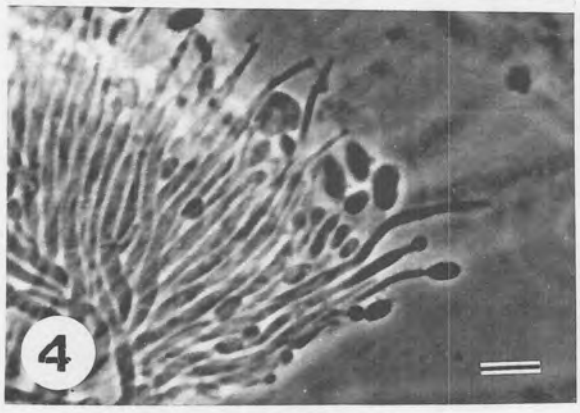
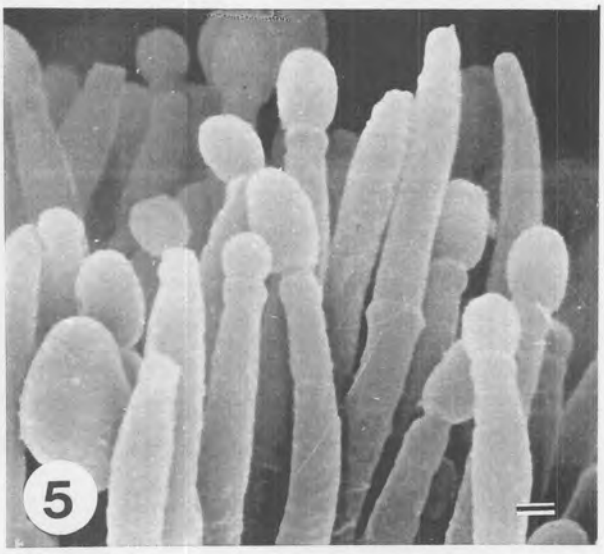
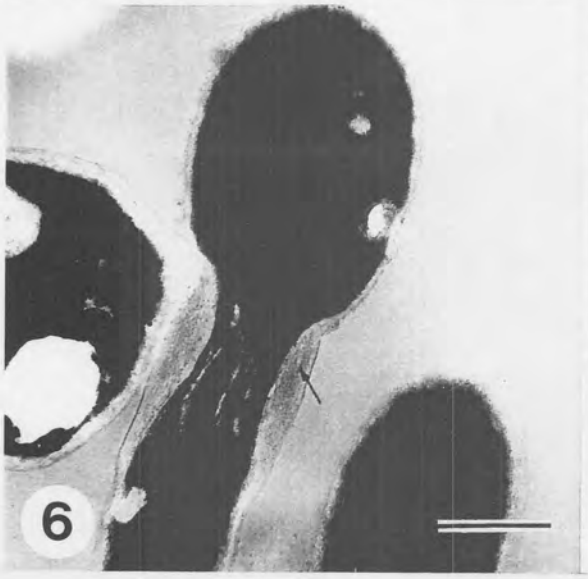
Fig. 2-7. Light, scanning and transmission micrographs of conidiophore and conidia of *Kendrickiella phycomycoides* (MUCL 38565). **Fig. 2.** Conidiophore (Bar =50 µm). **Fig. 3**. Scanning electron micrograph of the conidiogenous apparatus (Bar = 50 µm). **Fig. 4**. Light micrograph of the conidiogenous cells (Bar = 10 µm). **Fig. 5.** Scanning electron micrograph of the conidiogenous cell. Arrows indicate the inconspicuous collarettes (Bar = 5 µm). **Fig. 6.** Section through a conidiogenous cell. The arrow indicates periclinal thickening (Bar = 1 µm). **Fig. 7.** Conidia (Bar = 5 µm).









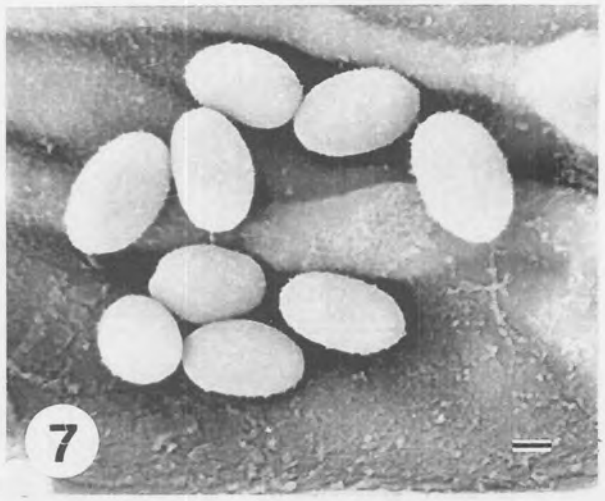
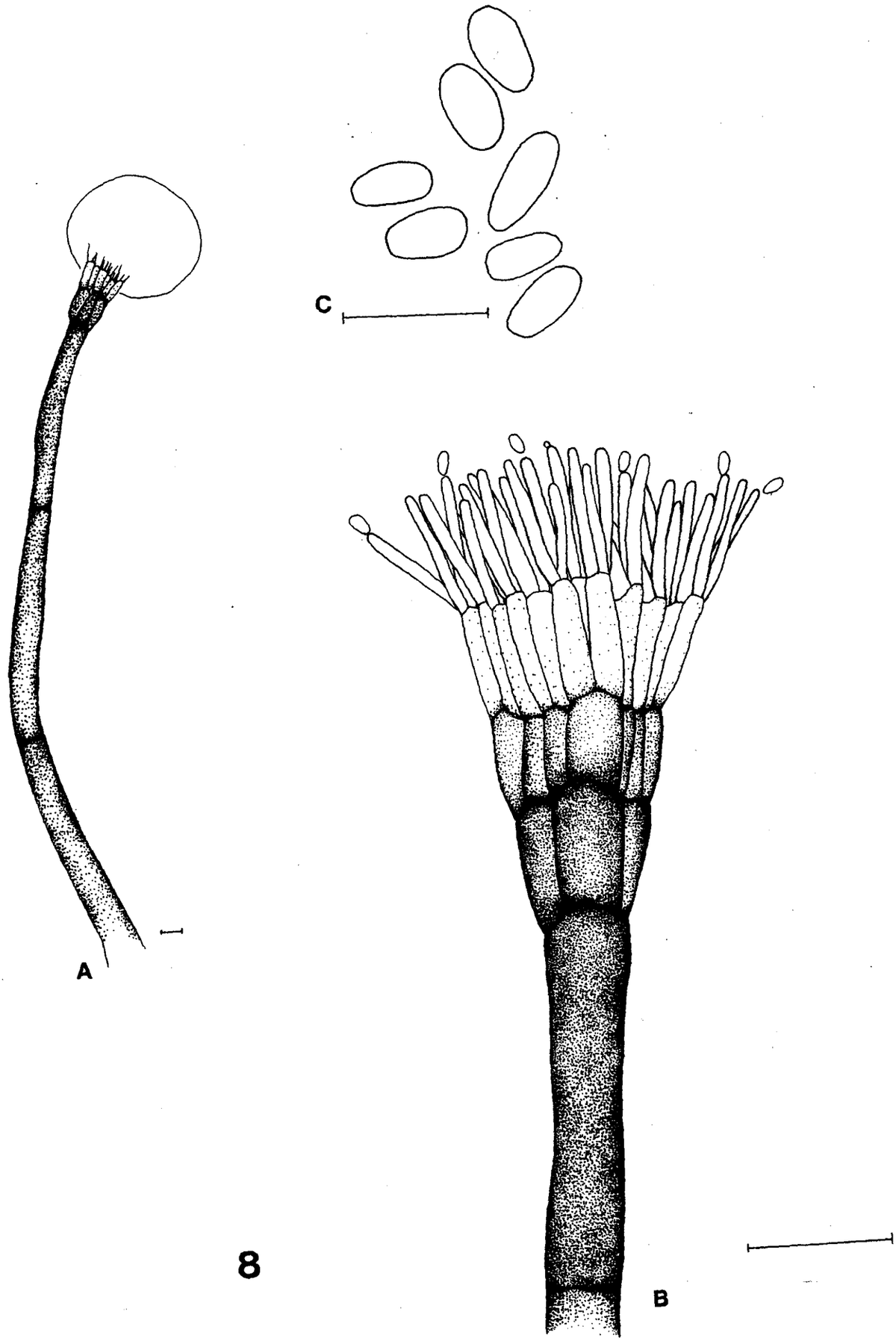


Fig. 8. Conidiophores and conidia of *Kendrickiella phycomycoides* (MUCL 38565). A. Conidiophore without rhizoids. B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).



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Chapter 5

Jacobs, K., Wingfield, M.J. and Roux, J. (1999). *Leptographium eucalyptophilum,* a new species from *Eucalyptus* in the Congo. South African Journal of Botany (in press).

Leptographium eucalyptophilum, a new species from *Eucalyptus* in the Congo

Leptographium spp. are known mostly from the Northern hemisphere where they have been described mainly from coniferous hosts. Few *Leptographium* spp. have been described from the Southern hemisphere and the tropics. During a recent survey of fungal diseases on *Eucalyptus* in the Republic of Congo, West Africa, an unidentified *Leptographium* sp. was isolated from stems of *Eucalyptus* hybrids. Comparison with known *Leptographium* spp. led us to conclude that this is a previously undescribed species. It is, therefore, described in this paper as *Leptographium eucalyptophilum* sp. nov.

Keywords: *Eucalyptus, Leptographium,* West Africa, Congo, fungal description

INTRODUCTION

Leptographium spp. are characterized by dark mononematous conidiophores with complex series of branches. These branches terminate in conidiogenous cells that produce conidia through percurrent proliferation (Kendrick, 1962; Wingfield, 1993). However, delayed secession of the conidia can create the impression of sympodial conidium development (Van Wyk *et al.,* 1988). Approximately half of all *Leptographium* spp. are known to be associated with an *Ophiostoma* teleomorph (Harrington, 1988; Wingfield, 1993). As in the case of *Ophiostoma, Leptographium* spp. are also known to be able to tolerate high concentrations of cycloheximide in culture media (Harrington, 1981).

Most *Leptographium* spp. occur on conifers (Lagerberg *et al.,* 1927; Kendrick, 1962; Harrington, 1988), although a few exceptions have been described (Davidson, 1942; 1958; 1971; 1976; Jooste, 1978; Weber *et al.,* 1996). *Leptographium* spp. are essentially saprotrophic (Harrington, 1988; Wingfield *et al.,* 1988) and are known to be causative agents of blue-stain on conifers (Lagerberg *et al.,* 1928; Morrison & Hunt, 1988; Solheim, 1995). In only a few instances, *Leptographium* spp. are known as primary pathogens, capable of causing considerable losses (Cobb, 1988; Harrington, 1993).

Leptographium spp. are well-adapted for insect dispersal (Nelson, 1934; Harrington, 1988; 1993). The most common insect associates of these fungi are bark beetles (Coleoptera: Scolytinae) in the genera *Hylastes* and *Hylurgops* (Harrington, 1988; Perry, 1991). These insects generally feed on roots of conifers, but the nature of the association is currently not clear.

Plantation forestry, based on exotic *Eucalyptus* spp., forms an important part of the export market of many countries (Turnbull, 1991). Currently approximately 8-9 million hectares of exotic *Eucalyptus* plantations exist in tropical and sub-tropical countries of the world (Turnbull, 1991; Wingfield & Wingfield, 1998). Although *Eucalyptus* is an unusual niche for *Leptographium* spp., recent surveys of diseased trees in the Republic of Congo, have resulted in the isolation of a *Leptographium* sp. of unknown identity. The aim of this study was to identify this *Leptographium* sp., and to consider its pathogenicity to *Eucalyptus*.

MATERIALS AND METHODS

A survey of the diseases of *Eucalyptus* trees in the Point Noire area of the Republic of Congo resulted in the consistent isolation of an unknown *Leptographium* sp. Isolates were found sporulating in the xylem of diseased *E. urophylla* X *E. pellita* hybrid trees from the Kissoko plantation. Spore masses were transferred from the apices of conidiophores to 2 % malt extract (MEA) plates (20g Biolab malt extract, 20g Biolab agar and 1000 ml distilled water) 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 were imbedded in lactophenol and mounted on glass slides for microscopic examination. Fifty measurements were taken of each relevant morphological structure and ranges and averages computed. Colours were determined with the aid of a colour chart (Rayner, 1970).

The optimal growth temperatures of representative isolates (PREM 56312, PREM 56313) were determined by inoculating eight MEA plates for each temperature (5 to 35 °C at 5 °C intervals) with a 6.0 mm diameter agar disk taken from the actively growing margin of a two week old isolate. Colony diameters were measured four and eight days after commencing the experiment. The colony diameter was 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. The material was dehydrated in a graded acetone series and dried using a critical-point drier. Specimens were mounted and coated with gold palladium alloy and examined using a Jeol JSM 840 scanning electron microscope.

Cycloheximide tolerance of isolates (PREM 56312, PREM 56313) was determined by placing them on 2 % MEA with different concentrations of cycloheximide (0, 0.05, 0.1, 0.5, 1, 2.5 and 5 g/l). Petri dishes were incubated in the dark at 25 °C for eight days. Five replicate plates were prepared for each concentration and the growth rate (mm/day) was determined based on the average of ten diameter readings.

To determine the possible role of the *Leptographium* sp. in disease development on *Eucalyptus* spp., an isolate (PREM 56312) was inoculated on to 20 clones of *Eucalyptus grandis* x E. *camaldulensis* hybrid saplings. The experiment was

conducted in a glass house with an average daily temperature of 25 °C with ambient day/night light periods. The test isolate was cultured on MEA agar for 14 days. The bark of approximately one-year-old trees was removed with a 4 mm diameter cork borer. An agar plug of equal size, overgrown with the test fungus, was inserted into the wounds. All wounds were sealed with parafilm to prevent desiccation of the wound and inoculum. Ten trees were inoculated in a similar fashion, using sterile agar plugs to serve as controls. Lesion development was assessed after 6 weeks by investigating both the outer bark and xylem.

RESULTS

The *Leptographium* isolates from *Eucalyptus* were characterized by an optimal growth temperature of 30 °C. Conidiophores were found to be long and slender and not as dark as those of other *Leptographium* spp. These isolates were further characterized by their long, oblong conidia. In some instances rhamoconidia were observed. Such structures have not been seen in other *Leptographium* spp. and they appear to be unique to the isolates from *Eucalyptus*.

Pathogenicity tests on a *Eucalyptus* hybrid indicated that the *Leptographium* sp. is not pathogenic to *Eucalyptus*. No external lesions were produced, but the fungus did prevent wounds from healing as quickly as those associated with the control inoculations. In the xylem, a blue discoloration was found in association with the *Leptographium* inoculations. This species is most probably a saprotroph and may be able to cause blue stain on dead wood. Comparison with other known species of *Leptographium*, revealed that this species has not been described previously 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 eucalyptophilum **K. Jacobs, M.J. Wingf. and J. Roux sp. nov.**

Teleomorph state: none observed.

Coloniae optime in temperatura 30°C crescentes; atroviride olivaceae. Hyphae immersae vel emersae in medio solido, cum myceliis aeriis abundantibus. Conidiophora singula vel ad terna, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (180-) 323 (-500) µm longa, cum 2 vel 3 seriebus ramorum cylindricorum; 2 - 3 ramis primariis; sine structuris rhizoidiformibus. Conidia aseptata, oblonga vel obovoidea, (6.0-) 8.0 (-9.0) x (3.0-) 3.0 (-5.0) µm.

Colonies with optimal growth at 30 °C on 2 % MEA, reaching 27 mm in diameter in 6 days. No growth below 10 °C or above 35 °C. Able to withstand high concentrations of cycloheximide with a 15 % reduction in growth on 0.1 g/l cycloheximide after days at 30 °C in the dark. Colonies dark green olivaceous (23''') with a smooth margin. Hyphae submerged and on top of solid medium with abundant aerial mycelia, light olivaceous to hyaline, smooth, not constricted at the septa, (2.0-) 3.0 (-5.0) µm diameter. Conidiophores occurring singly or in groups of up to three, arising directly from the mycelium, erect, macronematous, mononematous, (180-) 323 (-500) µm in length, rhizoid-like structures absent (Fig. 1, 7a). Stipe, light olivaceous, smooth, cylindrical, simple, 4-9 septate, (140-) 272 (-440) µm long from first basal septum to below primary branches, 4.0 – 5.0 µm wide below primary branches, apical cell of stipe not swollen; (5.0-) 6.5 (-10) µm wide at base, basal cell not swollen. Conidiogenous apparatus, excluding the conidial mass, (30-) 52 (-80) long, , with 2 to 3 series of cylindrical branches; 2-3 primary branches, light olivaceous to hyaline, smooth, cylindrical, aseptate, (12-) 17 (-26) µm long and (3.0-) 4.0 (6.0) µm wide. Secondary branches hyaline, aseptate, (7.0-) 10 (-13) µm long, (1.0-) 2.0 (-4.0) µm wide; tertiary branches hyaline, aseptate, (5.0-) 7.0 (-10) µm long, 2.0 – 3.0 µm wide (Fig. 2, 7b). Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (7.0-) 10 (-13) µm long and (1.0-) 1.5 (-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, Kirk and Sutton, 1982; 1983; Van Wyk, Wingfield and Marasas, 1988) (Fig. 3, 4). Conidia accumulating in slimy droplets at the apex of

conidiogenous apparatus, aseptate, oblong to obovoid and (6.0-) 8.0 (-9.0) x 3.0 – 5.0 µm (Fig. 5, 6, 7c).

Holotype: PREM 56312, isolated from the xylem of diseased *Eucalyptus urophylla* X *E. pellita* hybrid, collected: J. Roux, Kissoko plantation, Point Noire area, Republic of Congo, June 1998. **Paratypes**: PREM 56313, PREM 56314, PREM 56315, PREM 56316, PREM 56317, PREM 56318, PREM 56319 PREM 56320, isolated from the xylem of diseased *Eucalyptus urophylla* X *E. pellita* hybrid, collected: J. Roux, Kissoko plantation, Point Noire area, Republic of Congo, June 1998.

Dried cultures of the holotype and paratypes have been deposited in PREM.

DISCUSSION

Leptographium eucalyptophilum is characterized by its long, oblong conidia. Other species with similar long conidia, are *L. americanum* Jacobs, Wingfield & Bergdahl and the *Leptographium* anamorphs of *O. penicillatum* (Grosmann) Siemaszko and *O. dryocoetidis* (Kendrick & Molnar) Harrington (Grosmann, 1932; Kendrick & Molnar, 1965; Jacobs *et al.,* 1997). The conidia of *L. eucalyptophilum* can, however, easily be distinguished from *O. penicillatum* and *O. dryocoetidis* that are twice as broad as those in *L. eucalyptophilum* (Grosmann, 1932; Kendrick & Molnar, 1965). In addition, *O. penicillatum* and *O. dryocoetidis* are known to produce perithecia, which were never seen in the case of *L. eucalyptophilum*. Both *O. penicillatum* and *O. dryocoetidis* occur on conifers n the Northern hemisphere and are associated with severe staining of host tissue (Solheim, 1986; Molnar, 1965).

Leptographium americanum, has long and almost needle shaped conidia that are most similar to those of *L. eucalyptophilum* (Jacobs *et al.,* 1997). The conidia are, however, much longer than those found in *L. eucalyptophilum. Leptographium eucalyptophilum* is characterized by two to three primary branches on the stipe, in contrast to two branches that are consistently found in isolates of *L. americanum. Leptographium eucalyptophilum* and *L. americanum* can also be distinguished based on their host preferences and insect associations. *Leptographium americanum* is known only on larch in North America associated with the bark beetle *Dendroctonus*

simplex (Jacobs *et al.,* 1997). *Leptographium eucalyptophilum,* is found on *Eucalyptus* and no association with any insect has been observed.

Leptographium eucalyptophilum has an optimal growth temperature of 30 °C. This is unlike most other species in *Leptographium* with optimal growth temperatures of between 20 and 25°C. This phenomenon has also been observed in *Leptographium calophylli* that occurs in the Seychelles (Webber *et al.,* in press) and appears to be characteristic of *Leptographium* spp. from tropical areas.

Pathogenicity trials showed that *L. eucalyptophilum* most likely does not play a primary role in disease development on *Eucalyptus* trees. This fungus was found to occur on lesions caused by *Ceratocystis fimbriata* Ell. & Halst. (Roux *et al.,* 1999), a fungus that has recently been shown to be pathogenic to *Eucalyptus* spp. and was isolated in abundance from dying trees in the Republic of Congo (Roux *et al.,* 1999). *Ceratocystis fimbriata* is characterized by the production of fruity aromas and insects, carrying this fungus, might accidentally also serve as vectors of *L. eucalyptophilum.*

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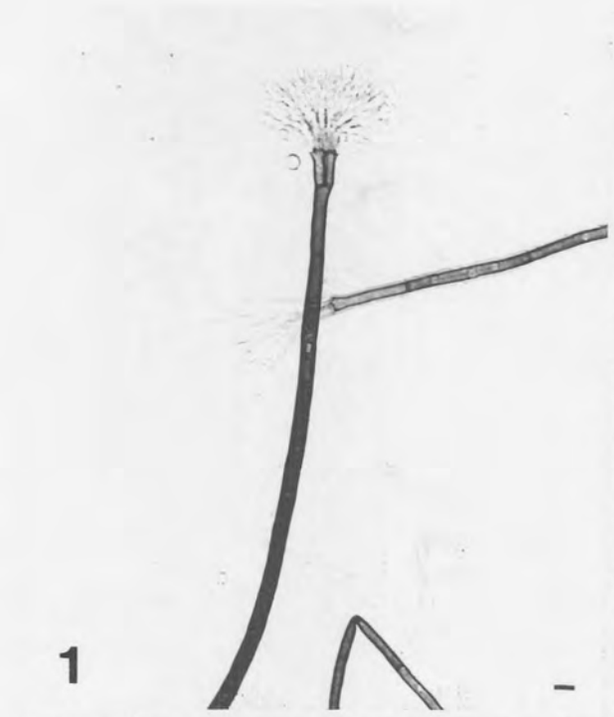
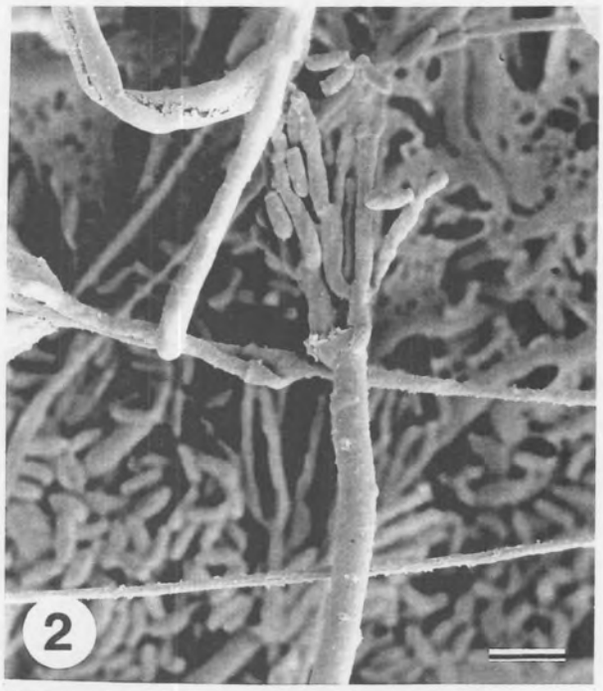
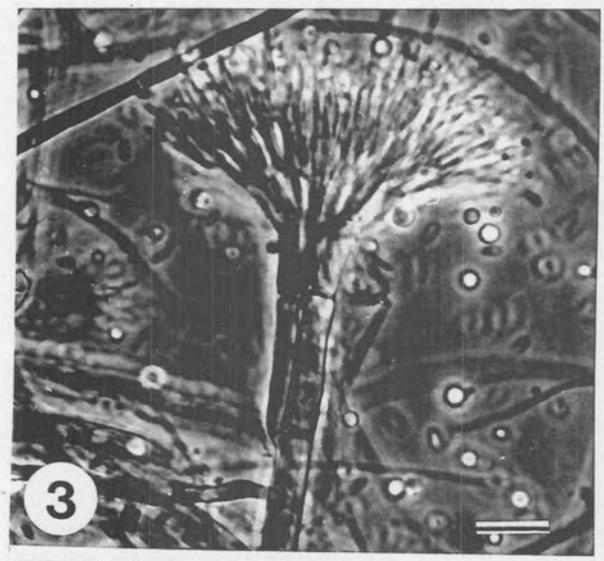
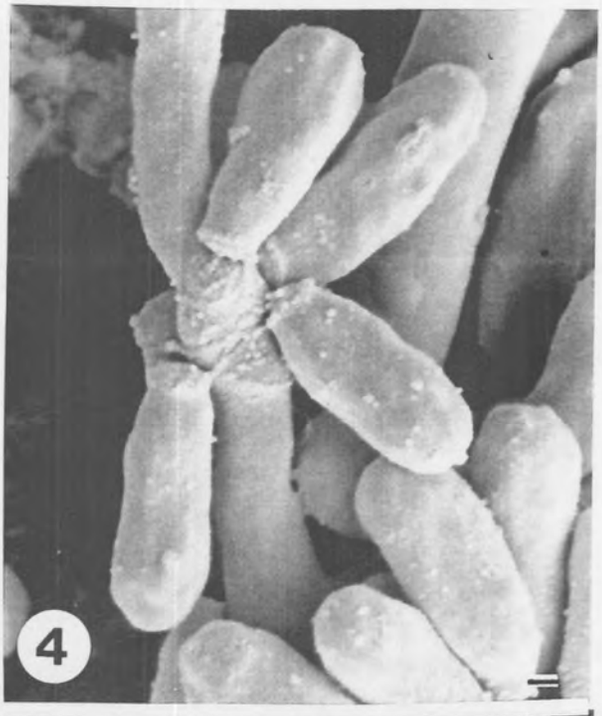
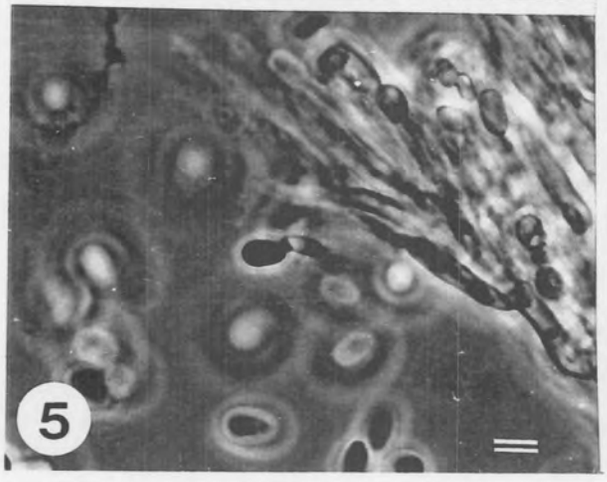
Fig. 1 - 6. *Leptographium eucalyptophilum* (PREM 56312). **Fig. 1.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 100 µm). **Fig. 2.** Scanning electron micrograph of the conidiogenous apparatus (Bar = 10 µm). **Fig. 3.** Light micrograph of the conidiogenous apparatus (Bar = 10 µm). **Fig. 4.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 5 µm). **5.** Light micrograph of conidiogenous cells (Bar = 10 µm). **Fig. 6.** Conidia with occasional rhamoconidia (Bar = 10 µm).











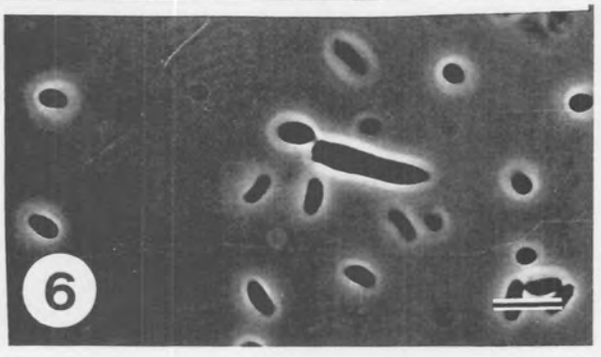
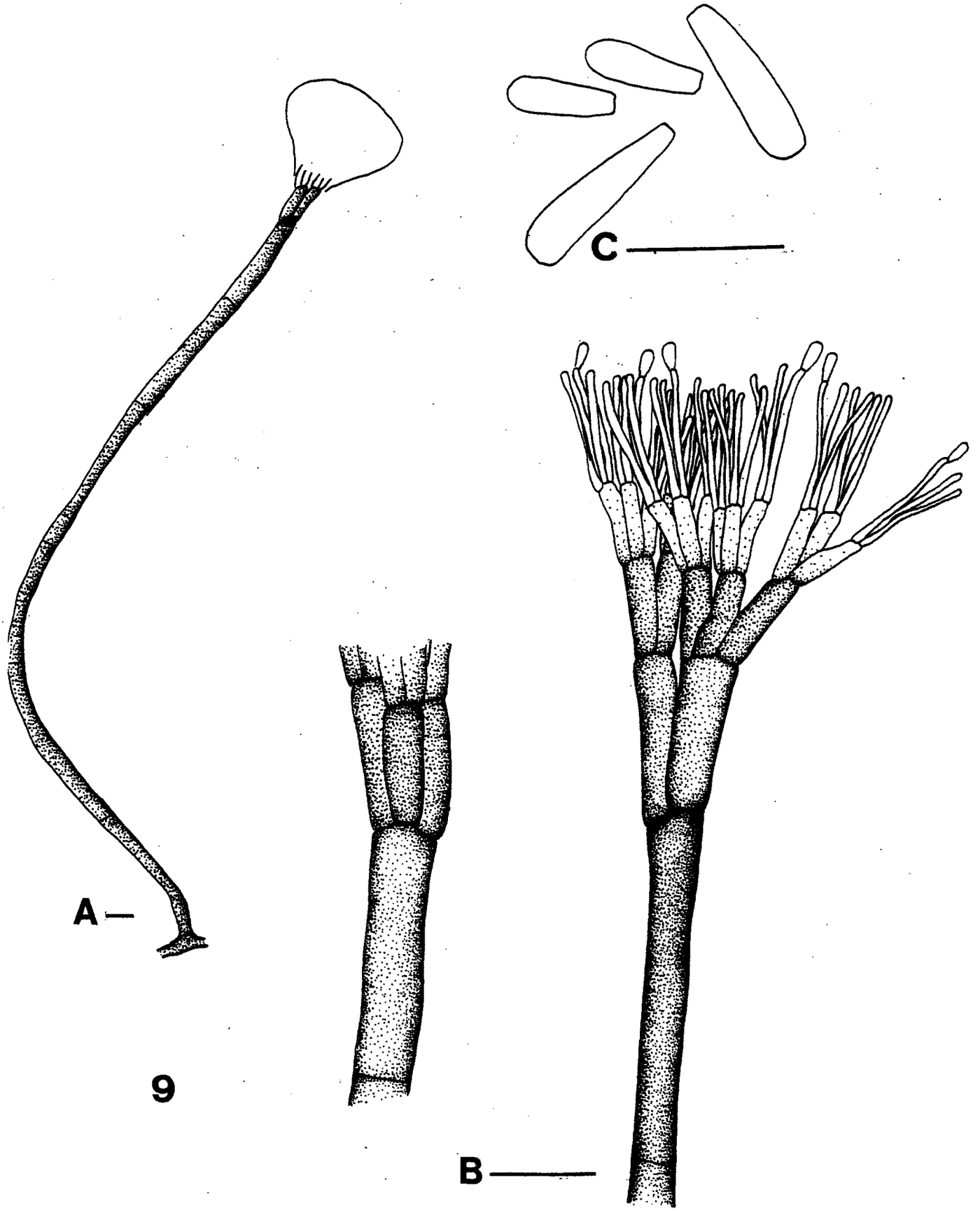


Fig. 7. *Leptographium eucalyptophilum* (PREM 56312). A. Habit sketch of the conidiophore. B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).



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Chapter 6

Jacobs, K, Wingfield, M.J., Uzunovic, A. and Frisullo, S. (1999). Three new species of *Leptographium* from pine, similar to *L. procerum.* Mycological Research (submitted)

Three new species of *Leptographium* from pine, similar to *L. procerum*

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

Keywords: *Leptographium procerum, Pinus* spp.

INTRODUCTION

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

Approximately half the described *Leptographium* spp. are known to have *Ophiostoma* states (Harrington, 1988; Wingfield, 1993). Those species for which teleomorphs are unknown are generally recognized as being related to *Ophiostoma* through their association with bark beetles and by a number of unusual physiological characteristics. For example, *Ophiostoma* spp. and their *Leptographium* anamorphs are characterized by an ability to tolerate high concentrations of the antibiotic cycloheximide in culture (Fergus, 1956; Harrington, 1988). They also have cellulose and rhamnose in their cell walls which makes them unlike most other ascomycetes (Rosinski & Campana, 1964; Spencer & Gorin, 1971; Weijman & de Hoog, 1975; Marais, 1996).

Ophiostoma and *Leptographium* species are well known as the causal agents of sap stain in lumber (Lagerberg, Lundberg and Melin 1927; Solheim, 1986). In this regard, they are generally considered to be saprophytes, although controversy surrounds their role in the biology of bark beetles (Harrington, 1988; Wingfield, Capretti & Mackenzie, 1988; Wingfield, Harrington & Solheim, 1995). Some *Ophiostoma* and *Leptographium* species are important pathogens of trees. Of these, the best known are *Ophiostoma ulmi* and *O. novo-ulmi,* the causal agents of Dutch elm disease (Brasier, 1979; 1986; 1991). The three varieties of *Leptographium wageneri* (Kendrick) Wingfield cause the important black stain root disease of conifers in the Western United States and are the one of two species of this genus that are unequivocally recognized as primary pathogens (Wagener & Mielke, 1961;

Cobb, 1988; Harrington & Cobb, 1987; Harrington, 1988; Wingfield *et al.,* 1988; Webber *et al.,* 1999).

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

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

In recent years, we have accumulated a large number of cultures from many parts of the world, that have tentatively been identified as *L. procerum.* Although these isolates peripherally resemble *L. procerum,* many differences in their morphology and physiology have been noted. The aim of this investigation was to undertake a detailed study of these cultures and to determine whether they can justifiably be retained in a single taxon.

MATERIALS AND METHODS

Isolates examined in this study were obtained from a wide range of hosts and geographic locations (Table 1). Comparisons with herbarium specimens, including the holotype, of *L. procerum* were also made. These include DAOM 63700, Canada, St. Paul, Quebec, *Pinus banksiana*, 4 September 1959, collected: W.B. Kendrick (holotype); DAOM 62093, New York, Montgomery County, *Pinus resinosa* (interior of roots with resinous lesions), February 1959, collected: D.S. Welch; DAOM 62094, New York, Newfield, *Pinus resinosa* (interior of roots with resinous lesions), Feb. 1959, collected: D.S. Welch; DAOM 62095, New York, Columbia County, Conoan, *Pinus resinosa* (interior of roots with resinous lesions), collected: Feb. 1959, D.S. Welch; DAOM 62096, New York Stockton, Chatauqua County, *Pinus resinosa* (interior of roots with resinous lesions), Feb. 1959, collected: D.S. Welch; DAOM 63686, Sweden, Järna, Södermanland, *Pinus* sp., Aug. 1959, collected: A. Mathiesen-Käärik; DAOM 33940, Sudbury, *Pinus strobus*, Nov. 1952, collected: S.N. Linszon.

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

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

Four morphological groups, including the isolates representing *L. procerum sensu stricto,* were identified. Optimal temperatures for growth of representative isolates of these groups [CMW 3766, CMW 3767 (*L. alethinum*); CMW 2840, CMW 2892, CMW 3047 (*L. pityophilum*); CMW 301, CMW 281(*L. euphyes*); CMW 2460, CMW12 (*L.*

procerum sensu stricto)] were determined by inoculating eight MEA plates for each temperature with a 6 mm diameter agar disk taken from the actively growing margin of a fresh isolate. The plates were incubated at temperatures ranging from 5 to 35 °C at 5 °C intervals. Colony diameters were measured in two directions perpendicular to each other on the fourth and the eighth day after commencing the experiment, and the diameters of colonies computed as an average of eight readings.

Cycloheximide tolerance of representative isolates of the four morphological groups representing *L. procerum sensu lato* 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 growth was measured. Five replicate plates were inoculated for each concentration and the growth was determined based on an average of ten readings with two readings perpendicular to each other, for each plate.

RESULTS

Four morphologically different groups arose from our detailed comparison of the larger set of isolates that had been designated as *L. procerum sensu lato.* One of these represents *L. procerum sensu stricto* which was confirmed through comparison with the herbarium type specimens. Isolates of *L. procerum s.s.* are characterized by long dark conidiophores with two to three primary branches and rhizoids at the bases of the conidiophores. Colonies can easily be recognized by the dark concentric rings formed by clusters of conidiophores. Conidia of *L. procerum* are small and obovoid.

The second morphological group of isolates was characterized by having long conidiophores and obovoid conidia that are considerably longer than those of *L. procerum s.s.* Conidiophores in this group also had rhizoids at their bases. However, the conidiogenous apparatuses in this group of isolates were not as darkly pigmented as those found in *L. procerum s.s.* The third sub-group of isolates was characterized by conidiophores with several short primary branches, similar to those of *L. serpens* (Goidanich) Von Arx. However, unlike *L. serpens,* the hyphae did not have a serpentine growth pattern on the surface of agar. The fourth sub-group of

isolates was characterized by short robust conidiophores, unlike those observed in *L. procerum*. This species resembles the *Leptographium* anamorph of *Ophiostoma grandifoliae* (Davidson) Harrington. Comparison with other known species of *Leptographium* revealed that the three groups of isolates previously accommodated in *L. procerum s.l.*, did not resemble any known *Leptographium* species. We, therefore, describe them as new species.

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 alethinum **K. Jacobs, M.J. Wingf. & A. Uzunovic sp. nov.**

Teleomorph state: not known.

Coloniae optime in temperatura 20°C crescentes; olivaceae; margine laevi. Hyphae immersae, sine myceliis aeriis. Conidiophora singula vel ad sena, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (560-) 1032 (-1270) µm longa, cum 3 vel 4 seriebus ramorum cylindricorum; 2 - 4 ramis primariis; sine structuris rhizoidiformibus. Conidia aseptata, obovoidea extremitatibus truncatis, (4.0-) 6.0 (-9.0) x 2.0 - 3.0 µm.

Colonies with optimal growth at 20 °C on 2 % MEA, reaching 23 mm in diameter in 6 days. There was a little growth below 5 °C and no growth 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 20 °C in the dark. Colony olivaceous (19"f). Colony margin smooth. Hyphae submerged with no aerial mycelium, olivaceous to light olivaceous (Rayner, 1970), smooth, not constricted at the septa, (2.0-) 6.0 (-12) µm diameter.

Conidiophores occurring singly or in groups of up to six, arising directly from the mycelium, erect, macronematous, mononematous, (560-) 1032 (-1270) µm in length, rhizoid-like structures occasionally present. Stipe dark olivaceous, smooth,

cylindrical, simple, 6 - 10 septate, (500-) 922 (-1150) µm long (from first basal septum to below primary branches), (10.0-) 11.5 (-12.5) µm wide below primary branches, apical cell not swollen, (10-) 13 (-15) µm wide at base, basal cell not swollen (Fig. 1, 7a). Conidiogenous apparatus (60-) 111 (-170) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches, 2-4 primary branches, olivaceous, smooth, cylindrical, aseptate, (25-) 37 (-55) µm long and (5.0-) 8.0 (-13) µm wide, secondary branches olivaceous to hyaline, aseptate, (12-) 20 (-33) µm long, (3.0-) 5.0 (-9.0) µm wide, tertiary branches hyaline, aseptate, (10-) 14 (-20) µm long, (2.0-) 3.0 (-5.0) µm wide, quaternary branches aseptate, hyaline, (8.0-) 12 (-17) µm long, (2.0-) 2.5 (-3.0) µm wide (Fig. 2, 7b). Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (12-) 17 (-23) 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, Wingfield and Marasas, 1988) (Fig. 3, 4). Conidia, aseptate, obovoid with truncate ends, (4.0-) 6.0 (-9.0) x 2.0 - 3.0 µm (Fig. 5, 6, 7c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Holotype: CMW 3766, *Hylobius abietis* galleries, England, collected A. Uzunovic.

Additional specimens: CMW 3767, *Hylobius abietis* galleries, England, collected A. Uzunovic, CMW 3765, *Hylobius abietis* galleries, England, collected A. Uzunovic; CMW 3764, *Hylobius abietis* galleries, England, collected A. Uzunovic; CMW 2159, Corsican pine, England, collected: J.N. Gibbs.

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 pityophilum **K. Jacobs, M.J. Wingf. & S. Frisullo sp. nov.**

Teleomorph state: not known.

Coloniae optime in temperatura 20°C crescentes; atro-olivaceae; margine laciniati. Hyphae immersae vel emersae in medio solido, sine myceliis aeriis. Conidiophora singula, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (142-) 381 (-626) µm longa, cum 3 vel 4 seriebus ramorum cylindricorum; 2 - 5 ramis primariis; sine structuris rhizoidiformibus. Conidia aseptata, obovoidea, (4.0-) 5.0 (-6.0) x 2.0 - 3.0 µm.

Colonies with optimal growth at 20 °C on 2 % MEA, reaching 25 mm in diameter in 6 days. No growth below 5 °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 mycelium, light olivaceous to dark olivaceous, surrounded by rough granular layer, not constricted at the septa, 2.0 - 3.0 µm diameter.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (142-) 381 (-626) µm in length, rhizoid-like structures absent. Stipe dark olivaceous, smooth, cylindrical, simple, 3 - 9 septate, (105.5-) 317 (-564) µm long (from first basal septum to below primary branches), (7.5-) 10 (-12.5) µm wide below primary branches, apical cell not swollen, (7.5-) 11 (-12.5) µm wide at base, basal cell not swollen (Fig. 8, 14a). Conidiogenous apparatus (37-) 66 (-99) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches, 2 - 5 primary branches, olivaceous, smooth, cylindrical to barrel-shaped, aseptate, (11-) 17 (-25) µm long and (5.0-) 7.0 (-11) µm wide, secondary branches light olivaceous to hyaline, aseptate, (8.0-) 12 (-17) µm long, (3.0-) 5.0 (-8.0) µm wide; tertiary branches hyaline, aseptate, (7.0-) 10.5 (-16) µm long, (2.0-) 3.0 (-5.0) µm wide, quaternary branches aseptate, hyaline, (8.0-) 10 (-12) 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, (14-) 16.5 (-21) µm long and (1.5-) 2.0 (-3.0) µm wide. Conidium development occuring 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, obovoid, (4.0-) 5.0 (-6.0) x 2.0 - 3.0 µm (Fig.

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

Holotype: CMW 2840, isolated from *Pinus nigra,* Italy, collected: S. Frisullo.

Additional specimens: CMW 2892, isolated from *Pinus nigra,* Italy, collected: S. Frisullo. CMW 3047, isolated from *Pinus nigra,* Italy, collected: S. Frisullo. CMW 2838, isolated from *Pinus nigra,* Italy, collected: S. Frisullo. CMW 3063, isolated from *Pinus nigra,* Italy, collected: S. Frisullo. CMW 2874, isolated from *Pinus nigra,* Italy, collected: S. Frisullo.

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

Teleomorph state: not observed.

Coloniae optime in temperatura 25 °C crescentes; olivaceae; margine laevi. Hyphae immersae vel emersae in medio solido, sine myceliis aeriis. Conidiophora singula, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (204-) 300 (-315) µm longa, cum 3 vel 4 seriebus ramorum cylindricorum; 2 - 3 ramis primariis; structurae rhizoidiformes adsunt. Conidia aseptata, obovoidea extremitatibus truncatis, aliquando oblonga, (4.0-) 5.0 (-6.0) x 2.0 - 3.0 µm.

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 19 mm in diameter in 6 days. No growth below 5 °C or above 30 °C Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.1 g/l cycloheximide after days at 25 °C in the dark. Colony olivaceous (19"f). Colony margin smooth. Hyphae submerged or on top of solid medium with no aerial mycelia, light olivaceous to hyaline, smooth, occasionally constricted at the septa, (2.0-) 3.0 (-5.0) µm diameter.

Conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (204-) 300 (-315) µm in length, rhizoid-like structures present. Stipe olivaceous, smooth, cylindrical, simple, 3 - 9 septate, (142.5-) 224 (-353.5) µm long (from first basal septum to below primary branches), (6.0-) 7.0 (-9.0) µm wide below primary branches, apical cell not swollen, (6.0-) 7.0 (-12.5) µm wide at base, basal cell not swollen (Fig. 15, 21a). Conidiogenous apparatus (31-) 73 (-93) long, excluding the conidial mass, with 3 to 4 series of cylindrical branches, 2 - 3 primary branches, light olivaceous, smooth, cylindrical, aseptate, (11-) 18.5 (-47) µm long and (5.0-) 6.0 (-8.0) µm wide, secondary branches light olivaceous to hyaline, aseptate, (8.0-) 12 (-18) µm long, (3.0-) 4.0 (-6.0) µm wide, tertiary branches hyaline, aseptate, (8.0-) 10.5 (-13) µm long, (2.0-) 3.0 (-5.0) µm wide, quaternary branches aseptate, hyaline, (7.0-) 10 (-12) µm long, 2.0 - 3.0 µm wide (Fig. 16, 21b). Conidiogenous cells discrete, 2-3 per branch, cylindrical, tapering slightly at the apex, (10-) 14.5 (-20) µm long and 1.0 - 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 with truncated ends, occasionally oblong, (4.0-) 5.0 (-6.0) x 2.0 - 3.0 µm (Fig. 19, 20, 21c). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus.

Holotype: PREM 45703, *Pinus strobus,* New Zealand, collected: M. Dick.

Additional specimens: CMW301, *Pinus strobus,* New Zealand, collected: M. Dick; PREM 45701, *P. radiata,* New Zealand, collected: M. Dick; CMW 259, *Pinus strobus,* New Zealand, collected: M. Dick.

DISCUSSION

Leptographium alethinum, L. pityophilum and *L. euphyes* can easily be distinguished from *L. procerum* based on a number of morphological differences. The most obvious distinguishing character in these species is the absence of the characteristic concentric rings typically formed in agar colonies of *L. procerum*. *Leptographium alethinum* can further be distinguished from *L. procerum* based on the absence of

rhizoids, whereas these structures are prominent in isolates of *L. procerum.* Furthermore, the conidia of *L. alethinum* are obovoid, but slightly longer (4 - 9 µm) than those of *L. procerum* (3 - 5 µm).

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

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

Leptographium pityophilum is similar to *L. serpens* and *L. wageneri.* It can be distinguished from *L. wageneri* based on its optimal growth temperature at 20 °C, compared with 15 °C for *L. wageneri.* *Leptographium pityophilum* can be distinguished from *L. serpens* based on its straight uncurved hyphae, compared to the distinctly serpentine hyphae of *L. serpens.* *Leptographium serpens* is further characterized by longer (250 - 1270 µm) conidiophores with rhizoids (Kendrick, 1962), compared to the shorter conidiophores (142 - 626 µm) without rhizoids in *L. pityophilum.* *Leptographium pityophilum* and *L. serpens* share a similar habitat as both have been isolated from *Pinus nigra* in Europe. Because of their morphological similarity, they might have mistakenly been treated as a single species. No insects are known to be associated with *L pityophilum* although these are most likely to exist.

Leptographium euphyes can be distinguished from *L. procerum* based on its short robust conidiophores, which were unlike the long conidiophores described for *L. procerum*. Both these species have rhizoids and conidia of similar shape and size (Kendrick, 1962). Of the three new taxa described here, *Leptographium euphyes* is most unlike *L. procerum*. Comparison with other *Leptographium* spp. revealed that it is morphologically most similar to the *Leptographium* anamorph of *Ophiostoma grandifoliae* (Davidson) Harrington. These species could, however, be distinguished based on the presence of a teleomorph in the latter species (Davidson, 1976) and its absence in the former species. In the absence of a teleomorph, *L. euphyes* can be distinguished from *O. grandifoliae* based on more complex conidiogenous apparatuses as well as larger conidia (4 - 6 µm) compared to *O. grandifoliae* (2.5 - 4 µm). Furthermore, *O. grandifoliae* occurs on oak (*Fagus grandifoliae*) in the USA, whereas *L. euphyes* originates from pine roots, which is introduced into New Zealand.

Leptographium euphyes is commonly isolated together with *L. procerum* in New Zealand. The fungus originates from a collection of isolates that were linked to a report of a root disease of *Pinus strobus* in New Zealand (Shaw & Dick, 1980). Later Wingfield and Marasas (1983) studied this collection of isolates and noted that it represented isolates having two distinct morphological forms. These included one group that was typical of *L. procerum* and another which were considered to be different. This latter group represents *Leptographium euphyes*.

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

This study has included a relatively large set of isolates of *L. procerum sensu stricto,* that have been defined through careful comparison with type specimens of this species. These will be useful in taxonomic studies based on DNA sequence data

that are planned for the future. They have also somewhat expanded the known geographic distribution of *L. procerum.* One of the interesting records includes that from South Africa, where the fungus has previously not been known. Various *Leptographium* spp. occur in this country where they are associated with root and the introduced pine root feeding bark beetles *Hylastes angustatus* and *Hylurgus ligniperda* (Wingfield & Marasas, 1980, 1983). These insects are native to Europe and we assume that *L. procerum* was introduced into South Africa with one or both of them.

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

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Table 1. Isolates used in this study

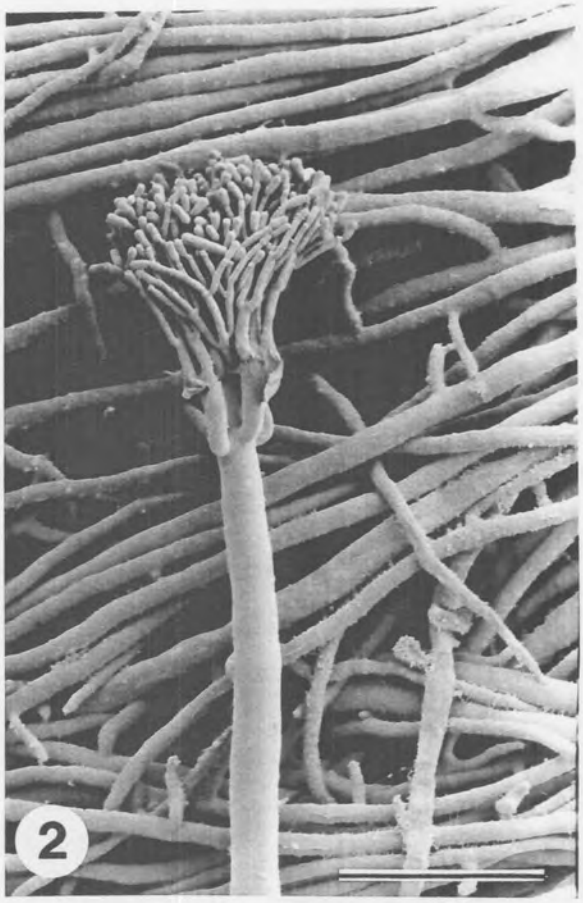
Number	Identification	Origin	Host	Collector
CMW 2460	*L. procerum*	Poland	*Pinus spp.*	T. Kawalski
CMW 3	"	USA	*P. strobus*	J. Altman
CMW 12	"	"	"	M.J. Wingfield
CMW 1831	"	"	*P. monticola*	P. Kulhavy
CMW 825	"	England	*Hylastes opacus*	J.N. Gibbs
CMW 828	"	"	*Hylurgops palliatus*	"
CMW 2172	"	"	*Hylobius* sp.	"
CMW 522	"	RSA	*Pinus* infested with *Hylastes* sp	G. Tribe
CMW 699	"	Italy	*P. pinea*	P. Capretti
CMW 3797	"	Norway	*Picea* sp.	M.J. Wingfield
CMW 25	"	Yugoslavia	*P. strobus*	M. Halambek
CMW 747	"	France	*Picea abies*	M. Morelet
CMW 261	"	New Zealand	*P. strobus*	M. Dick
CMW 20	"	Canada	*P. strobus*	Lincar
CMW 2159	*L. alethinum*	England	Corsican pine	J.N. Gibbs
CMW 3764	"	"	*Hylobius abietis*	A. Uzunovic
CMW 3765	"	"	"	"

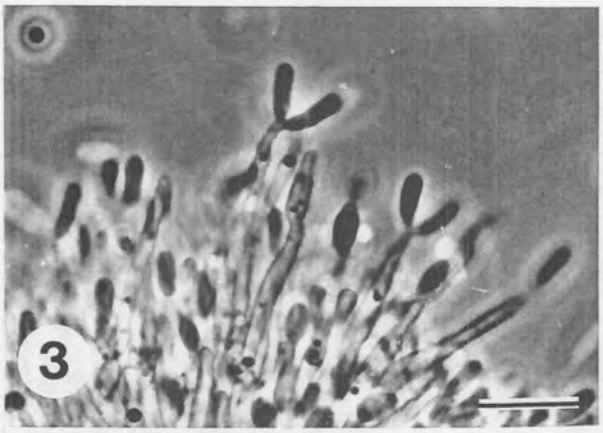
Table 1. cont.

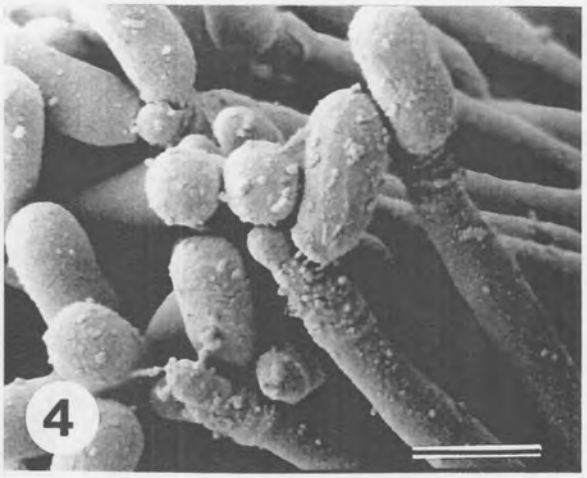
Number	Identification	Origin	Host	Collector
CMW 3766	"	"	"	"
CMW 3767	"	"	"	"
CMW 2892	*L. pityophilum*	Italy	*P. nigra*	S. Frisullo
CMW 2838	"	"	"	"
CMW 2840	"	"	"	"
CMW 2874	"	"	"	"
CMW 3047	"	"	"	"
CMW 3063	"	"	"	"
CMW 259	*L. euphyes*	New Zealand	*P. strobus*	M. Dick
CMW 264	"	"	*P. radiata*	"
CMW 291	"	"	*P. strobus*	"
CMW 301	"	"	*Pinus* sp.	"

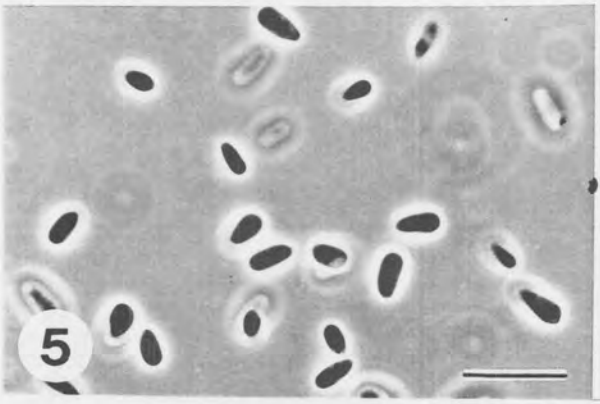
CMW: Culture collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, Republic of South Africa.

Fig. 1 - 6. *Leptographium alethinum* (CMW 3767). **Fig. 1.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 100 µm). **Fig. 2.** Complex conidiogenous apparatus (Bar = 100 µm). **Fig. 3.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 µm). **Fig. 4.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 5 µm). **Fig. 5-** Conidia (Bar = 10 µm). **Fig. 6.** Conidia (Bar = 1 µm).









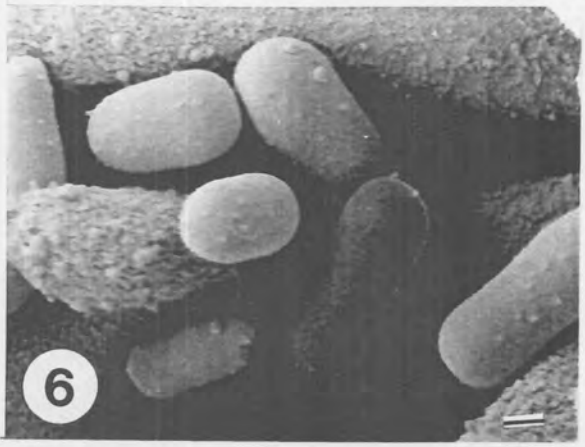
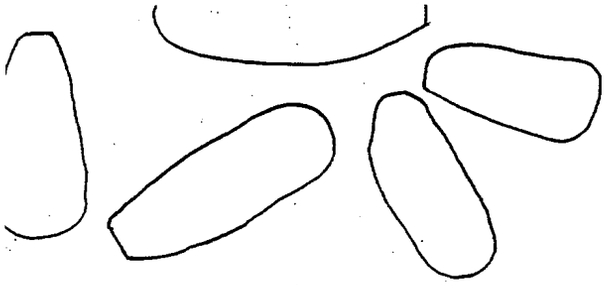


Fig. 7. *Leptographium alethinum* (CMW 3767). A. Habit sketch of the conidiophore (Bar = 50 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).



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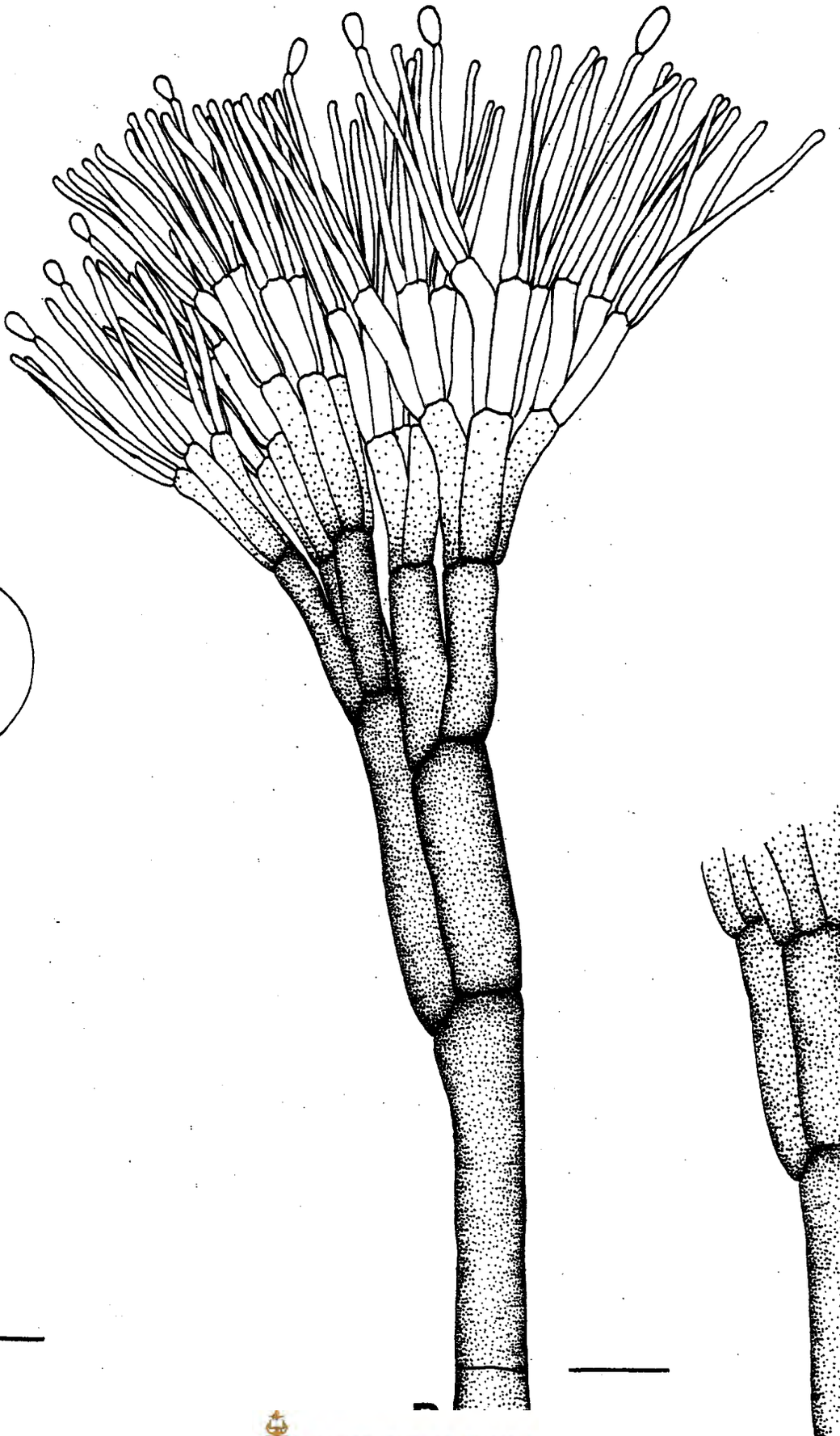

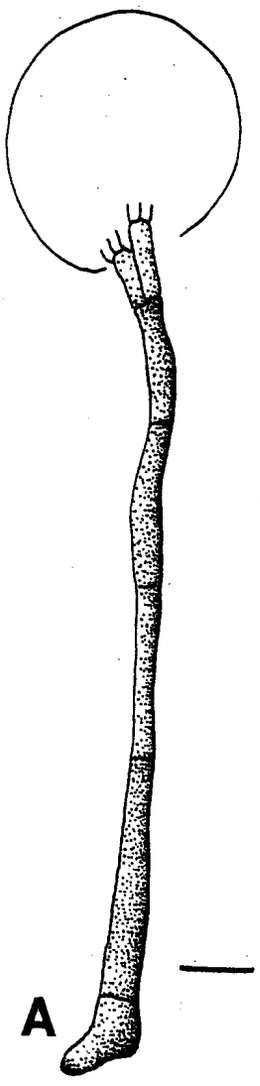

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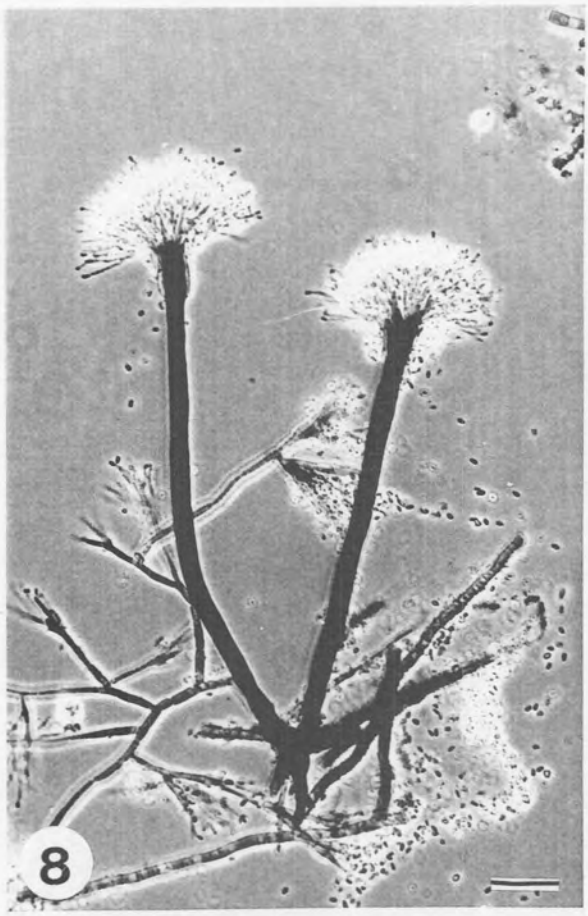
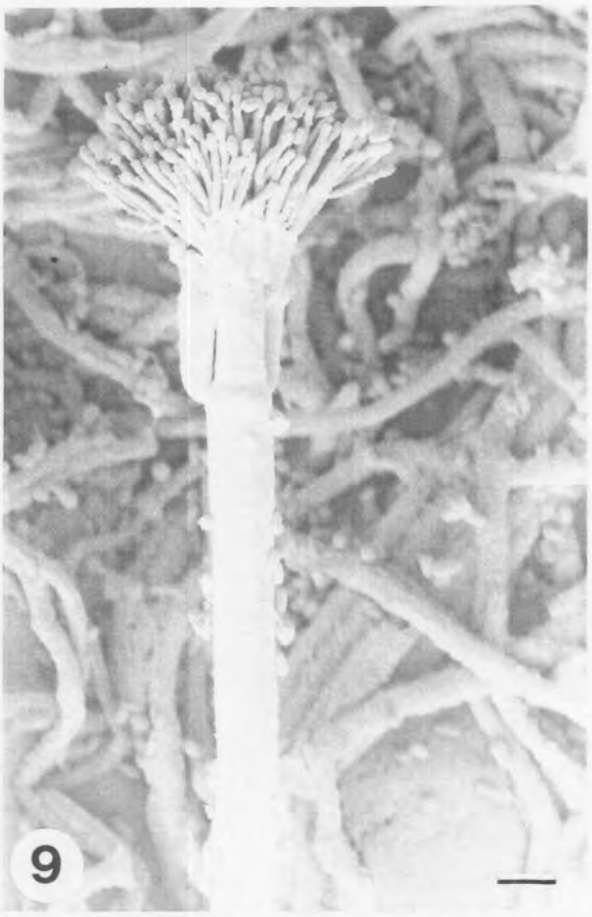
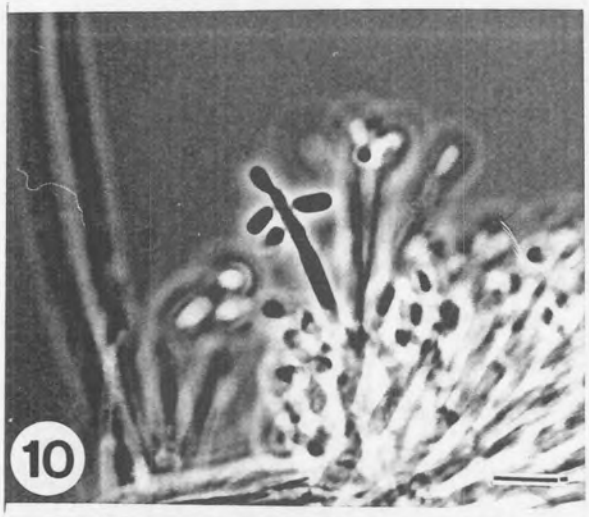
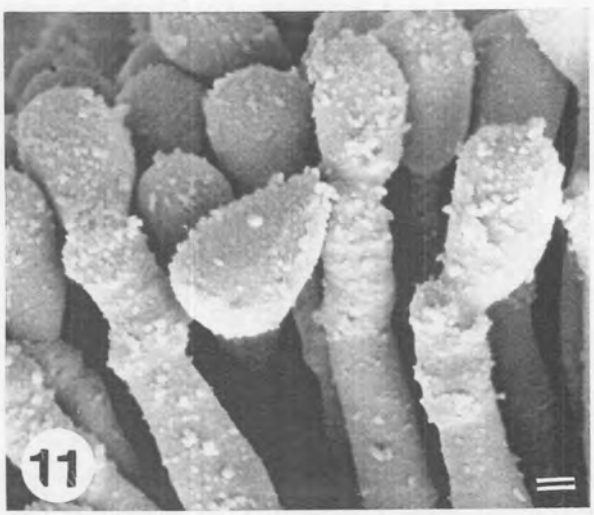
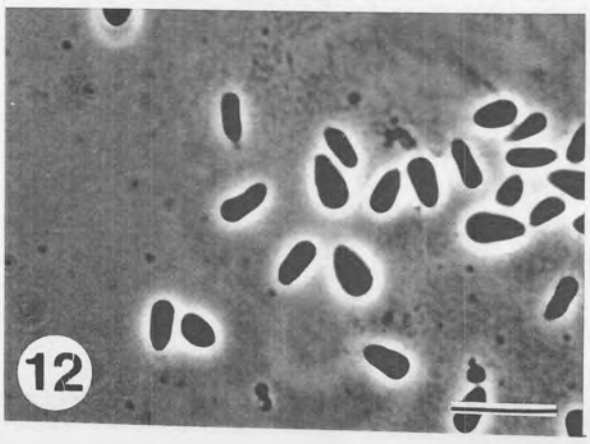
Fig. 8 - 13. *Leptographium pityophilum* (CMW 2892). **Fig. 8.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 20 µm). **Fig. 9.** Complex conidiogenous apparatus (Bar = 10 µm). **Fig. 10.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 µm). **Fig. 11.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 1 µm). **Fig. 12** Conidia (Bar = 10 µm). **Fig. 13.** Conidia (Bar = 5 µm).











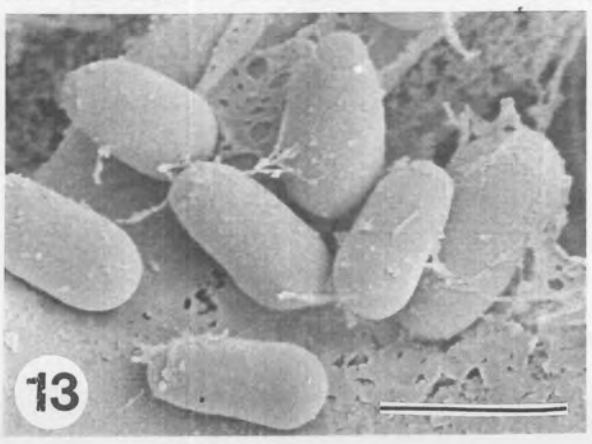


Fig. 14. *Leptographium pityophilum* (CMW 2892). A. Habit sketch of the conidiophore (Bar = 20 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).

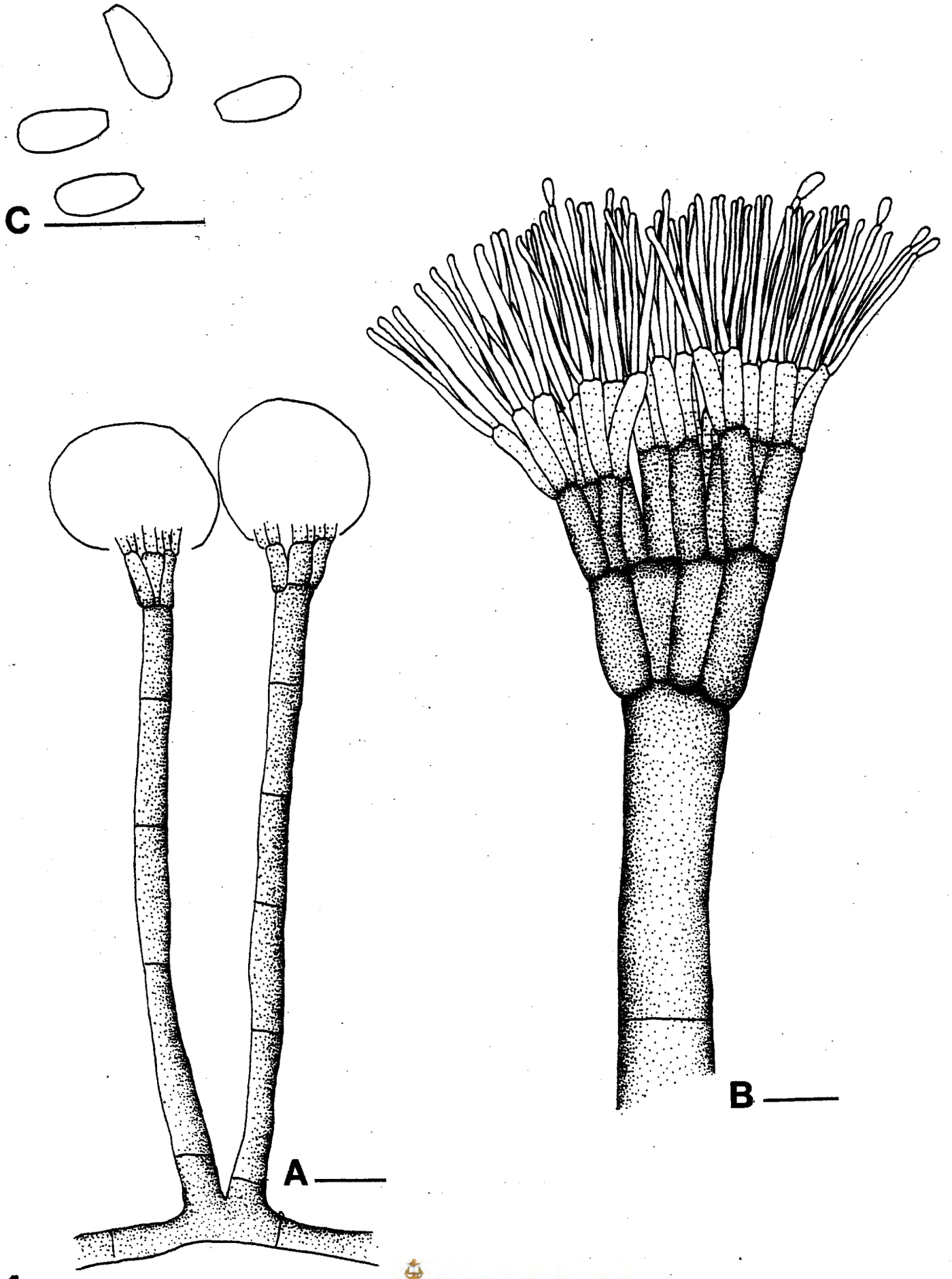
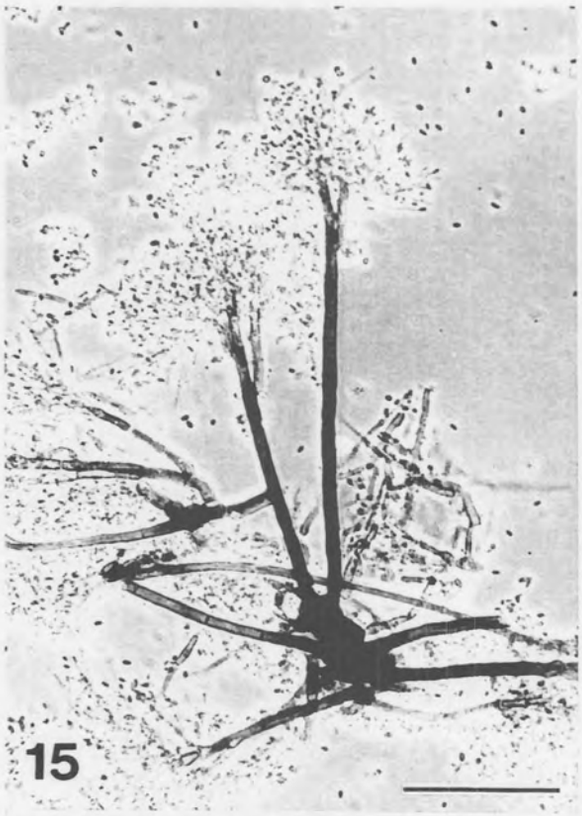


Fig. 15 - 20. *Leptographium euphyes* (CMW 259). **Fig. 15.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 µm). **Fig. 16.** Complex conidiogenous apparatus (Bar = 20 µm). **Fig. 17.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 µm). **Fig. 18.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 5 µm). **Fig. 19.** Conidia (Bar = 10 µm). **Fig. 20.** Conidia (Bar = 1 µm).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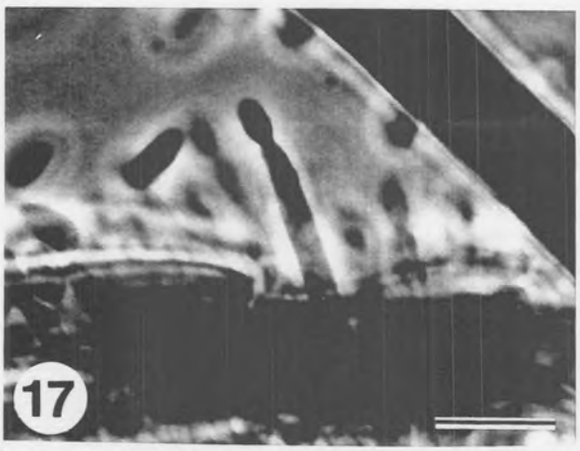
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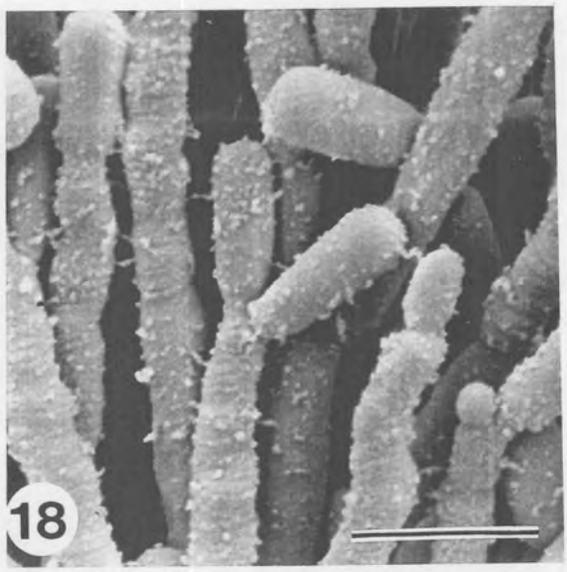
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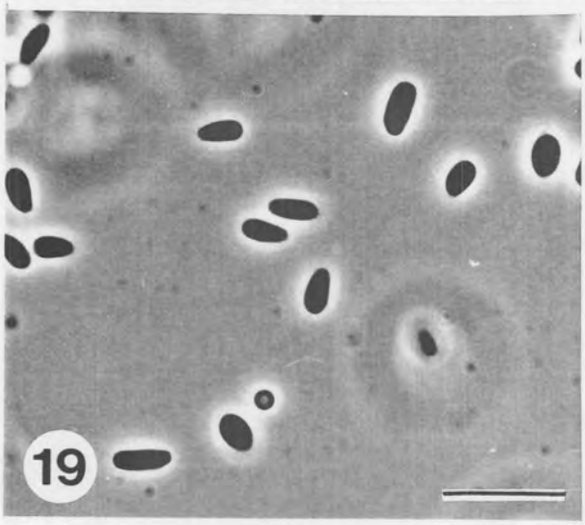
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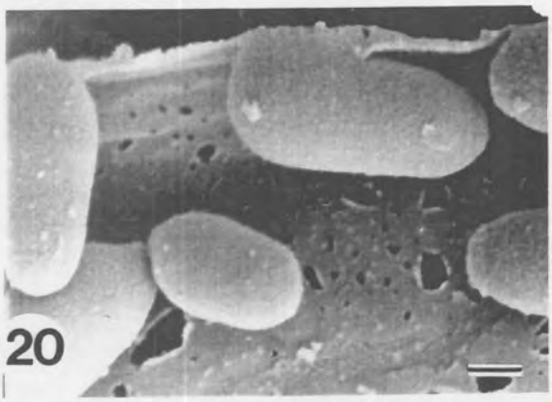
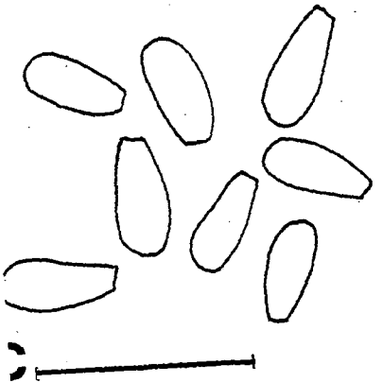
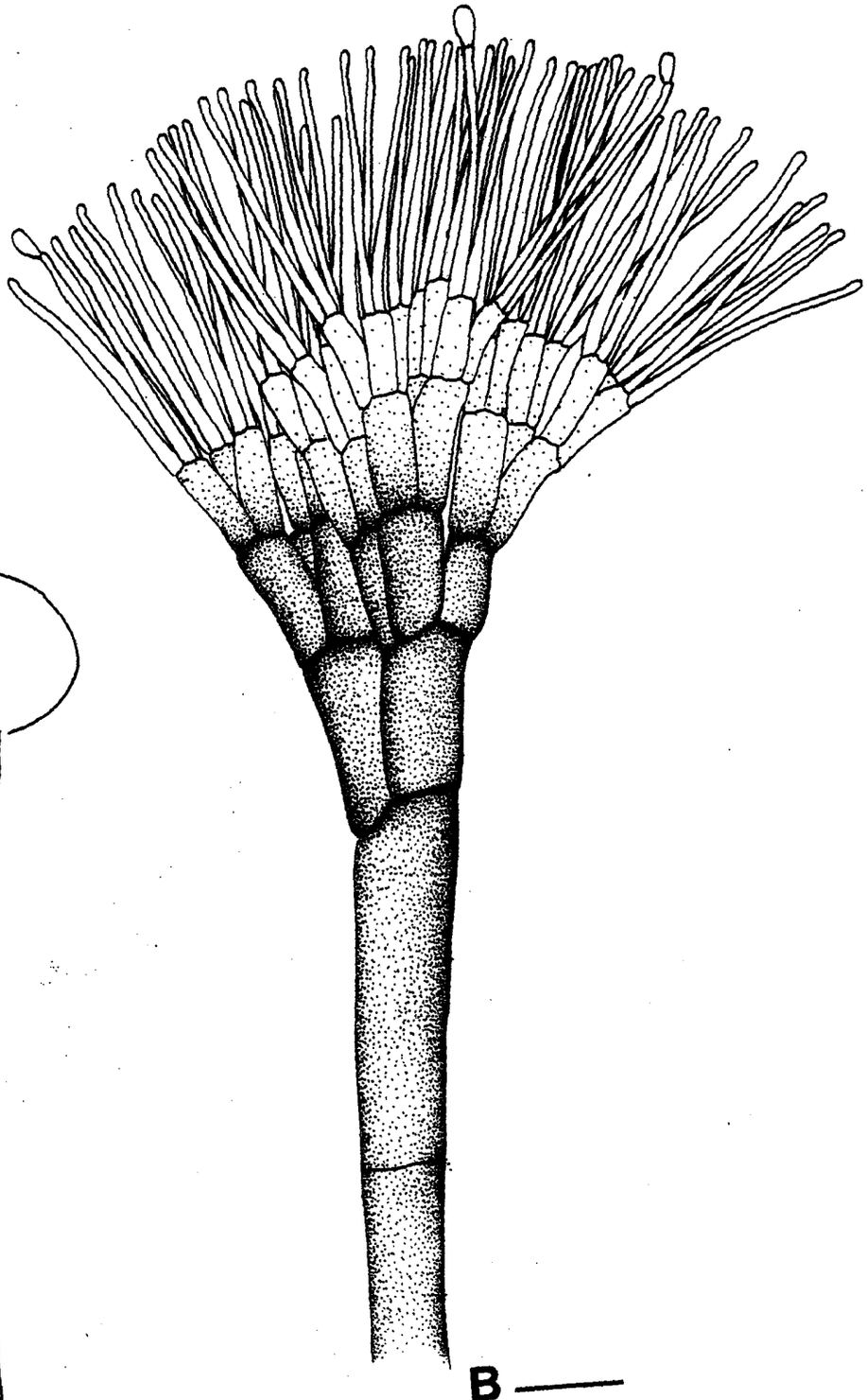
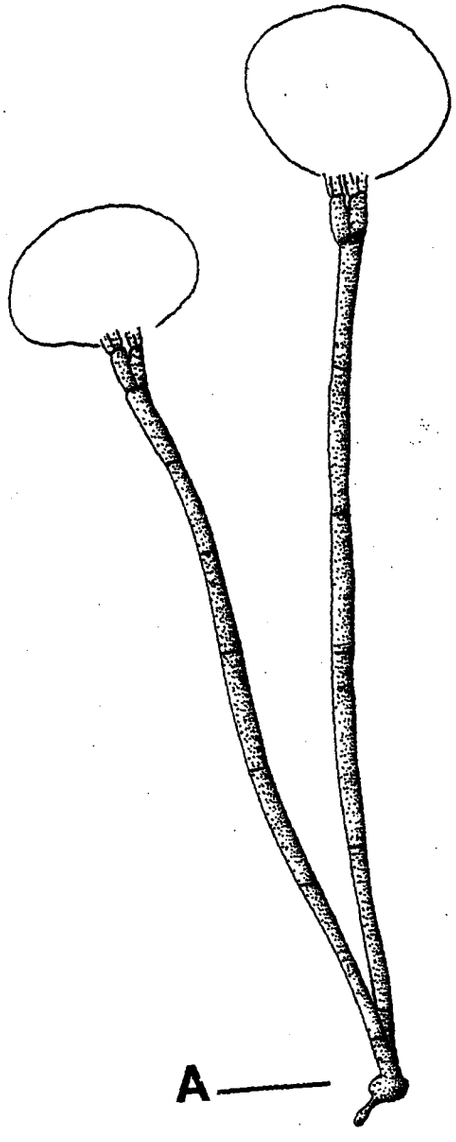

20

Fig. 21. *Leptographium euphyes* (CMW 259). A. Habit sketch of the conidiophore (Bar = 50 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).







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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).

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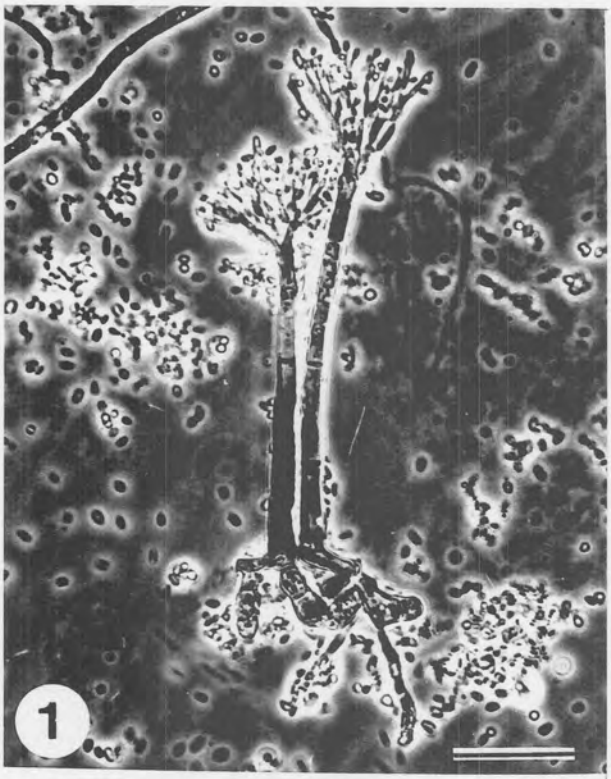
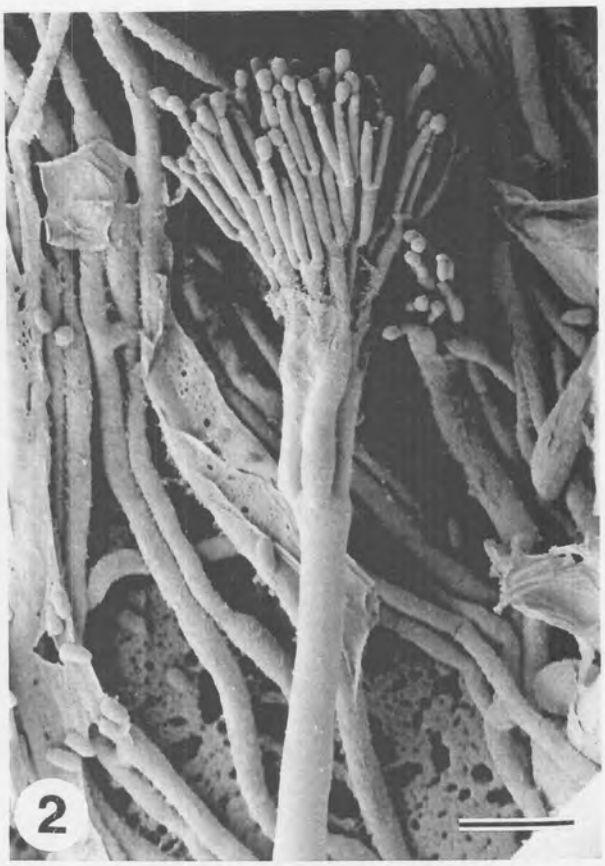
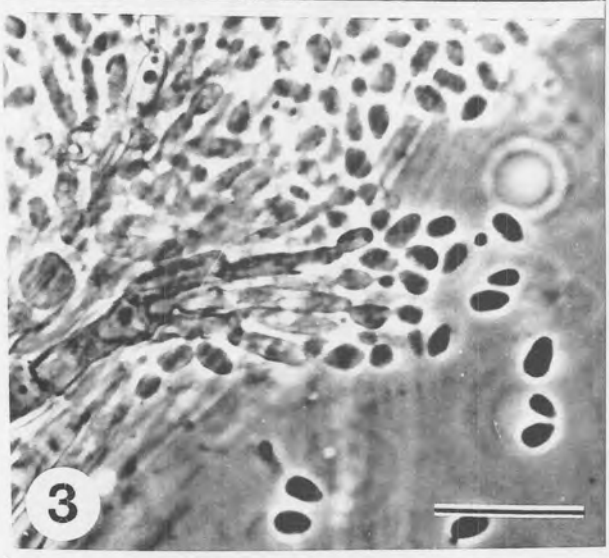
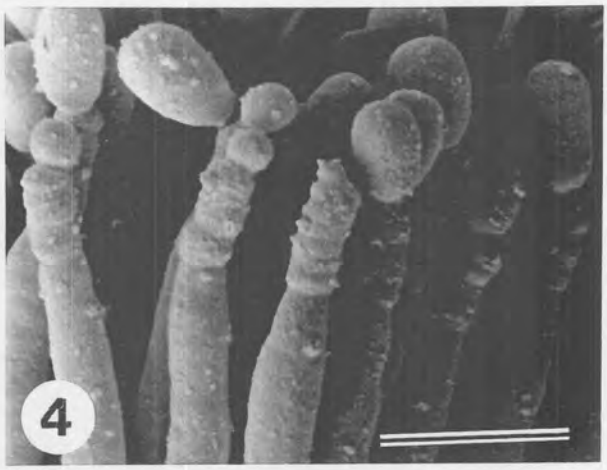
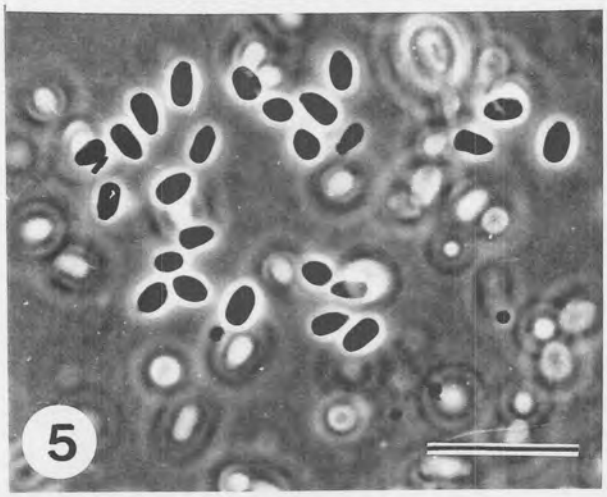
Fig. 1 - 6. *Leptographium pineti* (PREM 56391). **Fig. 1.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 µm). **Fig. 2.** Complex conidiogenous apparatus (Bar = 10 µm). **Fig. 3.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 µm). **Fig.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











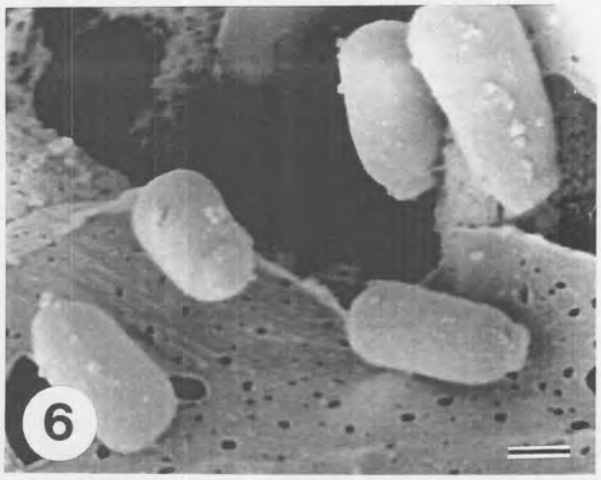
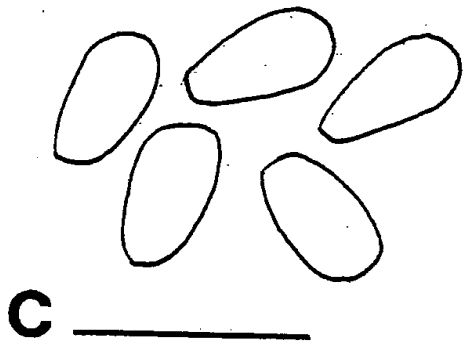


Fig. 7. *Leptographium pineti* (PREM 56391). A. Habit sketch of the conidiophore (Bar = 20 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).



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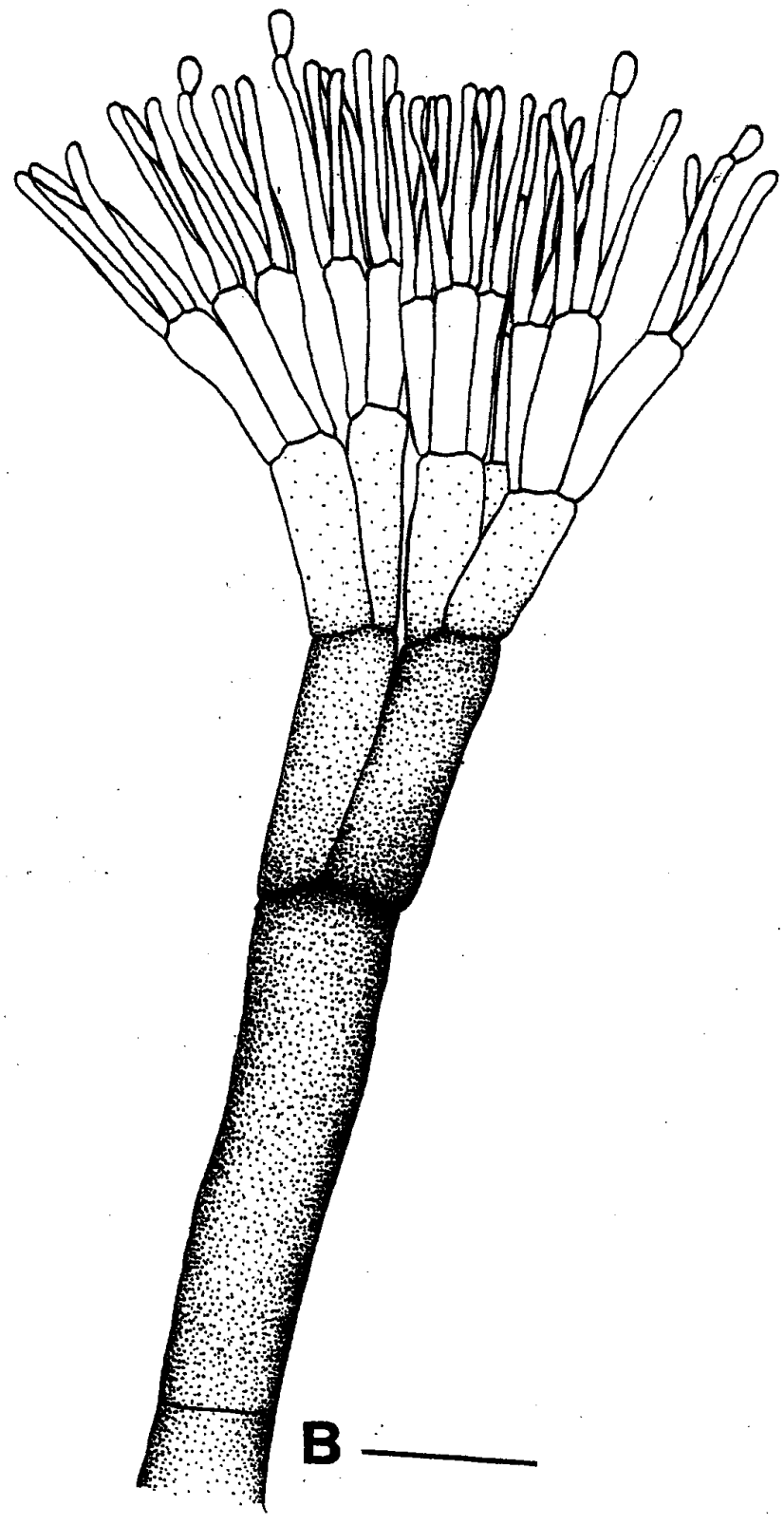

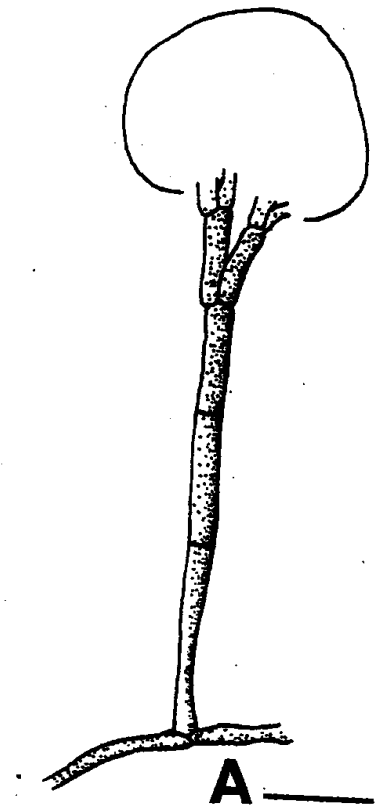

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B

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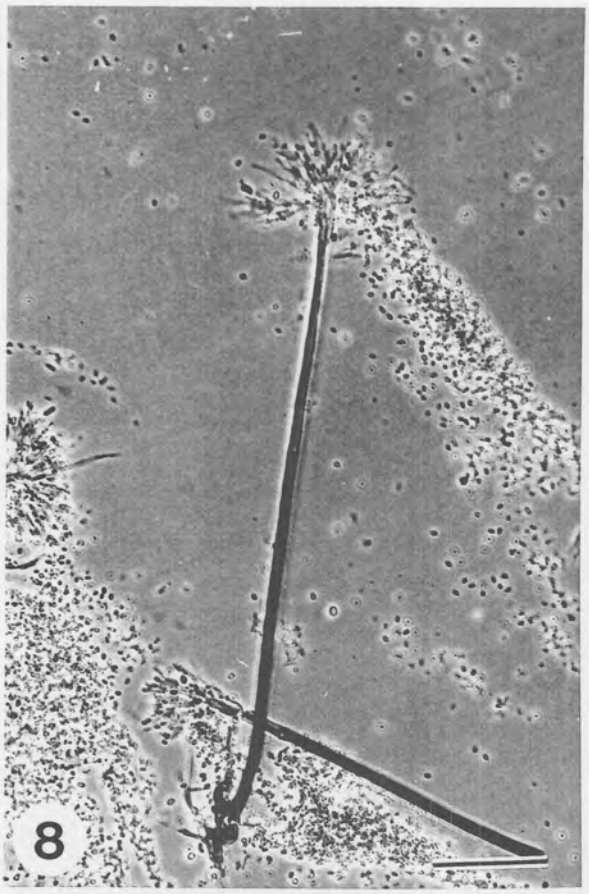
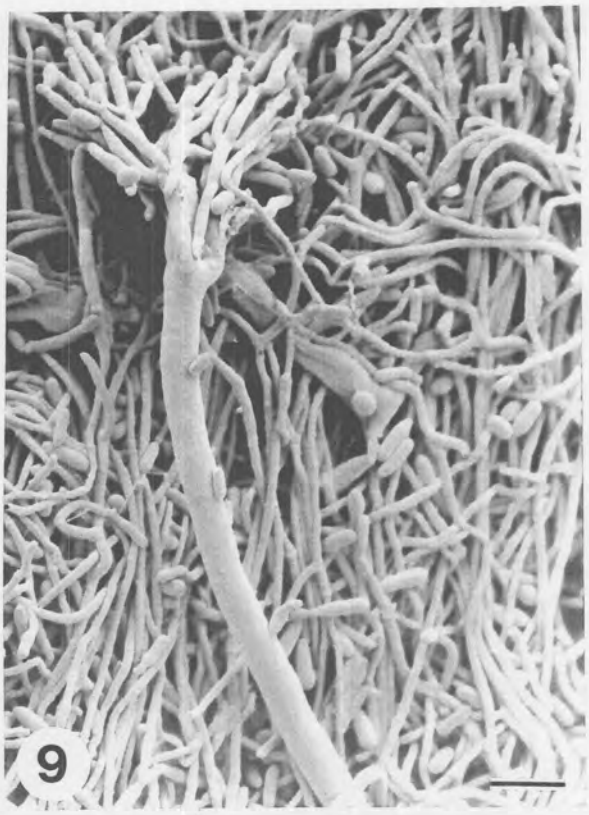
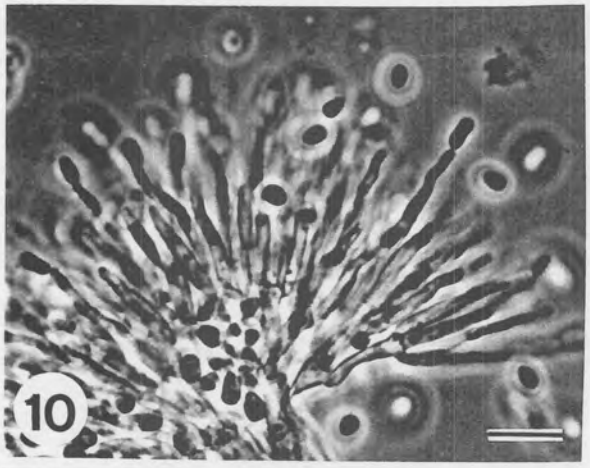
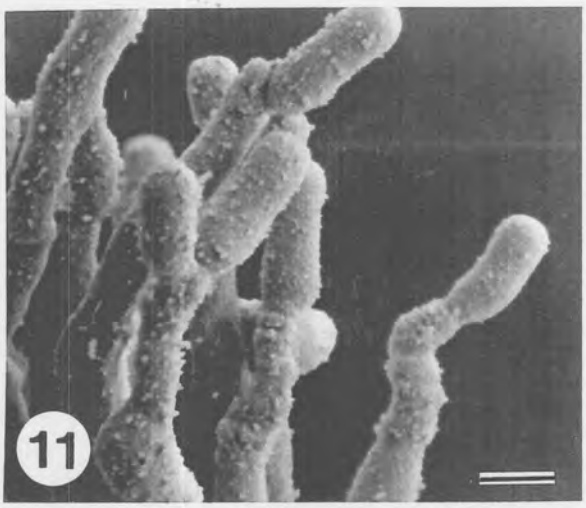
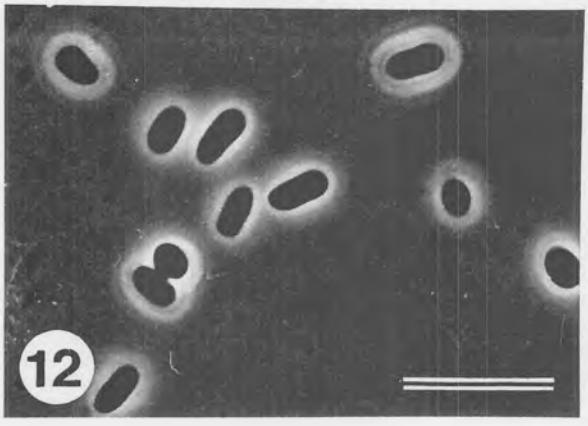
Fig. 8 - 13. *Leptographium abicolens* (CMW 2865). **Fig. 8.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 µm). **Fig. 9.** Complex conidiogenous apparatus (Bar = 10 µm). **Fig. 10.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 µm).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











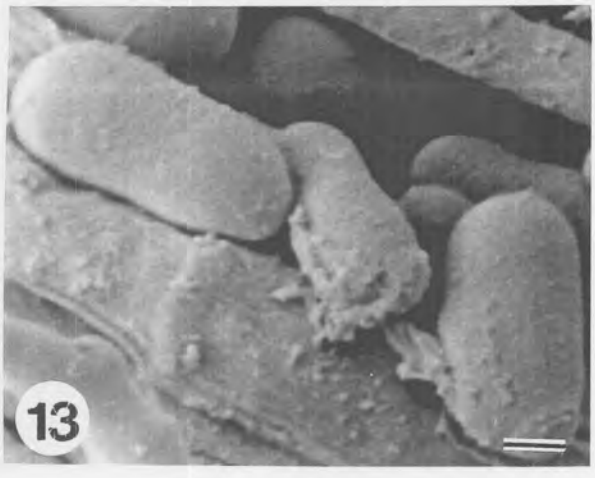


Fig. 14. *Leptographium abicolens* (CMW 2865). A. Habit sketch of the conidiophore

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

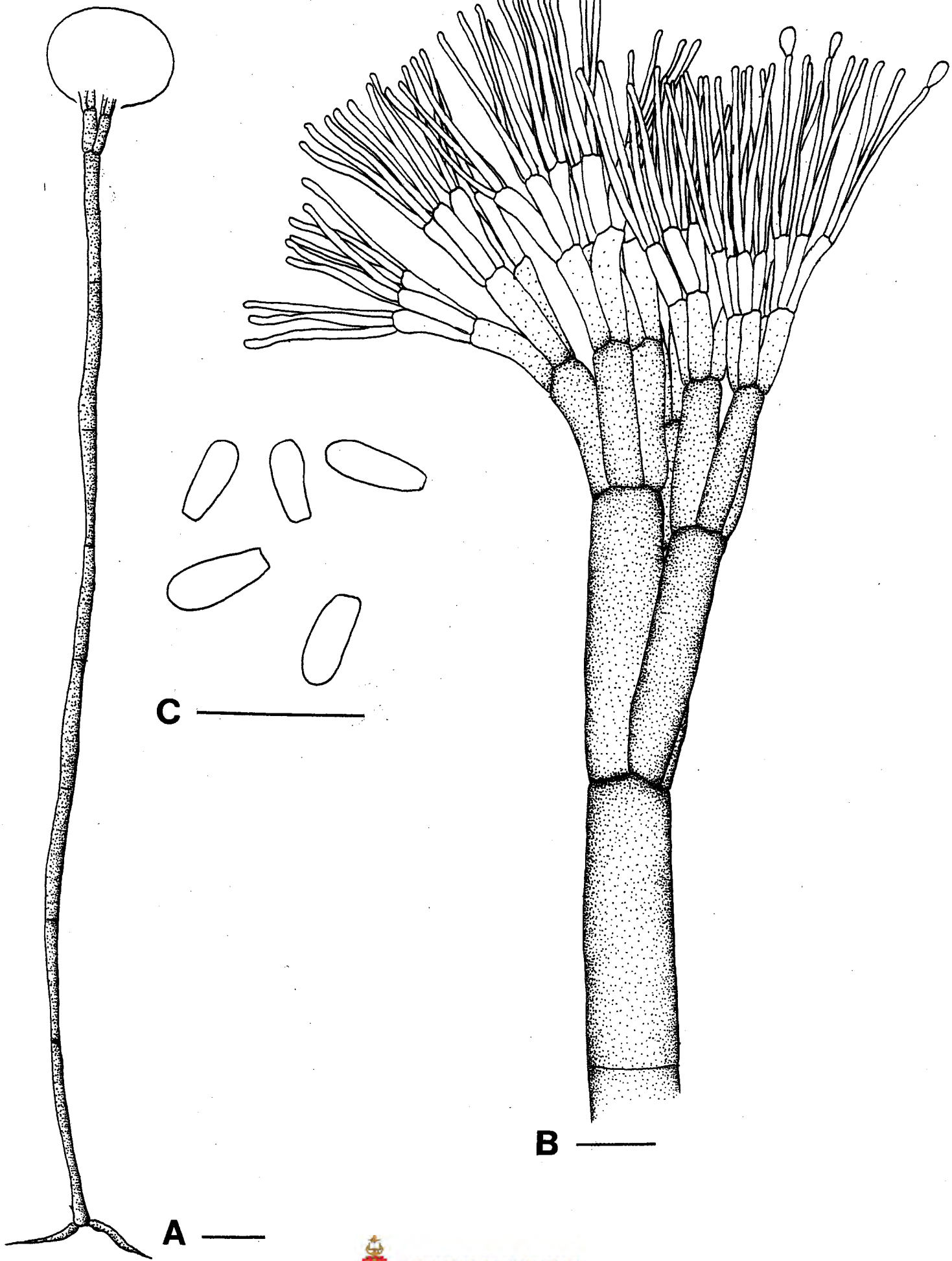


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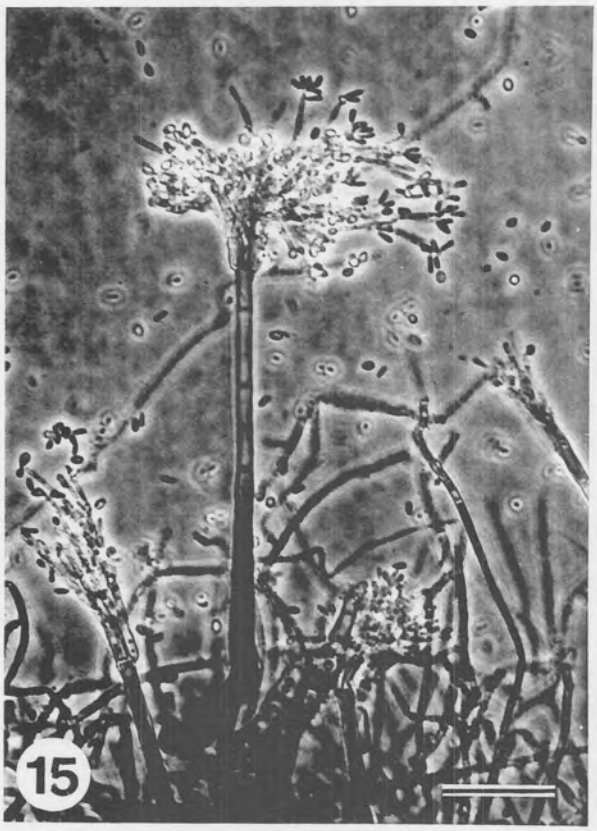
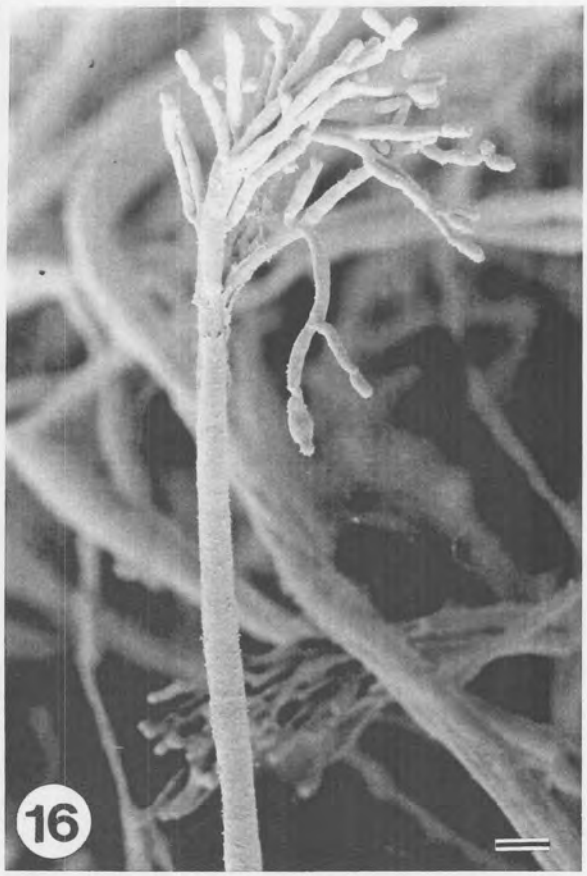
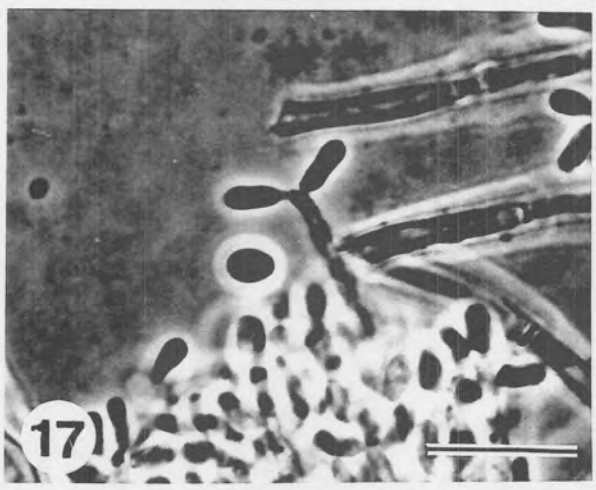
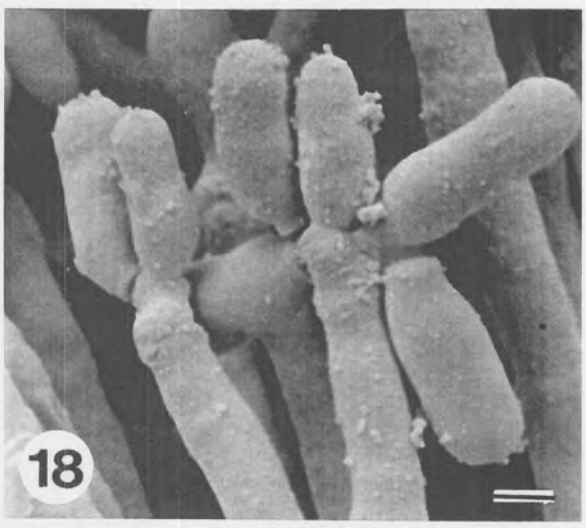
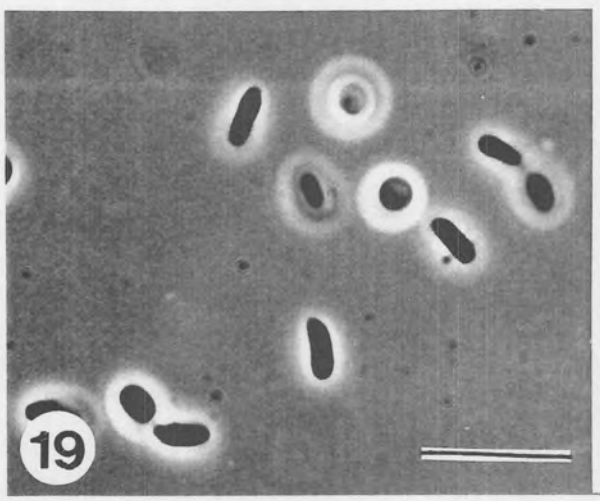
Fig. 15 - 20. *Leptographium peucophilum* (CMW 2876). **Fig. 15.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 50 µm). **Fig. 16.** Complex conidiogenous apparatus (Bar = 10 µm). **Fig. 17.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10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











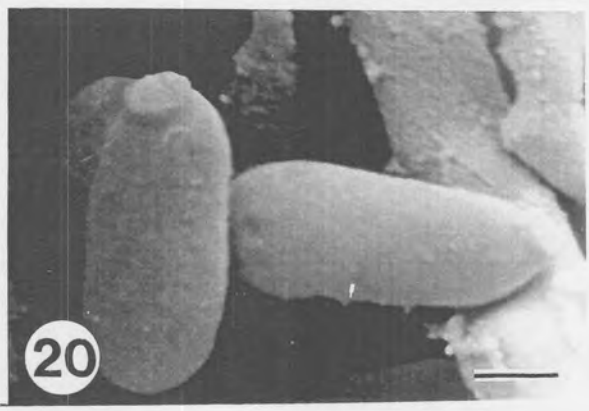
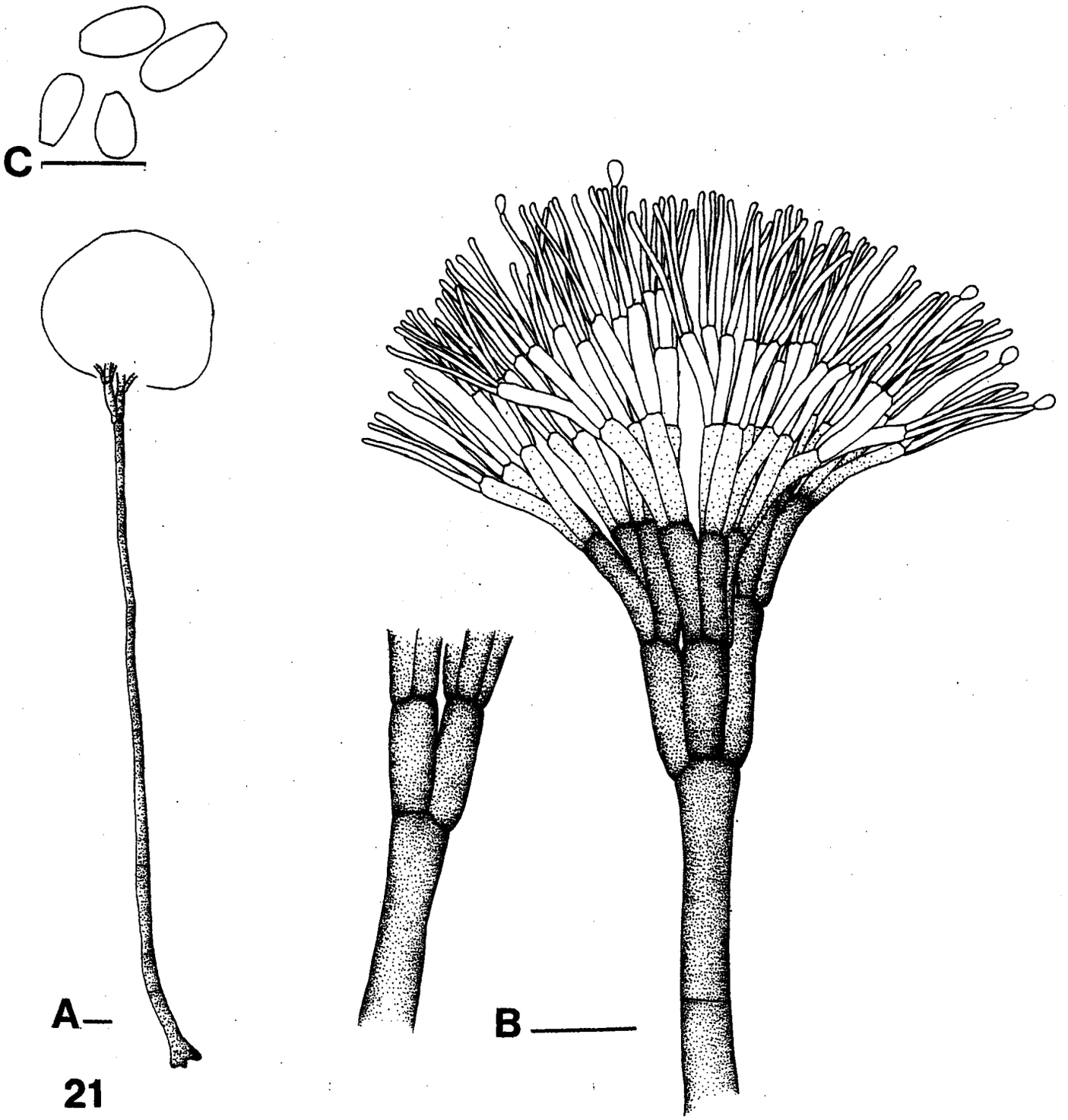


Fig. 21. *Leptographium peucophilum* (CMW 2876). A. Habit sketch of the conidiophore (Bar = 10 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 5 µm).



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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.

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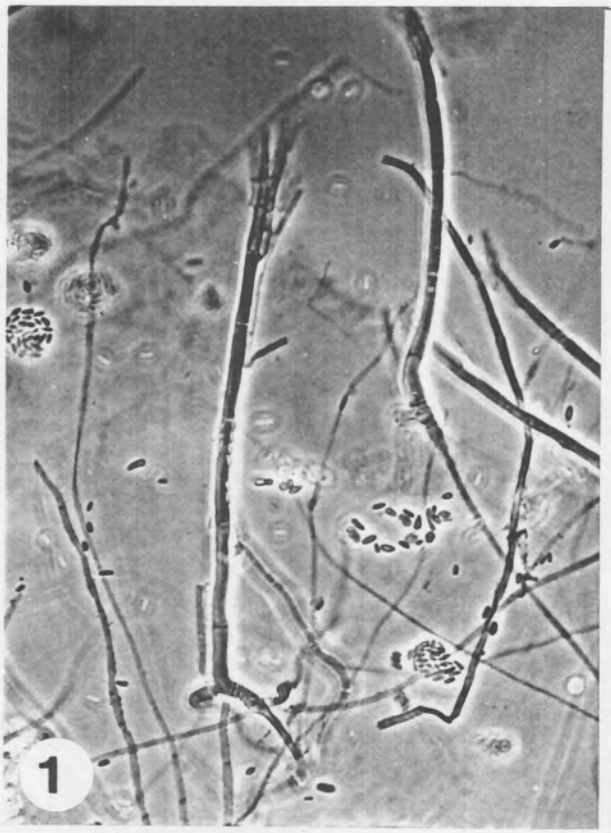
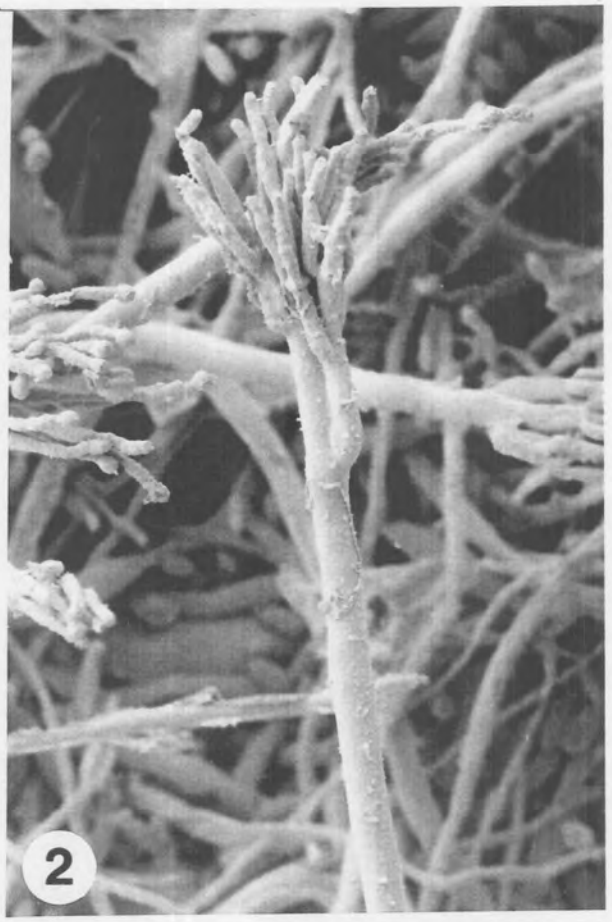
Table 1. Characteristics of *L. sibiricum* compared with those of morphologically similar species.

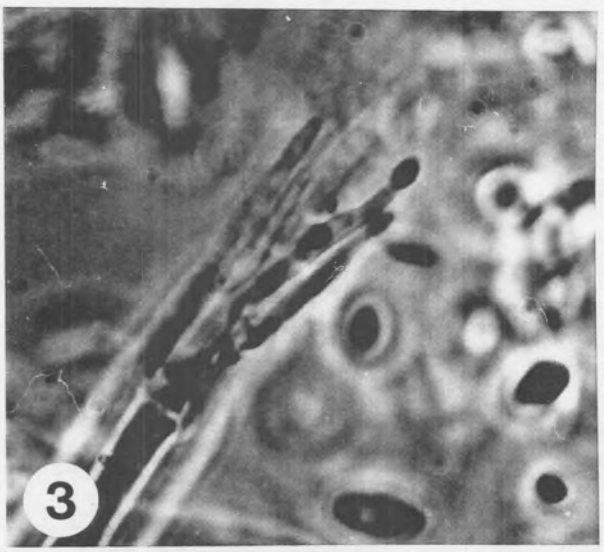
	L. sibiricum	***L. antibioticum***	***L. brachiatum***	***L. elegans***	***O. grandifoliae***	***O. leptographioides***
Substrate	*Abies sibirica*	*Pinus contorta, P. monticola, Abies lasiocarpa, Thuja plicata*[1]	*Psuedostuga menziesii, Picea mariana*[2]	*Chamaecyparis formosensis*[3]	*Fagus grandifolae*[4]	*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

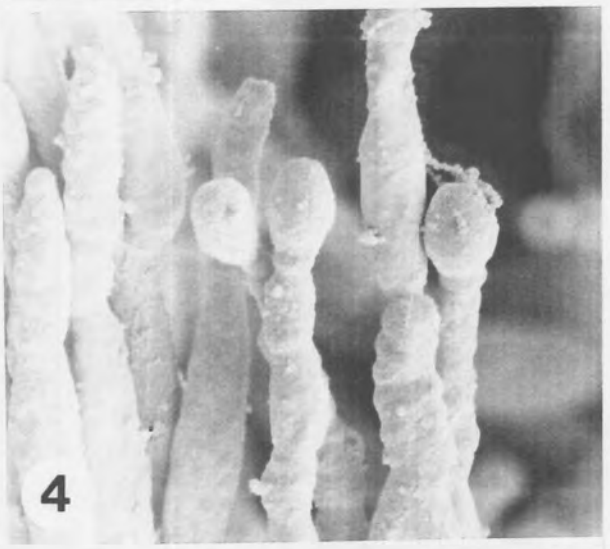
1. Kendrick, 1962; Mielke, 1979; Harrington, 1988. 2. Kendrick, 1962. 3. Wingfield, Crous & Tzean, 1994. 4. Davidson, 1976. 5. Davidson, 1942.

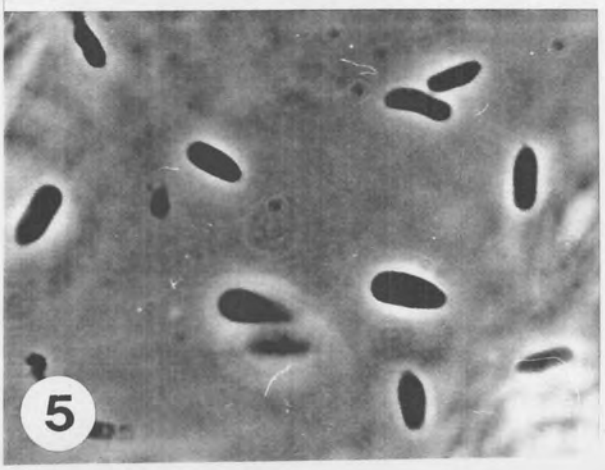
Fig. 1 - 6. *Leptographium sibiricum* (CMW 4484). **Fig. 1.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 10 µm). **Fig. 2.** Complex conidiogenous apparatus (Bar = 10 µm). **Fig. 3.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 10 µm). **Fig.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











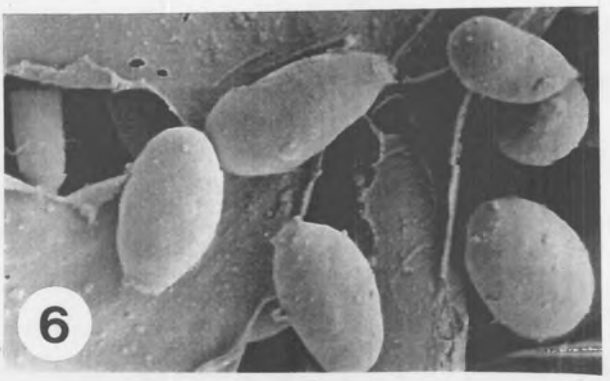
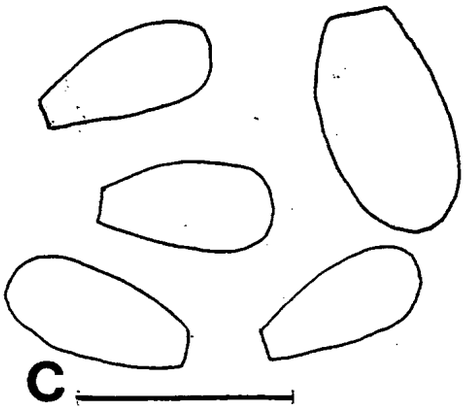
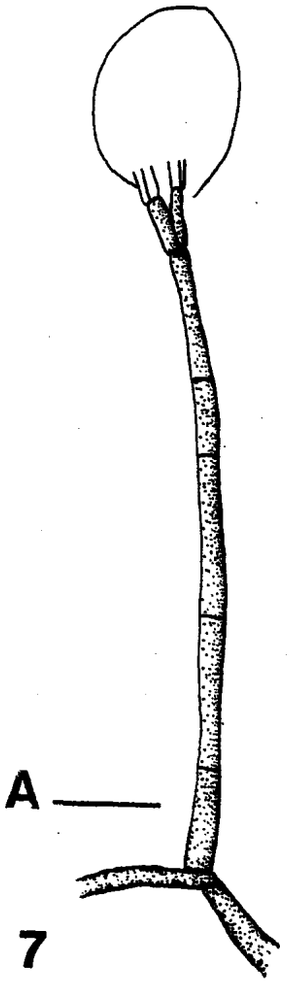
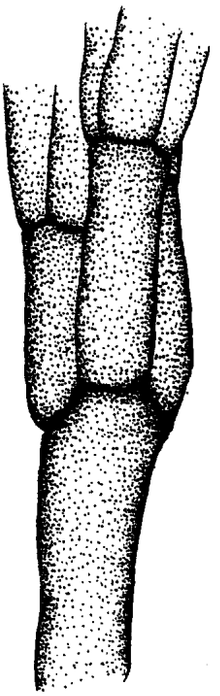
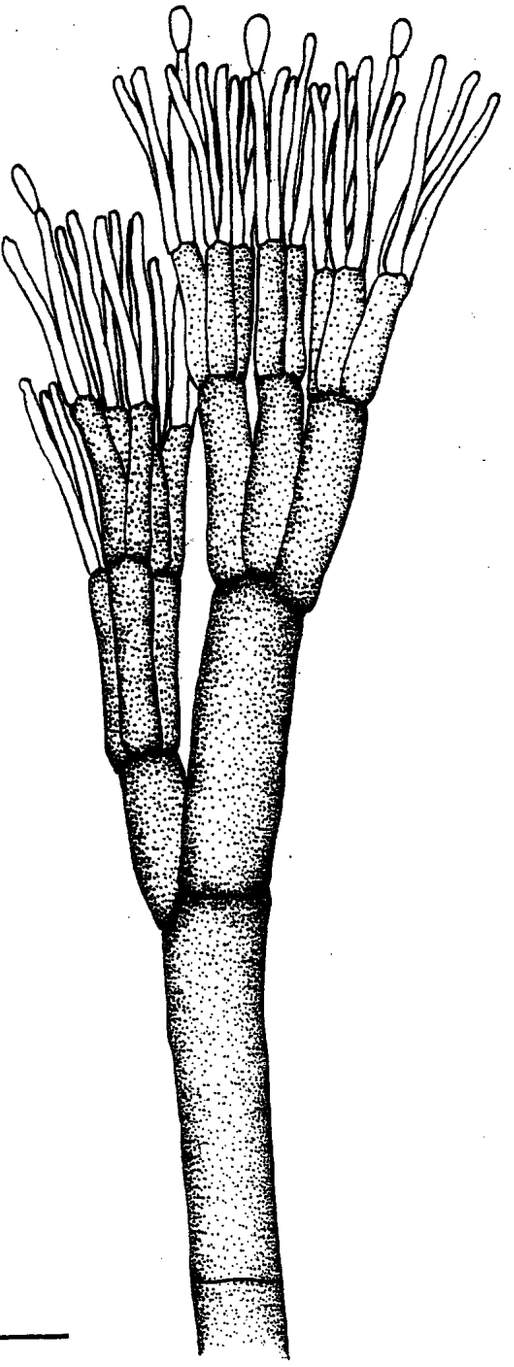


Fig. 7. *Leptographium sibiricum* (CMW 4484). A. Habit sketch of the conidiophore (Bar = 50 µm). B. Conidiogenous apparatus (Bar = 10 µm). C. Conidia (Bar = 10 µm).









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