

Three new *Leptographium* species associated with conifer roots in the United States

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Leptographium species are most commonly known as anamorphs of *Ophiostoma* and are usually associated with insects that infest trees. Three new species of *Leptographium* were isolated from conifer roots in various parts of the United States. These three species differ from described species both morphologically and on the basis of their allozyme banding patterns. *Leptographium albopini* occurs both in the eastern and western United States on white pine hosts, while *Leptographium douglasii* occurs commonly in the western United States and has been found only on *Pseudotsuga menziesii*. *Leptographium neomexicanus* occurs in the southwestern United States and has thus far only been collected from *Pinus ponderosa*.

Key words: *Leptographium albopini*, *Leptographium douglasii*, *Leptographium neomexicanus*, *Pinus*, *Pseudotsuga*, systematics, root infesting insects.

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Les espèces de *Leptographium* sont plus généralement connues par leurs anamorphes du genre *Ophiostoma* et sont généralement associées à des insectes qui vivent dans les arbres. Les auteurs ont isolé trois nouvelles espèces de *Leptographium* à partir de racines de conifères dans diverses parties des États-Unis. Ces trois espèces diffèrent des espèces déjà décrites, à la fois par leurs caractères morphologiques ainsi que par leurs patrons isozymiques. Le *Leptographium albopini* se retrouve aussi bien à l'est qu'à l'ouest des États-Unis sur le pin blanc, alors que le *Leptographium douglasii* se retrouve dans l'ouest des États-Unis et n'a été récolté que sur le *Pseudotsuga menziesii*. Le *Leptographium neomexicanum* se retrouve dans le sud-ouest des États-Unis et n'a été récolté jusqu'ici que sur le *Pinus ponderosa*.

Mots clés : *Leptographium albopini*, *Leptographium douglasii*, *Leptographium neomexicanus*, *Pinus*, *Pseudotsuga*, systématique, insectes colonisateurs des racines.

[Traduit par la rédaction]

Introduction

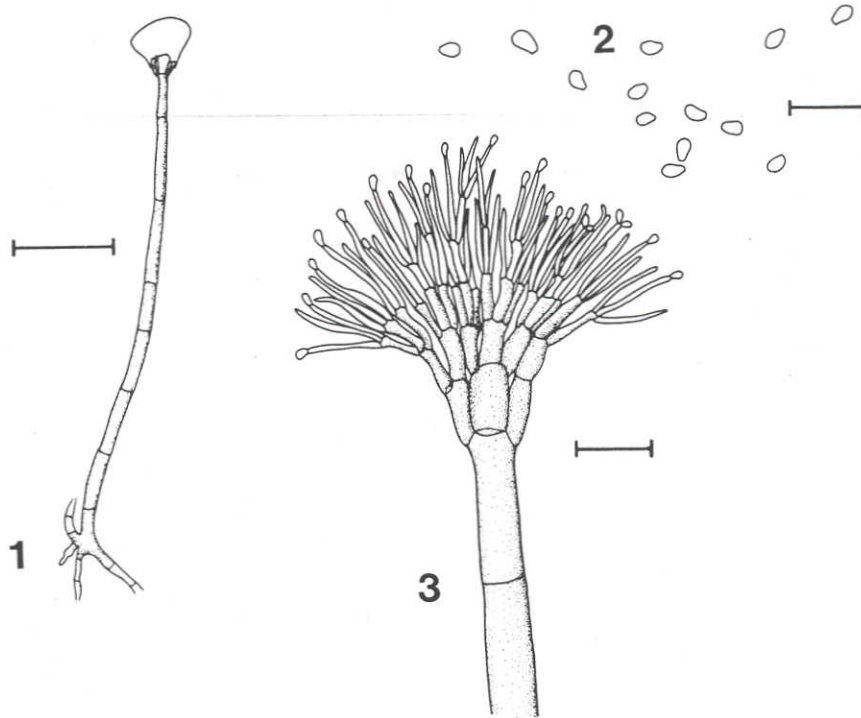
Species of *Leptographium* Lagerberg & Melin are dematiaceous Hyphomycetes characterized by dark, robust mononematous and macronematous conidiophores that terminate in a penicillately branched conidiogenous apparatus. Conidia are hyaline, aseptate, and produced in a slimy matrix through holoblastic extension of conidiogenous cells (Hughes 1953; Kendrick 1961, 1962; Wingfield 1993). Conidiation results in the accumulation of slimy conidial masses at the apices of conidiophores to facilitate insect dispersal (Wingfield 1993). Many species of *Leptographium* are anamorphs of *Ophiostoma* Sydow & Sydow (Upadhyay 1981; Wingfield 1985; Harrington 1987, 1988) and therefore are also characterized by being tolerant to high concentrations of cycloheximide in culture (Harrington 1981) and having rhamnose in their cells (Jewell 1974; Weijman and De Hoog 1975; Horner et al. 1986). These fungi are adapted to insect dispersal and commonly associated with scolytid bark beetles (Coleoptera: Scolytidae), many of which infest conifers (De Hoog and Scheffer 1984; Harrington and Cobb 1983; Harrington 1988, 1993a, 1993b). A number of species have been associated with root diseases of conifers (Hunt and Morrison 1979;

Mielke 1981; Harrington and Cobb 1983, 1987; Harrington 1993b), and *Leptographium wagneri* (Kendr.) Wingf. is the causal agent of the serious malady commonly known as black stain root disease in the western United States (Harrington 1993b).

Although generic circumscription of *Leptographium* is now reasonably well defined, species concepts are still clouded (Harrington 1988). Delimitation of species within the *Leptographium* complex is difficult, as there are only a few taxonomic criteria that can be used, and these may change with prolonged storage and subculturing (Zambino and Harrington 1992). Furthermore, many described species are poorly known. Indeed, even the type species *Leptographium lundbergii* Lag. & Melin lacks type material and requires further clarification (Wingfield and Gibbs 1991).

Harrington (1988) expressed the opinion that based on his collections over an extended time period, many species of *Leptographium* have yet to be described. Indeed, three undescribed species have commonly been collected in the United States in recent years. These three species have been subject to both morphological scrutiny as well as detailed comparisons based on allozyme analyses (Zambino and Harrington 1992) supporting the contention that they are new. The aim of this paper is to provide descriptions and names for these three *Leptographium* spp. associated with conifer roots.

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FIGS. 1–3. Conidiophores and conidia of *Leptographium neomexicanus*. Fig. 1. Mononematous, macronematous conidiophore with rhizoids at the base. Scale bar = 75 μm . Fig. 2. Oblong to obpyriform conidia. Scale bar = 15 μm . Fig. 3. Conidiogenous apparatus showing series of metulae terminating in conidiogenous cells. Scale bar = 15 μm .

TABLE 1. Comparison of the extent of growth of *L. albopini*, *L. douglasii*, and *L. neomexicanus* after 4 days at 25°C on malt extract agar emended with various concentrations of cycloheximide

Fungus	Concn. of cycloheximide (g/L)						
	0	0.005	0.1	0.5	1.0	2.5	5.0
<i>L. albopini</i>	32.0	28.6	27.8	24.7	21.5	15.4	11.4
<i>L. douglasii</i>	16.6	18.5	15.8	13.5	11.6	8.8	6.6
<i>L. neomexicanus</i>	65.4	62.9	60.6	50.7	47.2	24.9	16.0

NOTE: Measurements (mm) represent averages of colony diameter derived from six readings from three plates.

Materials and methods

Descriptions were based on cultures of the holotypes after growth at 25°C on 2% malt extract agar (MEA) (20 g Merck agar plus 20 g Merck malt extract per 1000 mL water) for approximately 3 weeks.

Growth rates were compared on MEA in Petri dishes after 4 days in the dark at temperatures ranging from 10 to 35°C in 5° intervals. Colony diameters were measured twice perpendicular to each other and growth rates were taken as an average of two readings on each of three Petri dishes.

Growth rates on various concentrations (0, 0.05, 0.1, 0.5, 1.0, 2.5, and 5.0 g/L) of cycloheximide added to 2% MEA were compared. Comparisons were made in Petri dishes at 25°C in the dark. Growth on cycloheximide emended MEA was determined on the basis of colony diameter by taking the average of two measurements from each of three Petri dishes after 4 days of growth.

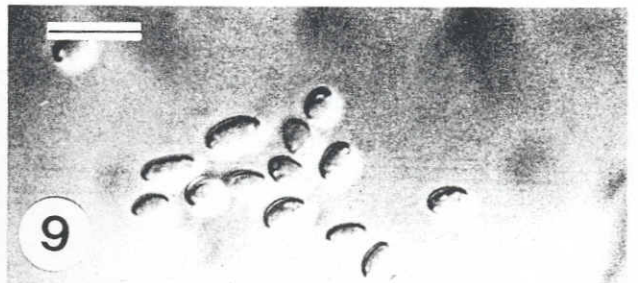
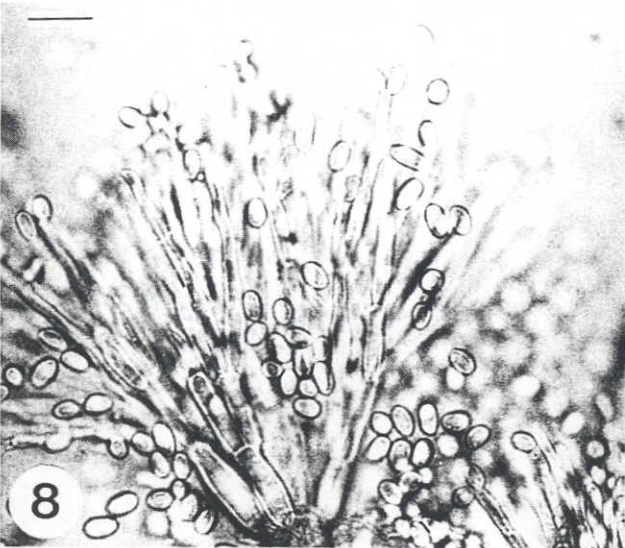
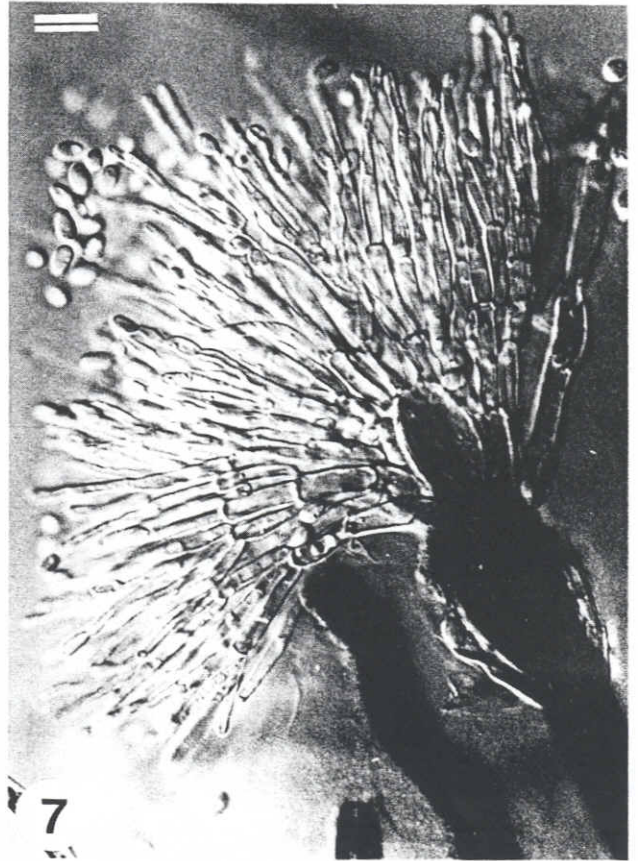
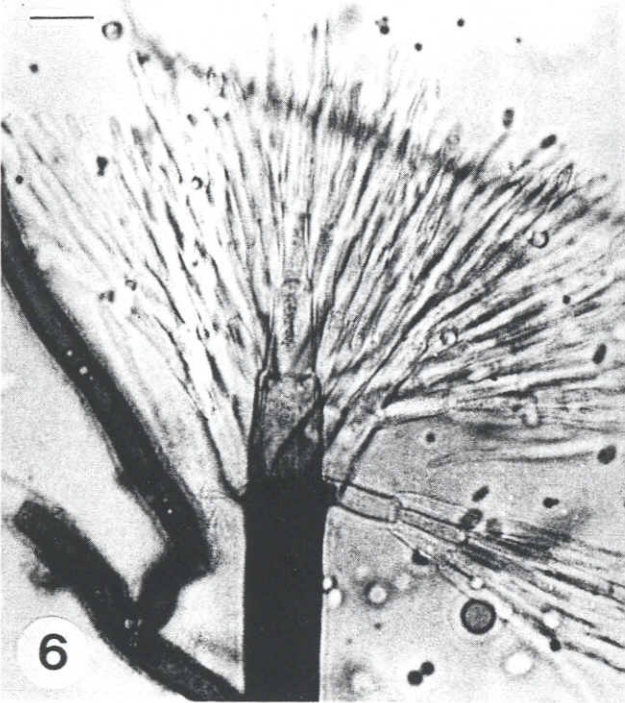
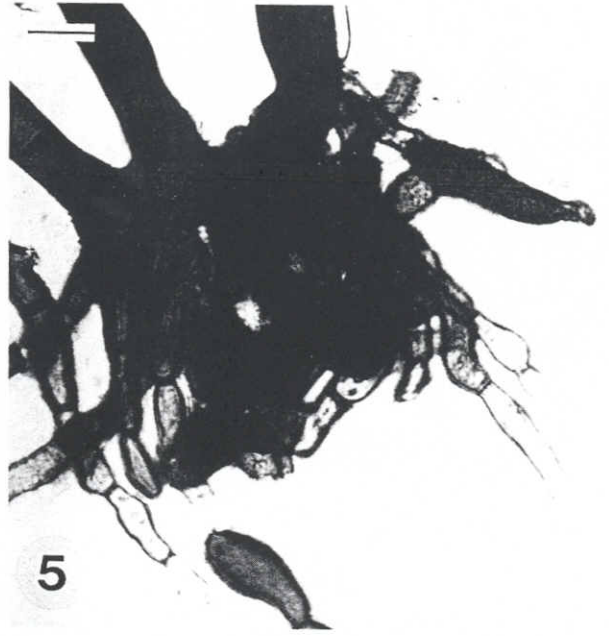
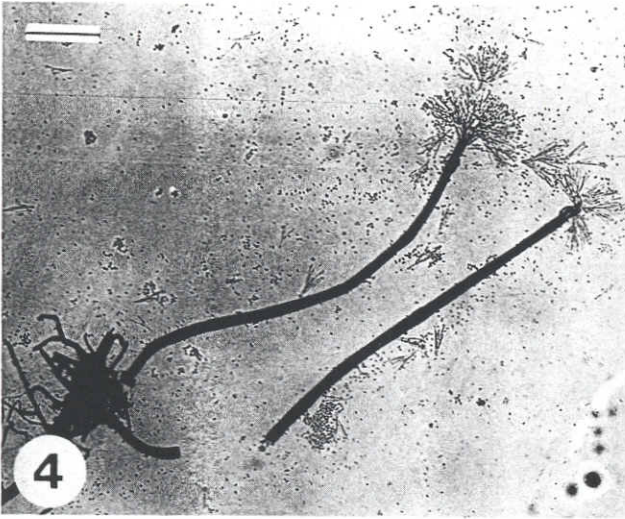
The three fungi were also examined using scanning electron microscopy to ascertain the mode of conidium development. Sporulating colonies on agar disks were fixed in 2.5% glutaraldehyde and 1.5% osmium tetroxide in a 0.1 M phosphate buffer, dehydrated in a graded acetone series, and critical point dried. Specimens were coated with gold-palladium and examined using a JSM 6400 scanning electron microscope.

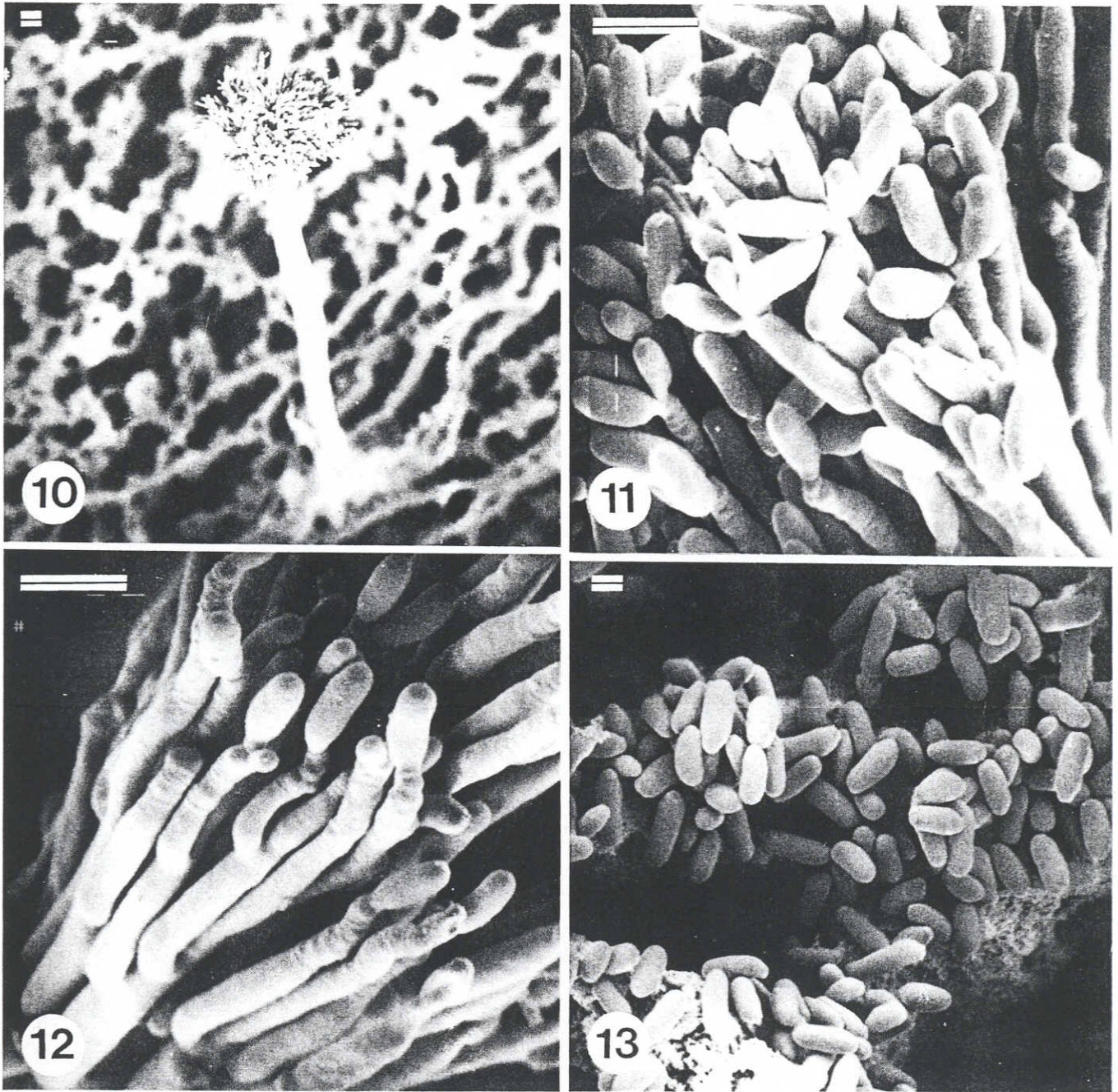
New species

Leptographium neomexicanus Wingfield, Harrington et Crous
sp. nov. Figs. 1–13

Coloniae primum hyalinae ad margines atro-olivacescentes in MEA dum senescent. Hyphae immersae in medio etsi mycelium sparsum aerium quoque adest, atro-olivaceae parietibus distincte asperis, 2–15 μm diametro. Conidiophora plerumque solitaria sed aliquando bina trinaque aggregata, macronemaosa, mononematosa, rhizoideis plerumque praesentibus bene evolutisque ad basim. Stipes erectus, olivaceus paulum pallescens ad apicem, 205–621 (\bar{x} = 395) μm longus 9–16 (\bar{x} = 13) μm latus ad basem paulumque tumidus ad apicem. Apparatus conidiogenus 50–84 (\bar{x} = 59 μm) longus massa conidica exclusa constans ex tribus usque quinque seriebus ramorum. Tres usque sex rami primae saepe ramus distinctus centralis paulo maiore quam ceteris, pallidiores colore, 11–30.5 (\bar{x} = 14) μm longae, 4–10 (\bar{x} = 6) μm lati. Cellae conidiogenae discretae, hyalinae, attenuatae a basi ad apicem, 12–20.5 (\bar{x} = 14) μm longae, 1.5–3.5 (\bar{x} = 2) μm latae. Auctus conidii constructio parietum ad restituendum ontogenie

FIGS. 4–9. Conidiophores, conidiogenous apparatus, conidiogenous cells, and conidia of *Leptographium neomexicanus*. Fig. 4. Mononematous, macronematous conidiophores. Scale bar = 70 μm . Fig. 5. Rhizoids at the base of conidiophores. Scale bar = 10 μm . Figs. 6 and 7. Conidiogenous apparatus showing arrangement of metulae. Scale bars = 10 μm . Fig. 8. Conidiogenous cells showing apparent sympodial proliferation. Scale bar = 10 μm . Fig. 9. Oblong to obpyriform conidia. Scale bar = 10 μm .





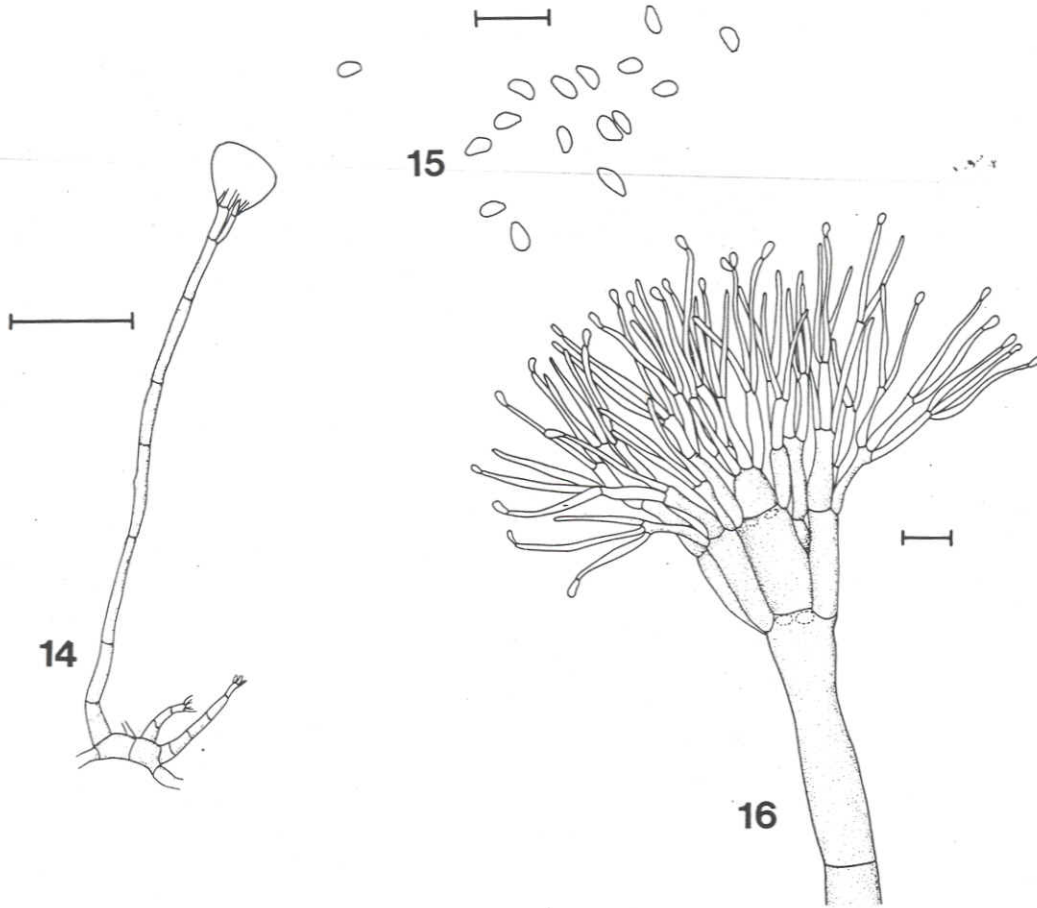
FIGS. 10–13. Conidiophore, conidiogenous cells, and conidia of *Leptographium neomexicanus* as seen using scanning electron microscopy. Fig. 10. Mononematous, macronematous conidiophore. Scale bar = 9 μm . Fig. 11. Conidiogenous cells with conidia appearing to have developed sympodially. Scale bar = 5 μm . Fig. 12. Conidiogenous cells showing distinct annellations. Scale bar = 5 μm . Fig. 13. Oblong to obpyriform conidia. Scale bar = 2.5 μm .

holoblastica percurrentique ontogenie, secessione retardata dante pseudo-aspectum sympodialis proliferationis. Conidia hyalina, oblonga vel obpyriformia paulumque curvata versus locum afficionis, 4.5–6.5 (\bar{x} = 5) μm longa, 2–4.5 (\bar{x} = 3) μm lata. Conidia accumulans circa apparatus conidiogenum in massa mucilaginoso quae cremicolorescit dum senescit.

HOLOTYPE: U.S.A., New Mexico, Mescalero, Pini ponderosae, T.C. Harrington, Aug. 1980, PREM 51443.

Colonies on MEA grew optimally at 25°C, reaching 65.5 mm in diameter in 4 days. Growth rate declined considerably at 20°C, and no growth occurred below 15°C or above 25°C.

The fungus was able to withstand high concentrations of cycloheximide with growth at 25°C reduced by approximately 50% on MEA containing 2.5 g/L of the antibiotic (Table 1). Colonies at first hyaline at the margins, becoming dark olivaceous, 23''''k (Rayner 1970) on MEA. Hyphae immersed in the medium, although a sparse aerial mycelium is also present, dark olivaceous with distinctly roughened walls, 2–15 μm in diameter. Conidiophores (Figs. 1, 3, 4) usually single but sometimes in groups of two or three, macronematous, mononematous with rhizoids usually present and well developed at the base (Figs. 1, 5). Stipe erect, olivaceous becoming



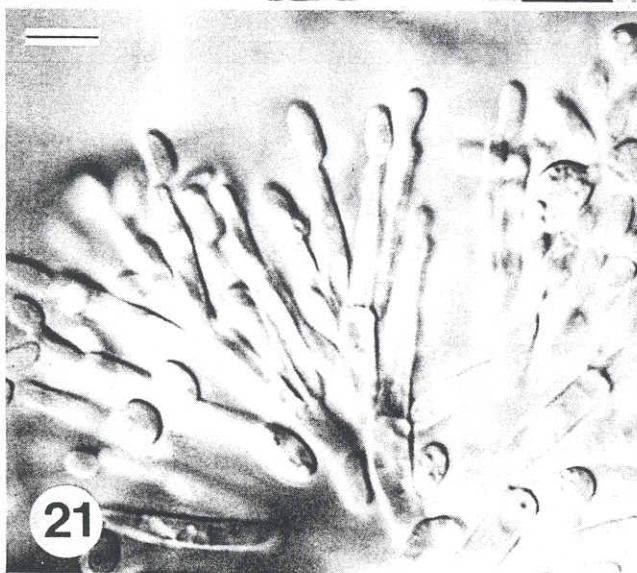
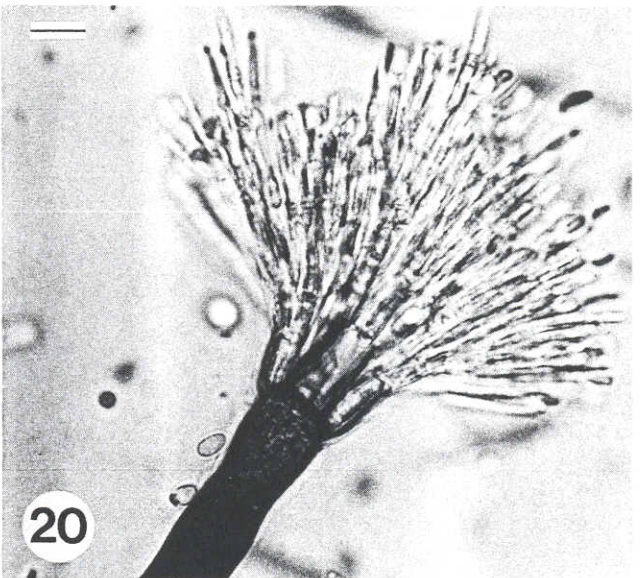
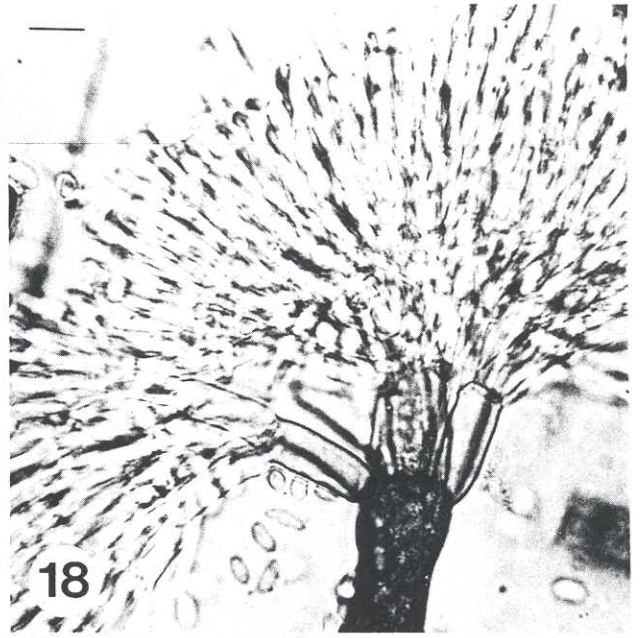
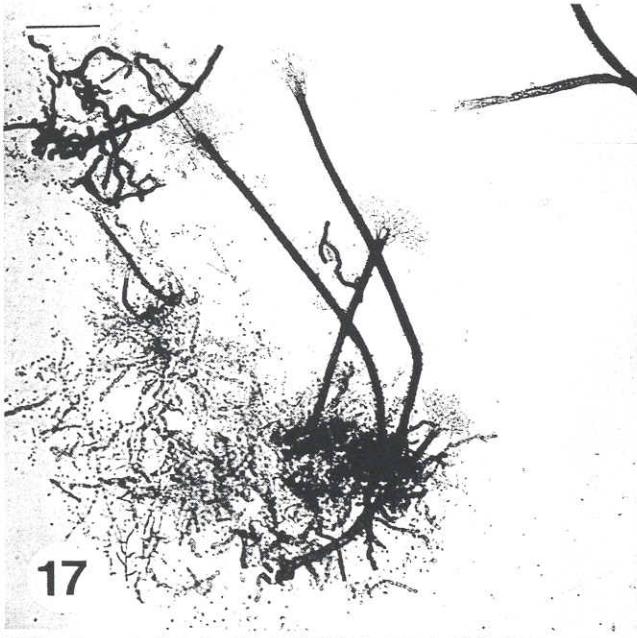
FIGS. 14–16. Conidiophores and conidia of *Leptographium douglasii*. Fig. 14. Single conidiophore. Scale bar = 80 μm . Fig. 15. Broadly ellipsoidal conidia. Scale bar = 13 μm . Fig. 16. Conidiogenous apparatus showing arrangement of metulae and conidiogenous cells. Scale bar = 10 μm .

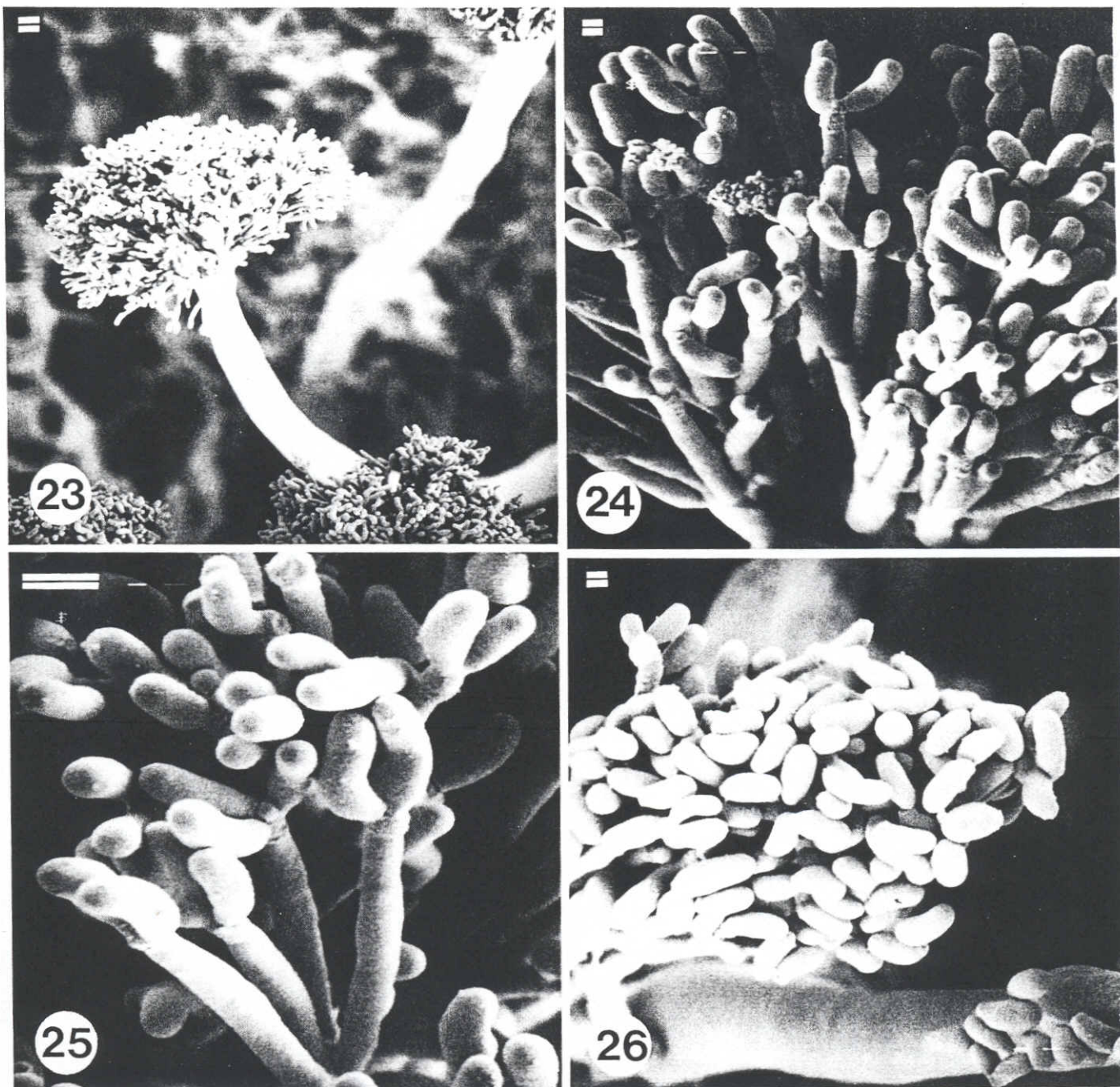
slightly lighter at the apex, 205–621 (\bar{x} = 395) μm long and 9–16 (\bar{x} = 13) μm wide at the base and slightly swollen at the apex. Conidiogenous apparatus (Figs. 3, 6–8, 12) 50–84 (\bar{x} = 59 μm) long (excluding conidial mass) consisting of between three and five series of branches. Three to six primary branches are present, often with a distinct central branch slightly larger than the others, paler in color than the stipe, 11–30.5 (\bar{x} = 14) μm long, 4–10 (\bar{x} = 6) μm wide. Conidiogenous cells (Fig. 12) discrete, hyaline, tapering from base to apex, 12–20.5 (\bar{x} = 14) μm long, 1.5–3.5 (\bar{x} = 2) μm wide. Conidium development replacement wall building with holoblastic ontogeny and percurrent ontogeny with delayed secession, giving a false appearance of sympodial proliferation. Conidia (Figs. 2, 9, 13) hyaline, oblong to obpyriform and slightly curved towards the point of attachment, 4.5–6.5 (\bar{x} = 5) μm long, 2–4.5 (\bar{x} = 3) μm wide. Conidia accumulating around the conidiogenous apparatus in a clear mucilaginous mass becoming pale yellow when dry.

CULTURES EXAMINED: U.S.A.: New Mexico: Mescalero, isolated from roots of *Pinus ponderosa*, T.C. Harrington, 1980, CMW 2079, C 32, NMP 102, ex-type culture at CBS, PREM 51443 HOLOTYPE; NEW MEXICO: Ruidoso, from roots of *Pinus ponderosa*, W.H. Livingston, 1979, CMW 2081, C 41, NMP 104, PREM 51444; New Mexico, Mescalero, from roots of *Pinus ponderosa*, W.H. Livingston, 1979, CMW 2080, C 46, NMP 103, ATCC 58571, PREM 51445 PARATYPES.

Leptographium douglasii Wingfield, Harrington et Crous
sp.nov. Figs. 14–26

Coloniae primum hyalinae atro-olivacescentes vel nigrescentes dum senescunt in MEA. Margines coloniarum effusae impares. Hyphae serpentinae, atro-olivaceae late ramosae crassis parietibus externis tectis atro exsudato, paene omnino immersae in illo medio, typice aggregatae in catervas inter se conglutinatas exsudatis parietum. Conidiophora solitaria sed saepe in catervis laxe dispositis in superficie agari, macronematosa, mononematosa rhizoideis plerumque praesentibus beneque evolutis at basim. Stipes erectus, atro-olivaceus paulum pallescens ad apicem, 148–650 (\bar{x} = 402.5) μm longus 11.5–28.5 (\bar{x} = 16.5) μm latus ad basim paulum tumidus ad apicem. Apparatus conidiogenous 46.5–119 (\bar{x} = 74.5) μm longus massa conidica exclusa, constans ex duabus usque quatuor seriebus ramorum. Usque sex rami principales saepe distincto ramo centrali paulo maiore quam ceteris, pallidiores colore quam stipes, 11–27 (\bar{x} = 19) μm longi, 5.5–10 (\bar{x} = 7) μm lati. Cellae conidiogenae discretatae, attenuatae a basi ad apicem, 10.5–24 (\bar{x} = 16) μm longae, 2–4.5 (\bar{x} = 3) μm latae mediam ad partem. Auctus conidii constructio parietum ad restituendum holoblastica ontogenie percurrentique ontogenie cum secessionem retardata danti pseudo-aspectum proliferationis sympodiorum. Conidia hyalina, late ellipsoidea late curvata ad extremum alterum, distincte curvata ad locum afficionis, 4.5–7 (\bar{x} = 6) μm longa, 2–4 (\bar{x} = 3) μm lata ad mediam par-





FIGS. 23–26. Conidiophore, conidiogenous cells, and conidia of *Leptographium douglasii*. Fig. 23. Mononematous, macronematous conidiophore subtending branching metulae and conidiogenous cells. Scale bar = 9 μ m. Figs. 24 and 25. Conidiogenous cells showing distinct annellations, and conidia appearing to have been produced sympodially owing to delayed secession. Scale bars = 5 μ m. Fig. 26. Broadly ellipsoidal conidia. Scale bar = 5 μ m.

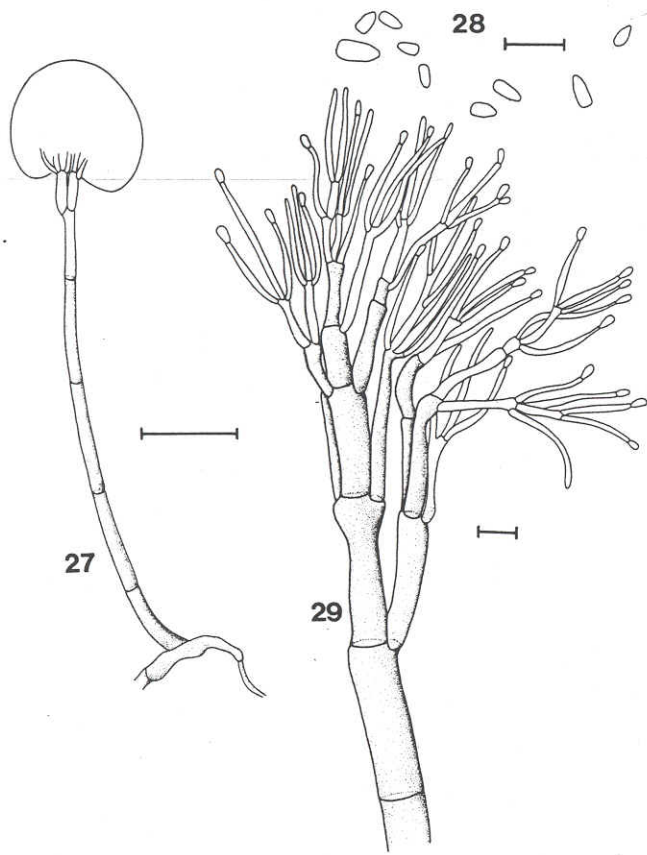
tem. Conidia accumulant circa apparatus conidiogenum in massa mucilaginoso.

HOLOTYPE: U.S.A., Oregon, Prospect, *Pseudotsugae menziesii*, D. Goheen, 1979, PREM 51446.

Colonies on MEA grew optimally at 20°C, reaching 22.5 mm in diameter in 4 days. Growth rate dropped consider-

ably at 15 and 25°C, and no growth occurred below 15°C or above 25°C. The fungus was able to withstand high concentrations of cycloheximide with growth at 25°C reduced by approximately 50% on MEA containing 2.5 g/L of the antibiotic (Table 1). Colonies hyaline, becoming dark olivaceous to black, 23''''k – 27''''m (Rayner 1970) on MEA. Colony

FIGS. 17–22. Conidiophores, conidiogenous apparatus, conidiogenous cells, and conidia of *Leptographium douglasii*. Fig. 17. Mononematous, macronematous conidiophores. Scale bar = 70 μ m. Figs. 18–20. Conidiogenous apparatus showing central primary metulae surrounded by slightly smaller metulae. Scale bars = 10 μ m. Fig. 21. Conidiogenous cells giving rise to conidia. Scale bar = 6 μ m. Fig. 22. Conidia. Scale bar = 6 μ m.



FIGS. 27–29. Conidiophores and conidia of *Leptographium albopini*. Fig. 27. Mononematous, macronematous conidiophores. Scale bar = 50 μm . Fig. 28. Oblong conidia. Scale bar = 10 μm . Fig. 29. Conidiogenous apparatus showing branching of the primary metulae. Scale bar = 8 μm .

margins effuse and uneven. Hyphae serpentine, dark olivaceous, extensively branched with thick outer walls, covered by a dark exudate; almost entirely immersed in the medium, typically aggregated in groups, cemented together by the wall exudates. Conidiophores (Figs. 14, 16, 17, 23) single but often also in loosely arranged groups on the agar surface, macronematous, mononematous, with rhizoids usually present and well developed at the base. Stipe erect, dark olivaceous, becoming slightly lighter at the apex, 148–650 (\bar{x} = 402.5) μm long and 11.5–28.5 (\bar{x} = 16.5) μm wide at the base and slightly swollen at the apex. Conidiogenous apparatus (Figs. 16, 18–21, 24) 46.5–119 (\bar{x} = 74.5) μm long (excluding conidial mass), consisting of between two and four series of branches. Up to six primary branches present, often with a distinct central branch slightly larger than the others, paler in color than the stipe, 11–27 (\bar{x} = 19) μm long, 5.5–10 (\bar{x} = 7) μm wide. Conidiogenous cells (Figs. 21, 25) discrete, hyaline, tapering from base to apex, 10.5–24 (\bar{x} = 16) μm long, 2–4.5 (\bar{x} = 3) μm wide at the middle. Conidium development replacement wall building with holoblastic ontogeny and percurrent ontogeny with delayed secession giving a false appear-

ance of sympodial proliferation. Conidia (Figs. 15, 22, 28) hyaline, broadly ellipsoidal, broadly rounded at one end with distinct taper, curving towards the point of attachment, 4.5–7 (\bar{x} = 6) μm long, 2–4 (\bar{x} = 3) μm wide at the center. Conidia accumulating around the conidiogenous apparatus in a mucilaginous mass.

CULTURES EXAMINED: U.S.A.: OREGON: Prospect, isolated from roots of *Pseudotsuga menziesii*, D. Goheen, 1979, CMW 2078, C 21, ex-type culture at CBS, PREM 51446 HOLOTYPE; NEW MEXICO: Cloudcroft, near Mescalero Indian Reserve, from roots of *P. menziesii*, M. Mielke, 1980, CMW 2076, C 36, PREM 51447; CALIFORNIA: Trinidad, from adult *Hylastes nigrinus*, collected from roots of *Pseudotsuga menziesii*, T.C. Harrington, 1980, CMW 2075, C 47, PREM 51460, and CMW 2073, C 6, PREM 51448; COLORADO: San Juan National Forest, from roots of *Pseudotsuga menziesii*, R. James, 1979, CMW 2074, C 40, COD 101, ATCC 58572, PREM 51449 PARATYPES.

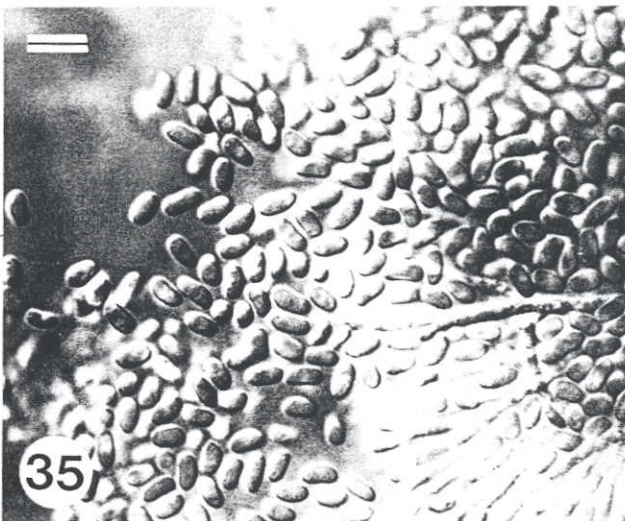
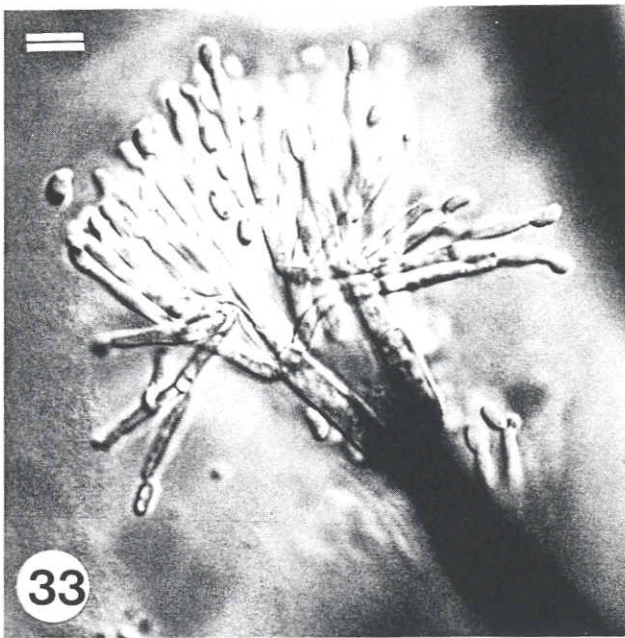
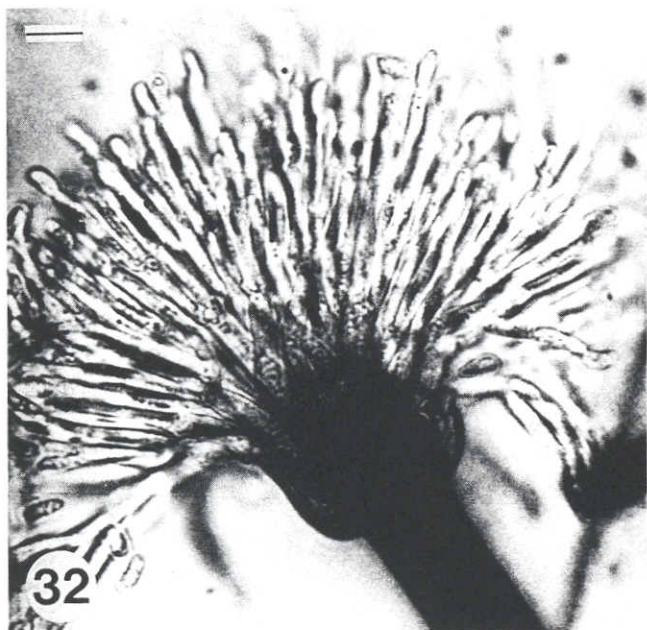
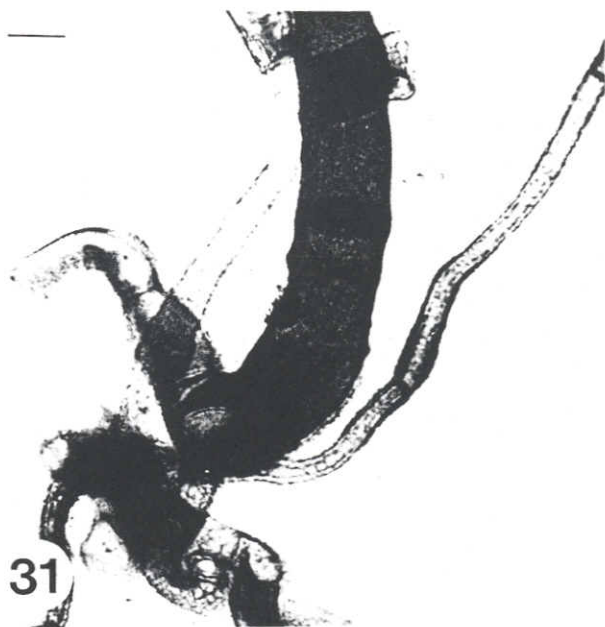
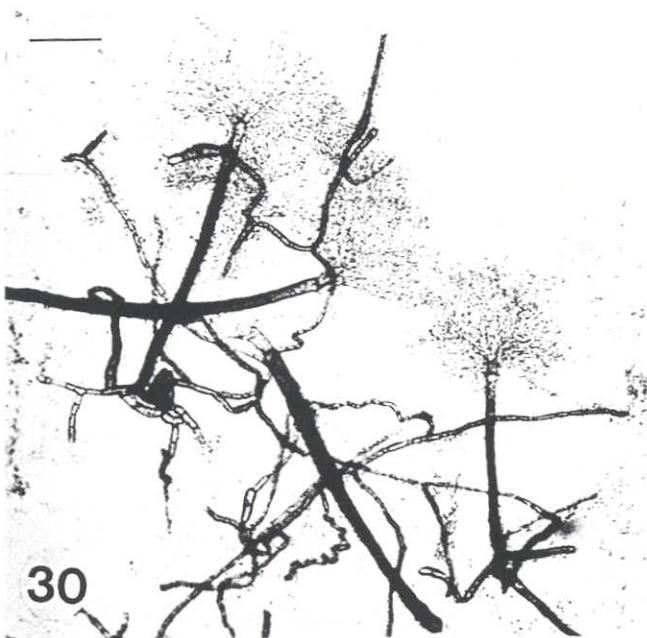
Leptographium albopini Wingfield, Harrington et Crous
sp. nov. Figs. 27–39

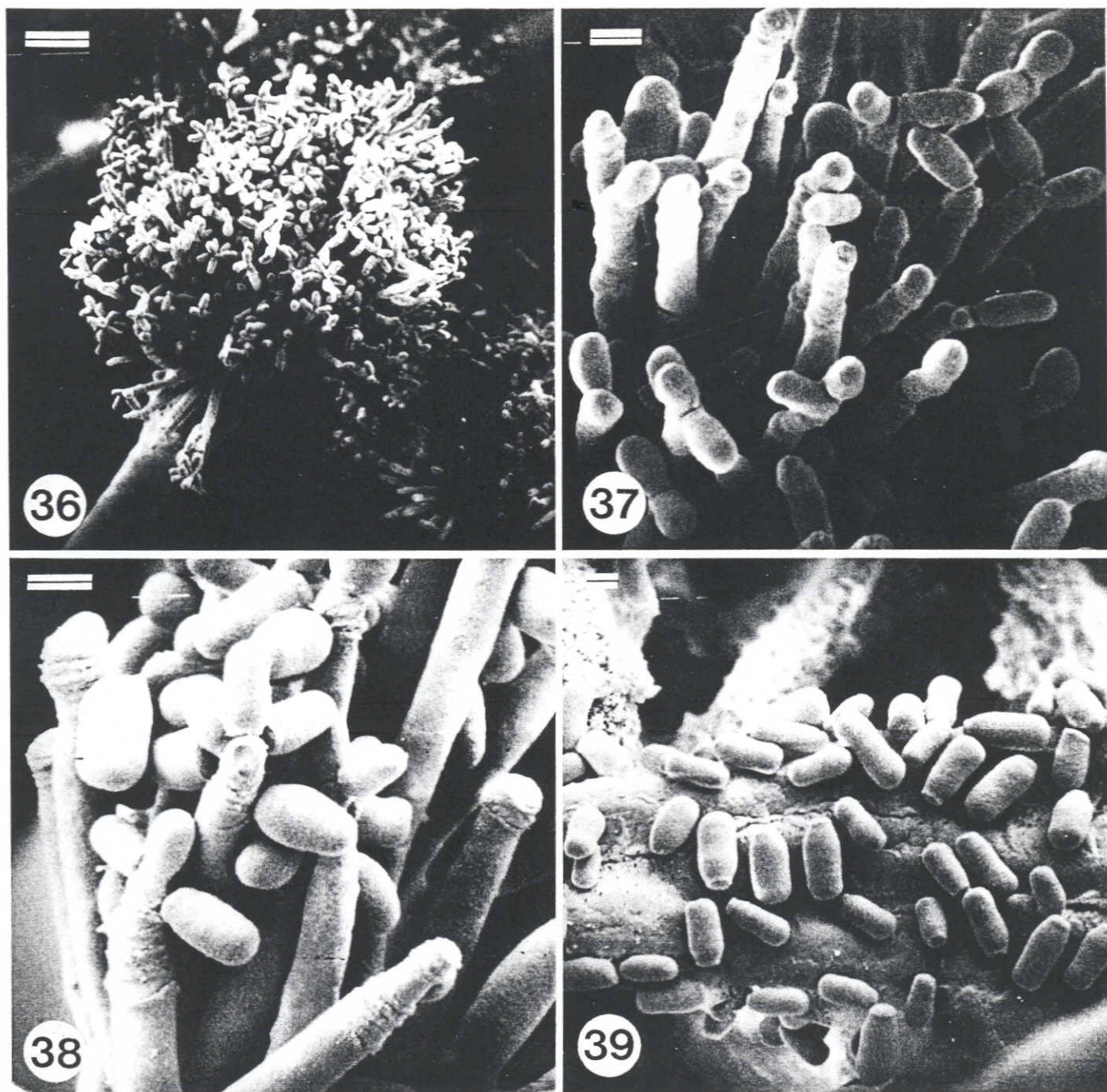
Coloniae primum hyalinae pallido-olivacescentes colore in MEA. Hyphae immersae in illo medio parietibus distincte asperis crassisque, erectae satis late separatae inter se, 3–12 (\bar{x} = 6.5) μm diametro. Nullum mycelium aerium formatum est. Conidiophora solitaria, macronematosa, mononematosa rhizoideis plerumque dense intertextis in nodo. Stipes erectus, pallido-olivaceus paulum pallescens ad apicem, 70–960 (\bar{x} = 323) μm longus 8–14 (\bar{x} = 10.5) μm latus ad basim paulumque tumidus ad apicem. Apparatus conidiogenus 40–92 (\bar{x} = 67) μm longus conidica massa exclusa constans ex tribus usque sex sed plerumque tribus vel quattuor seriebus ramorum. Primi rami dui usque quattuor sed plerumque dui inter se adjacentes, nullus ramus distinctus centralis, 12–30 (\bar{x} = 24) μm longi 5–11 (\bar{x} = 7.5) μm lati. Cellae conidiogenae discretae, hyalinae, attenuatae a basi ad apicem, 9–52 (\bar{x} = 20.5) μm longae, 1.5–3 (\bar{x} = 2) μm latae. Auctus conidii constructio parietum ad restituendum ontogenie holoblastica proliferationeque percurrenti, secessionone retardata dante pseudo-aspectum auctus sympodialis. Conidia hyalina, oblonga apice obtuso, attenuata distincte versus locum afficionis 3.5–8 (\bar{x} = 4.5) μm longa 1.5–4 (\bar{x} = 2) μm lata. Conidia accumulans circa apparatus conidiogenum in massa mucilaginoso cremicolorescens in senectute.

HOLOTYPE: U.S.A., Virginia, Blue Ridge Parkway, Pini strobi, A. Lackner, 1979, PREM 51450.

Colonies on MEA grew optimally at 25°C, reaching 33 mm diameter in 4 days. Growth rate was almost the same at 20°C and decreased slightly to 25 mm at 15°C. Minimal growth occurred at 10°C, and no growth was observed above 30°C. The fungus was able to withstand high concentrations of cycloheximide with growth at 25°C reduced by approximately 50% on MEA containing 2.5 g/L of the antibiotic (Table 1). Colonies at first hyaline becoming light olivaceous, 23''''b (Rayner 1970) (as for *L. neomexicanus* but lighter in color) on MEA. Hyphae immersed in the medium with distinctly roughened

FIGS. 30–35. Conidiophores, conidiogenous apparatus, conidiogenous cells, and conidia of *Leptographium albopini*. Fig. 30. Mononematous, macronematous conidiophores. Scale bar = 70 μm . Fig. 31. Base of conidiophore showing slightly developed rhizoidal hyphae. Scale bar = 10 μm . Figs. 32 and 33. Conidiogenous apparatus showing variation in arrangement of primary metulae. Scale bars = 10 μm . Fig. 34. Conidiogenous cells apparently proliferating sympodially. Scale bar = 10 μm . Fig. 35. Oblong conidia. Scale bar = 10 μm .





FIGS. 36–39. Conidiogenous apparatus, conidiogenous cells, and conidia of *Leptographium albopini* as viewed with a scanning electron microscope. Fig. 36. Conidiogenous apparatus with conidia apparently developing sympodially. Scale bar = 10 μm . Figs. 37 and 38. Conidiogenous cells showing well developed annellations. Scale bars = 2.5 μm . Fig. 39. Oblong conidia. Scale bar = 10 μm .

and thick walls, straight and reasonably broadly separated from each other, 3–12 (\bar{x} = 6.5) μm in diameter. No aerial mycelium formed. Conidiophores (Figs. 27, 29, 30, 36) single, macronematous, mononematous with rhizoids usually densely interwoven in a knot (Figs. 30, 31). Stipe erect, light olivaceous becoming slightly lighter at the apex, 70–960 (\bar{x} = 323) μm long and 8–14 (\bar{x} = 10.5) μm wide at the base and slightly swollen at the apex. Conidiogenous apparatus (Figs. 29, 32–34) 40–92 (\bar{x} = 67) μm long (excluding conidial mass) consisting of between three and six but mostly three or four series of branches. There are usually two to four primary branches, but mostly only two adjacent to each other

without a distinct central branch, 12–30 (\bar{x} = 24) μm long and 5–11 (\bar{x} = 7.5) μm wide. Conidiogenous cells (Figs. 37, 38) discrete, hyaline, tapering from base to apex, 9–52 (\bar{x} = 20.5) μm long, 1.5–3 (\bar{x} = 2) μm wide. Conidium development replacement wall building with holoblastic ontogeny and percurrent proliferation with delayed secession giving a false appearance of sympodial development. Conidia (Figs. 28, 35, 38, 39) hyaline, oblong with an obtuse apex and distinctly tapered to the point of attachment, 3.5–8 (\bar{x} = 4.5) μm long and 1.5–4 (\bar{x} = 2) μm wide. Conidia accumulating around the conidiogenous apparatus in a clear mucilaginous mass becoming cream colored when dry.

CULTURES EXAMINED: U.S.A.: VIRGINIA: Blue Ridge Parkway, from insect-infested roots of *Pinus strobus*, L., A. Lackner, 1979, CMW 2065, C 154, ex-type culture at CBS, PREM 51450 HOLOTYPE; COLORADO: Mesa Verde, from roots of *Pinus edulis* Engelm. infested by a *Hylastes* sp., R. Wagener, 1983, CMW 2072, C 156, PREM 51451, and CMW 2082, C 157, PREM 51452, and CMW 2071, C 155, PREM 51453; NORTH CAROLINA: Coweeta Hydrological Experiment Station near Otto, from roots of *Pinus strobus*, T.D. Leininger, 1985, CMW 2070, C 184, PREM 51454, and CMW 2068, C 182, PREM 51455 PARATYPES.

Discussion

Leptographium albopini, *L. douglasii* and *L. neomexicanus* (referred to as species I, F, and E in Zambino and Harrington 1992) are well defined and based on numerous collections over an extended period of time. All three species are associated with roots of conifers, which we consider to be an important distinguishing characteristic. Furthermore, these species can also be distinguished from other similar *Leptographium* spp. based on their allozyme banding patterns (Zambino and Harrington 1992).

Leptographium albopini appears to be found on the white pines *Pinus strobus* and *Pinus edulis*, and unlike *L. douglasii* and *L. neomexicanus*, has been collected both in the eastern and western United States. Superficially, *L. albopini* resembles *Leptographium procerum* (Kendr.) Wingf., which also occurs commonly on *Pinus strobus* in the eastern United States. It is, however, easily distinguished from *L. procerum* primarily by the fact that conidiophores are produced singly and never in groups. Although conidia of *L. albopini* are more or less the same shape as those of *L. procerum*, they tend to be larger in the former species. Mycelium of *L. albopini* is also typically surrounded by a thick verrucose sheath, which is absent in *L. procerum*. *Leptographium albopini* has also been found to be morphologically similar to *Leptographium serpens* (Goid.) von Arx (Zambino and Harrington 1992). However, it was found to have narrower conidiogenous cells (Harrington 1988) and undulating, curved hyphae (Zambino and Harrington 1992), which are different from the serpentine hyphae of *L. serpens*. These results were also supported by distinct zymograms of isolates of *L. albopini*.

Leptographium douglasii, which is apparently restricted to Douglas-fir, occurs commonly in the western United States. It is commonly associated with *Hylastes nigrinus* (Mann.) (Byler et al. 1983; Harrington and Cobb 1983), a root-feeding bark beetle known to occasionally vector *Leptographium wageneri* var. *pseudotsugae* Harrington & Cobb (Harrington et al. 1985). *Leptographium douglasii* was previously confused with *Leptographium wageneri* var. *pseudotsugae* as both species form serpentine hyphae. Indeed, a culture of this fungus was incorrectly used as *L. wageneri* in comparisons with *L. serpens* and *L. procerum* in a previous study (Wingfield and Marasas 1980). Although *L. douglasii* occurs alongside *L. wageneri* var. *pseudotsugae* in nature, it can easily be distinguished from the latter species. *Leptographium douglasii* has a more robust conidiogenous apparatus with more primary metulae than that of *L. wageneri* var. *pseudotsugae*. Conidia are also narrower and longer in *L. douglasii* and have a distinct curve towards the point of attachment, unlike *L. wageneri* var. *pseudotsugae*. Also, *L. douglasii* grows well at 25°C, whereas *L. wageneri* grows little or not at all at this temperature.

Leptographium neomexicanus was found only in the southwestern United States on *Pinus ponderosa* Laws. Unlike other *Leptographium* spp., it produces an abundance of grey aerial mycelium in culture. It is superficially similar to *L. douglasii* but is easily distinguished from the latter species in having distinctly shorter conidiophores with shorter primary metulae. Although conidia are slightly curved in this species, they are much less so than those of *L. douglasii*. *Leptographium neomexicanus* is also considerably faster growing in culture and has a higher optimum temperature for growth than *L. douglasii*. Finally, hyphae of *L. neomexicanus* are less serpentine than hyphae of *L. douglasii*.

Both *L. neomexicanus* and *L. douglasii* were shown to be weakly pathogenic to *P. ponderosa* and Douglas-fir seedlings but much less pathogenic than *L. wageneri* (Harrington and Cobb 1983). Neither *L. neomexicanus* nor *L. douglasii* gave rise to notable lesion development in inoculations, nor were they restricted to the tracheids. They can, therefore, also easily be distinguished from *L. wageneri* based on their lower virulence and mode of host colonization. The zymograms of *L. douglasii* and *L. neomexicanus* were similar to each other and *L. wageneri* but were distinct from all other *Leptographium* species compared by Zambino and Harrington (1992), showing them to represent distinct taxa within the *L. serpens* cluster.

These three *Leptographium* species can be reasonably distinguished from other described *Leptographium* species on conifers, but there are many other morphologically similar species that await description. The *Leptographium* species with small, more or less ellipsoidal conidia are particularly problematic. As with the species described above, allozyme and molecular analyses will help to clearly delineate new taxonomic units for morphological description.

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