

Pseudohalonectria (Lasiosphaeriaceae), an antagonistic genus from wood in freshwater

C. A. SHEARER

Department of Plant Biology, University of Illinois, Urbana, IL 61801, U.S.A.

Received June 20, 1988

SHEARER, C. A. 1989. *Pseudohalonectria* (Lasiosphaeriaceae), an antagonistic genus from wood in freshwater. Can. J. Bot. 67: 1944–1955.

Pseudohalonectria lignicola and five new species of *Pseudohalonectria* are described and illustrated from submerged wood in freshwater. Species are similar in overall morphology and produce bright yellow to brown perithecia, asci with thimble-shaped apical apparatuses and that separate from ascogenous hyphae at maturity, long, septate paraphyses attached to ascogenous hyphae, and hyaline to lightly pigmented phragmosporous ascospores. An anamorphic state was found for only one species. *Pseudohalonectria* has features common to both the Sordariales and Diaporthales, but is best placed in the Sordariales. Species are inhibitory to other filamentous fungi and yeasts in paired culture, and form soft-rot cavities in balsa and beech wood.

SHEARER, C. A. 1989. *Pseudohalonectria* (Lasiosphaeriaceae), an antagonistic genus from wood in freshwater. Can. J. Bot. 67: 1944–1955.

L'auteur décrit et illustre le *Pseudohalonectria lignicola* et cinq nouvelles espèces de *Pseudohalonectria* récoltées à partir de bois submergé en eau douce. Les espèces sont dans l'ensemble morphologiquement semblables et produisent des périthèces de jaune-brillant à brun, des asques munies d'un appareil apical en forme de dé à coudre et qui se séparent de l'hyphe ascogène à maturité, des paraphyses longues et septées attachées aux hyphes ascogènes, et des ascospores phragmosporiques hyalines ou faiblement pigmentées. Un stade anamorphe n'a été identifié que pour une seule espèce. Le genre *Pseudohalonectria* possède des caractères communs à la fois aux Sordariales et aux Diaporthales, mais correspond mieux aux Sordariales. Les espèces sont inhibitrices pour d'autres champignons filamenteux et levures en cultures de confrontation; elles forment de plus des cavités de pourriture molle dans le bois de balsa et hêtre.

[Traduit par la revue]

Introduction

Pseudohalonectria Minoura & Muroi was established in 1978 for *P. lignicola*, an ascomycete found on balsa wood submerged in a Japanese lake (Minoura and Muroi 1978). Characteristics of the genus include: colored perithecia with protruding, cylindrical, periphysate beaks; unitunicate, cylindrical to clavate asci with an IKI negative apical apparatus; presence of paraphyses; and cylindrical, smooth, hyaline to slightly colored ascospores. During investigations of the fungi colonizing wood in aquatic habitats, *P. lignicola* and five undescribed, related species were collected. These species are described and illustrated.

Materials and methods

Fruit bodies from natural substrata were crushed in 5-mL sterile distilled water and the resulting ascospore suspension was plated on antibiotic water agar (agar, 18 g L⁻¹, streptomycin sulfate and penicillin G, 0.5 g L⁻¹ each (Difco) and distilled water, 1 L). Single germinated ascospores were transferred to cornmeal agar (CMA, Difco) plates. Cultures were maintained at 4°C on slants of half-strength Emerson's yeast soluble starch agar (Emerson 1958) overlaid with balsa wood strips.

To induce sexual reproduction, fungi were grown on alfalfa stems (5–10 stems ~5 cm long) autoclaved for 1 h in 50 mL distilled water in 125-mL Erlenmeyer flasks. Flasks were shaken at 100 rpm at 22°C under alternating light:dark (8:16) conditions for 2 weeks and colonized stems were transferred to glass Petri dishes containing three pieces of sterile filter paper (Whatman No. 1) moistened with sterile distilled water. Alfalfa stems were incubated under the same conditions described above until reproduction was observed.

To determine soft-rot activity, strips of balsa (*Ochroma* sp.) and beech (*Fagus grandifolia* Ehrh.) wood (~60 × 10 × 2 mm) were autoclaved in distilled water and then placed on the surface of CMA agar (30 mL/dish) in plastic Petri dishes. Wood strips were inocu-

lated centrally with disks of mycelium (9 mm diam.). Wood cultures were incubated as above until colonization of wood was evident. Wood cells were scraped from the surfaces of sticks, placed in a drop of water on a glass slide, and examined with polarized light for the presence of soft-rot cavities. Uninoculated wood served as a control.

Taxonomy

Pseudohalonectria lignicola Minoura et Muroi

Figs. 1–6, 33E–33H

Colonies on CMA floccose, appressed, white at first, becoming pale to lemon yellow, pale yellow in reverse, colony margin even. Hyphae septate, smooth walled, 1–2.5 µm diam., hyaline. Ascomata (Fig. 33E) solitary to aggregated, immersed to superficial, pale yellow at first, becoming darkened with age, rostrate; venter globose to flattened globose, 227–497 × 243–524 µm, peridium membranous, tissue of textura angularis in face view, 13–15 cell layers thick, outer cells pseudoparenchymatic, darkened yellow and compressed laterally, inner cells pseudoparenchymatic, pale yellow (Fig. 30). Beaks long, cylindrical, periphysate, composed of parallel hyphae, outer hyphae ending in enlarged, outwardly directed, subglobose cells (Fig. 27), 170–621 × 65–221 µm. Paraphyses (Figs. 1, 2) longer than asci, wide at base, tapering to a rounded point, thin walled, attached to ascogenous hyphae, 108–198 × 3.6–8.4 µm at base, 1- to 5-septate. Asci (Figs. 3–5, 33F) unitunicate, cylindrical, straight or sigmoidal, with IKI-negative, thimble-shaped apical apparatus (Fig. 33G) which stains bright blue in acidic aqueous cotton blue, containing eight ascospores in two overlapping fascicles of four, 90–132 × 11–17.6 µm, separating at the basal septum from ascogenous hyphae and lying free in venter (Figs. 4, 5), lining the base and sides of ascomata. Ascospores (Figs. 6, 33H) yellow in mass, becoming orange brown with age,

hyaline to pale orange brown in transmitted light, cylindrical with bluntly rounded apices, sometimes allantoid, $38.4-74.8$ ($\bar{x} = 62.6$) \times $3.6-6.6$ ($\bar{x} = 5.1$) μm , 5- to 11-septate, not constricted at the septa, discharged forcibly or accumulating in a mass at the beak opening.

SPECIMENS EXAMINED: From Jordan Creek, a tributary of the Salt Fork of the Vermilion River, Vermilion County, Illinois, on *Platanus occidentalis* L. twigs submerged from 4 June 1978 to 1 November 1978, J-13-1, ATCC 44623, 10 July 1978, J-13-3, 11 March 1979, J-13-11, 8 March 1980, J-13-5; on *Prunus serotina* Ehrh. twigs submerged from 4 June 1978 to 30 July 1979, J-13-2, 5 November 1979, J-13-4, 9 January 1979, J-13-19, 7 November 1980, J-13-20; on *Acer saccharinum* L. twigs submerged 4 June 1978 to 5 May 1979, J-13-6, 11 March 1979, J-13-7, 10 July 1978, J-13-12, 4 September 1978, J-13-13, 7 November 1980, J-13-16, 1 November 1978, J-13-18; from Lake Pentwater, Michigan, on unidentified submerged twig, 26 September 1980, J-13-9; from the Salt Fork of the Vermilion River, Vermilion County, Illinois, on unidentified submerged twig, April 1983, J-13-14; from the Patuxent River at Lower Marlboro, Calvert County, Maryland, salinity 6.1–3.7 ppt, on balsa wood, 19 June 1969, CS-182-1; from the Spoon River, Fulton County, Illinois, September 1983, J-13-21; from the Sangamon River at Hart Woods, Champaign County, Illinois, on submerged twigs, 26 November 1986, J-13-22, 9 September 1987, J-13-23, 13 December 1987, J-13-27; from a stream in Shades State Park, Montgomery County, Indiana, on submerged twig, J-13-24; from a backwater of the Illinois River at Beardstown, Morgan County, Illinois, on submerged twig, J-13-25; from Hill Lake, Minnesota, on submerged wood, August 1987, J-13-26.

Repeated attempts to obtain the type specimen of *P. lignicola* were unsuccessful. All of the specimens cited here, however, agree well with the protologue of *P. lignicola*. Because of the large number of collections and because they were collected from a variety of geographical locations, the range of measurements is somewhat greater. Measurements of all collections are included in the foregoing description. Although Minoura and Muroi (1978) indicate the presence of an IKI-negative apical apparatus, they do not characterize it any further. The apical apparatus is thimble or barrel shaped and the tip of the ascus is retracted into this structure (Figs. 3, 5, 33G). Although the apical apparatus is IKI negative, it stains bright blue in a slightly acidified aqueous solution of cotton blue. The ascus apparatus does not, however, stain blue in lactic acid containing cotton blue. Other features not reported by Minoura and Muroi include the tendency for asci to separate from ascogenous hyphae prior to ascospore discharge and the attachment of paraphyses to ascogenous hyphae (Figs. 1–3). The ascospores are filled with lipid droplets (Fig. 6) and germinate readily on water agar.

Pseudohalonectria lignicola is one of the most commonly occurring species on wood in freshwater in central Illinois. It formed soft-rot cavities on both balsa and beech wood in this study and has been reported to form soft-rot cavities on ash (*Fraxinus pennsylvanica* Marsh.) and cottonwood (*Populus deltoides* Marsh.) (Zare-Maivan and Shearer 1988). It is also antagonistic at a distance to other filamentous fungi when grown in paired culture (Shearer and Zare-Maivan 1988).

***Pseudohalonectria adversaria* Shearer sp. nov.**

Figs. 7–9, 33I–33L

Ascomata superficialia vel partim immersa, brunneo-aurantiaca, membranacea, rostrata; venter globosus vel subglobosus, $445-614$ μm diametro; peridium e textura angulari constitutum; rostrum breve, cylindricum, $79-248 \times 109-158$ μm periphysatum. Asci hyalini vel pallide aurantiaci, cylindrici vel clavati, $120-150 \times 13-20$ μm , apparatu apiculari digitaliformi, liquore iodato haud tincto, $2.5-5 \times 2.5-3$ μm , apice ad porum apicale ab lacuna signato, ab hyphis ascogenis separati et in ventre libri jacentes. Paraphyses septatae, $133-230$ μm longae, $4.0-7.8$ μm latae ad basim, ad acumen rotundatum attenuatae. Ascosporae hyalinae, in cumulo aurantiacae vel pallide aurantiacae, ellipsoidales, 5-septatae vel nonnumquam 6- ad 7-septatae, $33.5-49$ ($\bar{x} = 41.9$) \times $4.5-7$ ($\bar{x} = 5.9$) μm .

HOLOTYPE: NY.

Colonies on cornmeal agar floccose, with regular margin, aerial hyphae white, immersed hyphae creamy yellow, reverse dull yellow. Ascomata (Fig. 33I) solitary to clustered, superficial or immersed, orange brown, membranous, rostrate; venter globose, $445-614$ μm diam., outer wall layer brownish orange, peridium tissue of textura angularis in face view, inner layer pseudoparenchymatic, light orange, outer layer pseudoparenchymatic, brownish orange; beaks short, cylindrical, $79-248 \times 109-158$ μm , periphysate. Asci (Figs. 9, 33J) unitunicate, containing eight ascospores in two overlapping fascicles of four, hyaline to pale orange, cylindrical or clavate, $120-150 \times 13-20$ μm , with IKI-negative, thimble-shaped apical apparatus (Fig. 33K) $2.5-5 \times 2.5-3.0$ μm which stains bright blue in slightly acidic aqueous cotton blue, apex indented at apical pore, separating from ascogenous hyphae and lying free in venter cavity. Paraphyses septate, $133-230$ μm long, $4-7.8$ μm wide at base, tapering to a rounded point, attached to ascogenous hyphae (Fig. 8). Ascospores (Figs. 7, 33L) hyaline to pale orange, orange in mass, ellipsoidal, 5-septate, occasionally 6 or 7 septate, $33.5-49$ ($\bar{x} = 41.9$) \times $4.5-7$ ($\bar{x} = 5.9$) μm .

HOLOTYPE: A dried culture (CS-603-1) grown on alfalfa stems. Culture isolated from a single ascospore from an ascoma on submerged woody debris from Deer Pond (Elvira Cypress Swamp), Johnson County, Illinois, 8 August 1977.

TYPE CULTURE: ATCC 56670.

ETYMOLOGY: From Latin *adversarius* meaning an antagonist, opponent, or adversary, and referring to the antagonistic activity of this species against other fungi.

Pseudohalonectria adversaria formed soft-rot cavities on balsa and beech wood. This isolate (as *Lasiosphaeria* sp. 1) was found to be inhibitory to and to resist inhibition by other aquatic Ascomycetes and Fungi Imperfecti (Shearer and Zare-Maivan 1988).

***Pseudohalonectria falcata* Shearer sp. nov.**

Figs. 10–15, 33A–33D

Ascomata in ligno immersa, luteo-brunnea, membranacea, a hyphis strictis textis, luteis obducta, rostrata; venter globosus vel subglobosus, $277-535$ μm diametro; peridium e textura angulari constitutum; rostrum cylindricum, apice rotundato, pallide luteum vel brunneo-luteum, periphysatum, $81-162 \times 81-108$ μm . Asci hyalini, cylindrici vel cymbiformes, $106-244 \times 14.4-21.6$ μm , apparatu apicali applanato, in liquore iodato haud tincto. Paraphyses 2–5-septatae, ad septa nunc constrictae, nunc non, $94-209 \times 4.8-12$ μm . Ascosporae hyalinae, falcatae 6- ad 16-septatae, $97-166$ ($\bar{x} = 135$) \times $4.2-7.2$ ($\bar{x} = 5.8$) μm .

HOLOTYPE: NY.

Colonies on cornmeal agar floccose, white at first, becoming yellow, yellow in reverse. Hyphae mostly immersed, hyaline to pale yellow, smooth walled, 1.5–3 μm diam. Ascomata (Fig. 33, A) on wood immersed, yellowish brown, membranous, rostrate; venter globose to subglobose, 277–535 μm diam.; peridium of tissue textura angularis in face view, pseudoparenchymatic in longitudinal section with an outer covering of loosely interwoven hyphae; beak cylindrical with rounded apex, light yellow or light brownish yellow, periphysate, 81–162 \times 81–108 μm . Asci (Figs. 10, 33C) hyaline, cylindrical to cymbiform, sometimes sigmoidal, 106–244 \times 14.4–21.6 μm , with IKI-negative, flattened apical apparatus (Figs. 12, 33D) which stains bright blue in slightly acidic aqueous cotton blue, separating from or remaining attached to ascogenous hyphae, containing eight ascospores in a single fascicle. Paraphyses (Figs. 13–15) septate, tortuous at first with or without constrictions at the septa, of large, thin-walled cells, 94–209 \times 4.8–12 μm , attached to ascogenous hyphae (Fig. 15). Ascospores (Figs. 11, 33B) hyaline, falcate, sometimes slightly sigmoid, 6- to 16-septate, 97–166 (\bar{x} = 135) \times 4.2–7.2 (\bar{x} = 5.8) μm .

HOLOTYPE: A culture fruiting on beechwood, CS-617-2, derived from a single ascospore isolated from an ascoma on a submerged twig collected from the Illinois River at Beardstown, Brown County, Illinois, 7 November 1987.

ETYMOLOGY: From Latin *falcatus* meaning curved or scythe shaped, referring to the curved ascospores.

ADDITIONAL MATERIAL EXAMINED: On submerged twig collected from Quiver Creek, a tributary of the Illinois River, Mason County, Illinois, 10 August 1978. NY. CS-617-1, ATCC 56673.

Pseudohalonectria falcata has several features which distinguish it from the other *Pseudohalonectria* species. The ascus apex apparatus is more flattened than thimble shaped and the developing paraphyses are markedly tortuous at first (Fig. 13) and often appear as chains of pseudoparenchymatic cells (Fig. 14). The mature paraphyses are shorter and broader than those of the other *Pseudohalonectria* species. The base of the ascus appears to be more deliquescent than those of the other species and ascospores are discharged through the deliquesced basal end. The ascospores frequently germinate from both spore apices while in the ascus. The asco-

spores, when discharged in water, often assume a sigmoidal shape with one end of the spore more curved than the other.

Pseudohalonectria falcata formed soft-rot cavities on balsam and beechwood, and isolate CS-617-1 was found to inhibit the growth of other aquatic Ascomycetes and Fungi Imperfecti in paired culture (Shearer and Zare-Maivan 1988).

Pseudohalonectria longirostrum Shearer sp. nov.

Figs. 16–18, 33U–33X

Ascomata in ligno partim immersa, vivide lutea, in brunneo-lutea furcescentes, rostrata; venter globosus vel applanato-globosus, 178–396 \times 267–396 μm ; rostrum praelongum, 1683–3712 \times 118–168 μm , cylindricum, periphysatum, a capillis brevibus in cellulam nodosam terminatis atque ab areolis capillarum longarum instar hypharum tectum. Asci cylindrici, recti vel sigmoidales, apparatu apicali digitaliformi in liquore iodato haud tincto, brevi-stipitati, 94–120 \times 8.4–12 μm , ad septum basale a hyphis ascogenis separati et in ventre liberi jacentes. Paraphyses acuminatae, 1- ad 4-septatae, 144–240 \times 7.2–9.6 μm . Ascosporae hyalinae vel pallide luteae, in cumulo luteae vel aurantiacae, 4- ad 8-septatae, filiformes, rectae vel sigmoideae, 84–105.6 (\bar{x} = 91.3) \times 3.8–4.0 (\bar{x} = 3.6) μm .

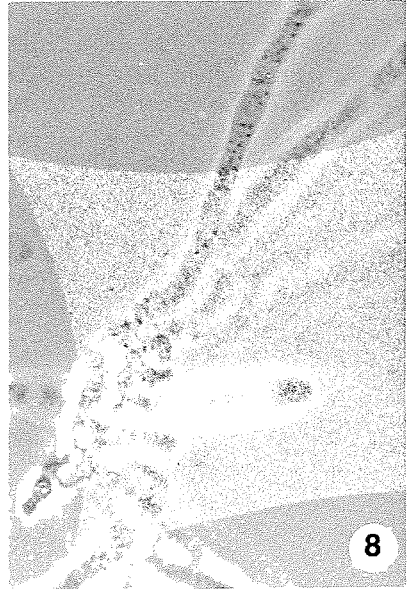
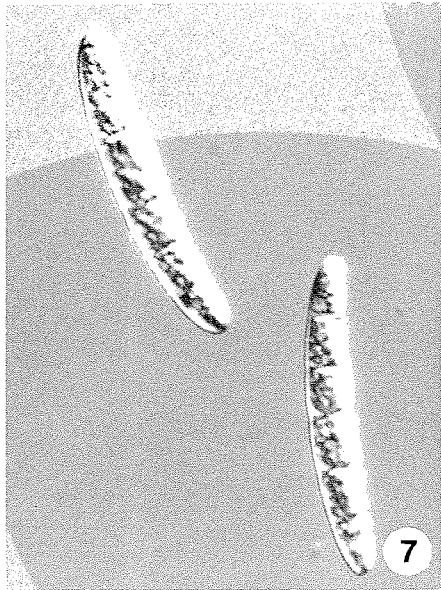
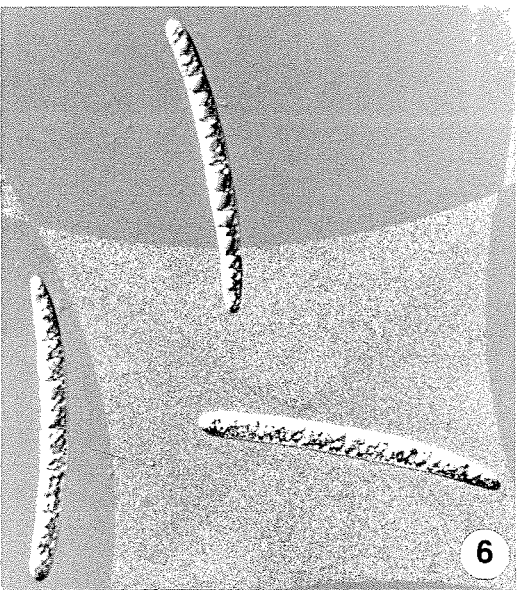
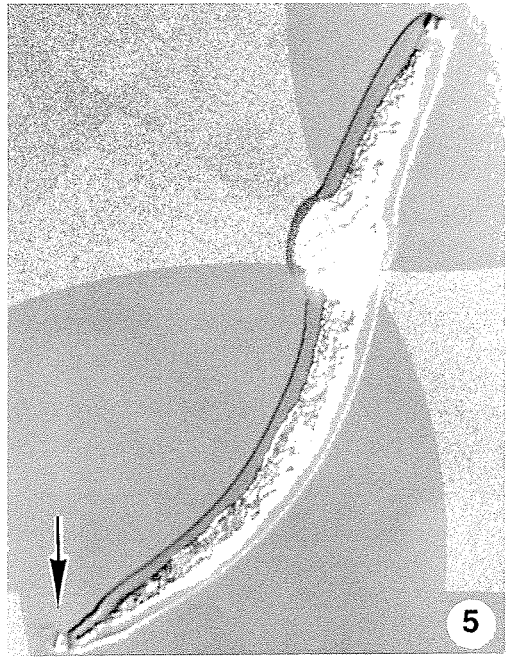
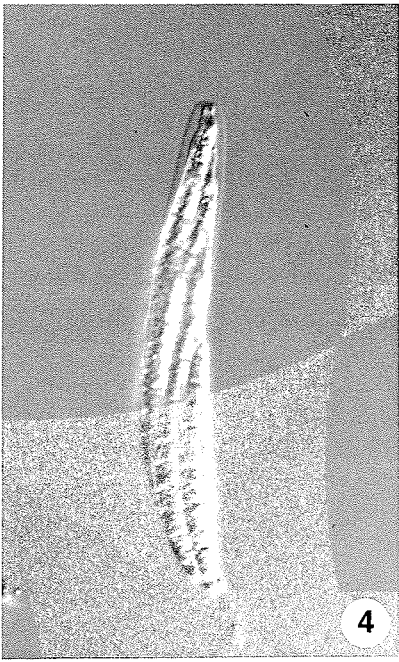
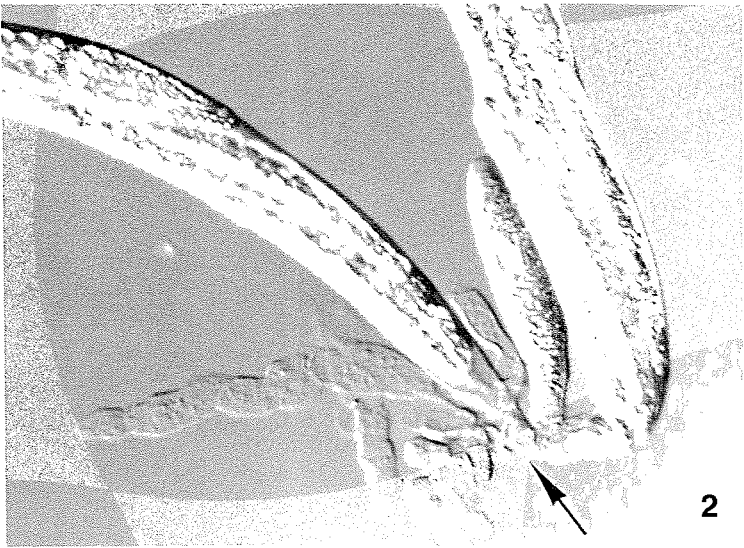
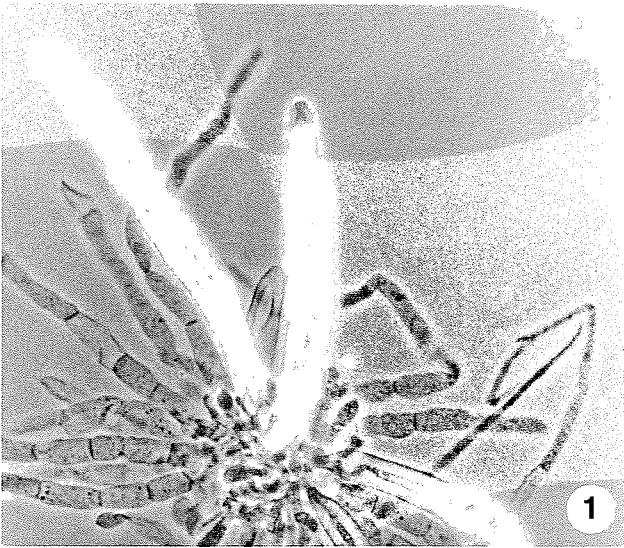
HOLOTYPE: NY.

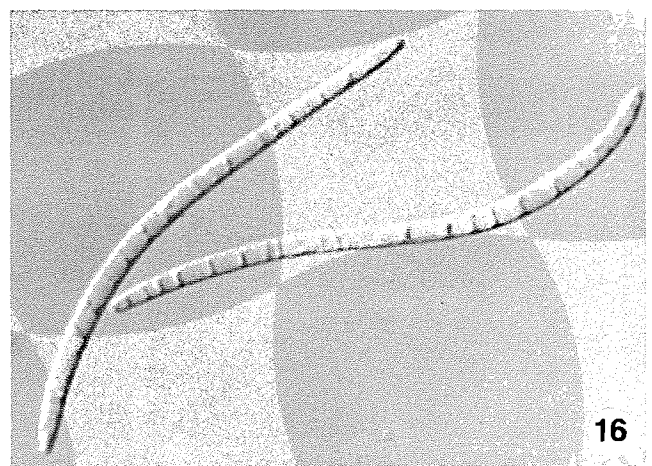
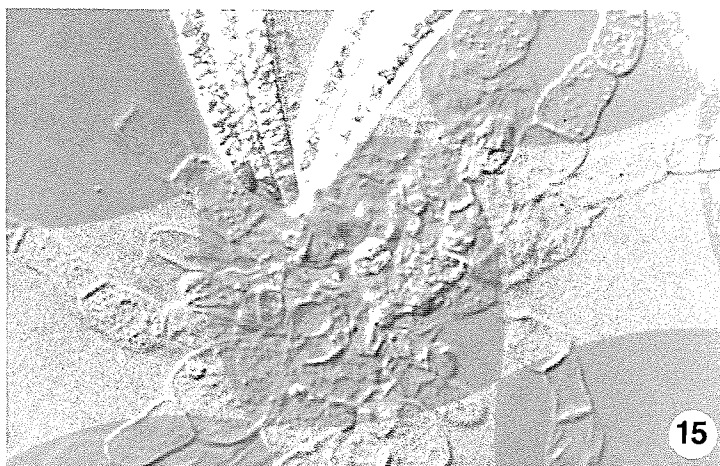
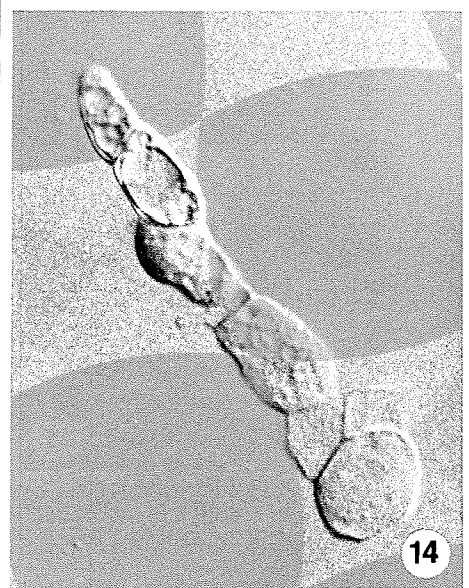
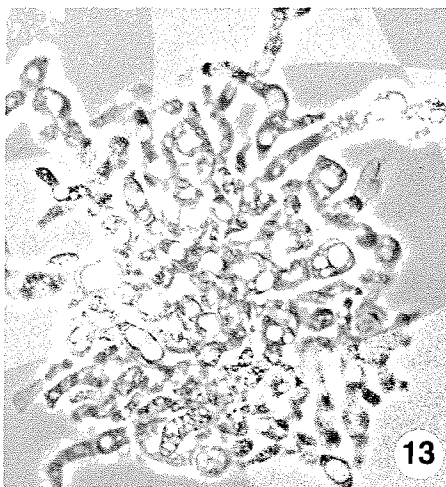
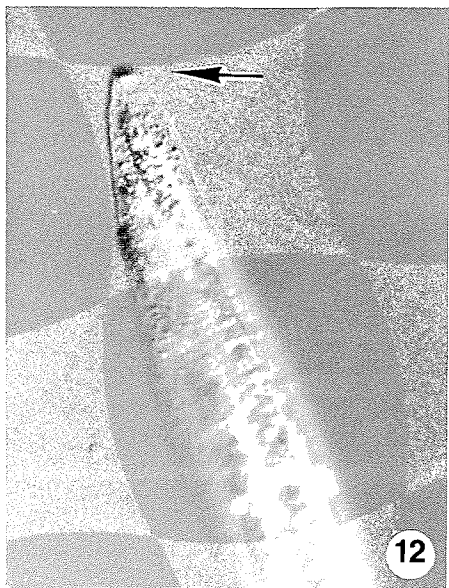
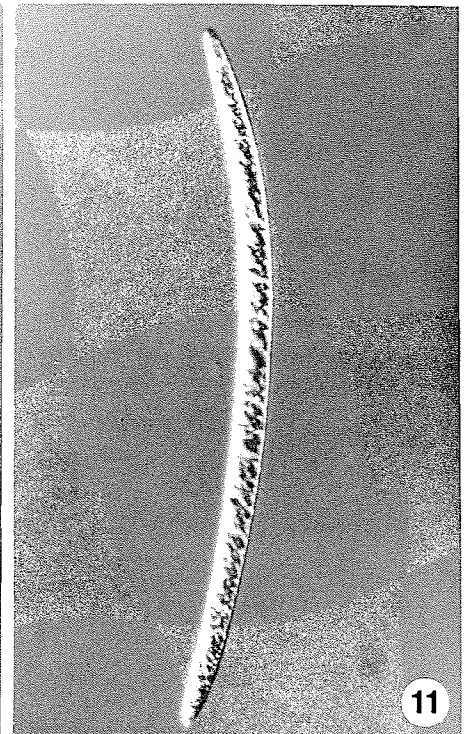
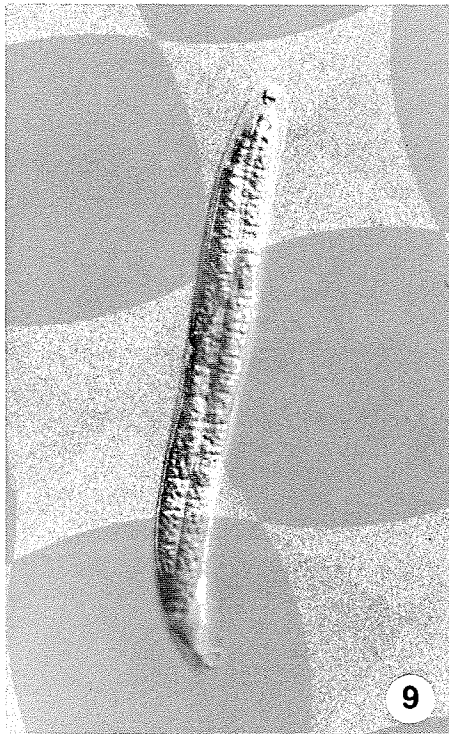
Colonies on cornmeal agar floccose, mostly immersed, hyaline at first, becoming intensely bright yellow; older regions of colony darkening, yellow pigment diffusing into the medium, colony reverse intensely yellow, colony margin regular. Ascomata (Fig. 33U) on wood immersed or partially immersed, bright yellow, becoming brownish yellow, rostrate; venter globose to flattened globose, 178–396 \times 267–396 μm , membranous, peridium of tissue textura angularis of very small-sized cells in face view, outer cells darkened yellow, inner cells pale yellow, pseudoparenchymatic; beaks very long, cylindrical, comprising parallel hyphae terminating in enlarged knoblike cells and covered with patches of long, hyphalike hairs, golden brown at base, pale yellow at the apex, periphysate, 1683–3712 \times 118–168 μm . Asci (Figs. 17, 33V) cylindrical, straight or sigmoidal, containing eight ascospores in a single fascicle, with IKI-negative thimble-shaped apical apparatus, 3–5 \times 2 μm (Figs. 18, 33W), short stalked, 94–120 \times 8.4–12 μm , separating from ascogenous hyphae at basal septum of ascus prior to ascospore

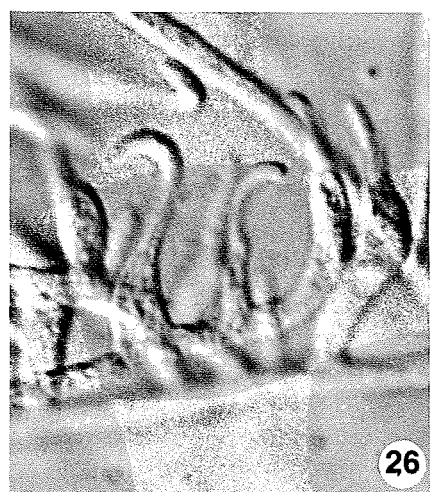
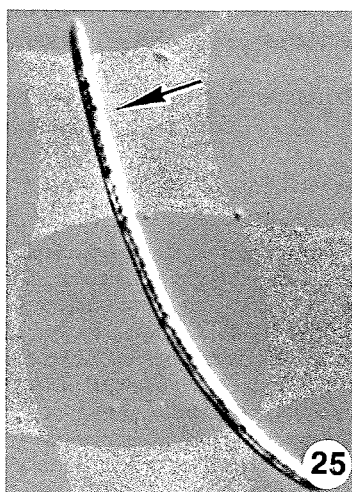
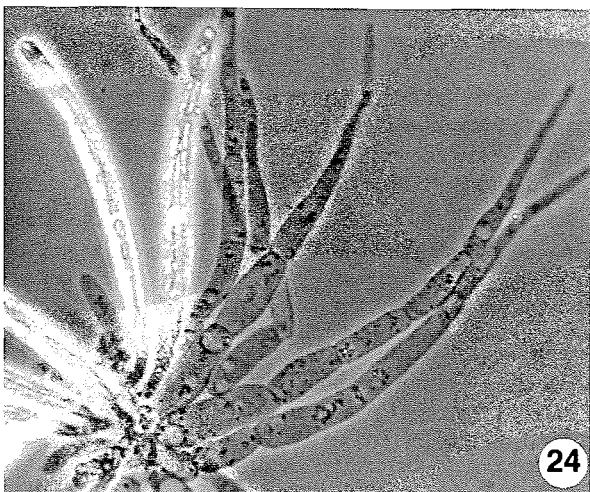
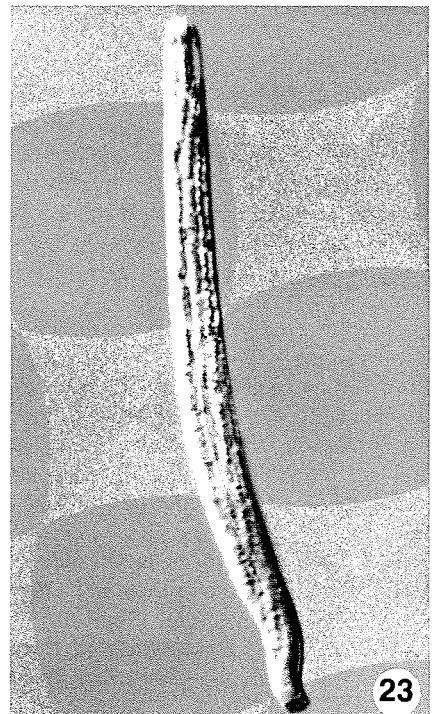
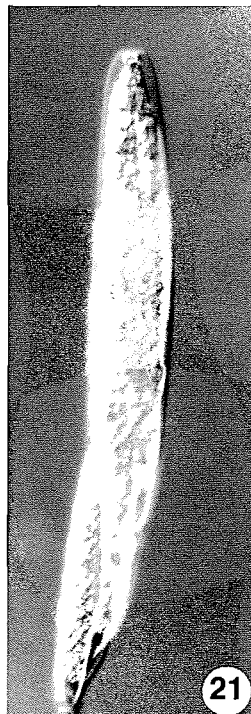
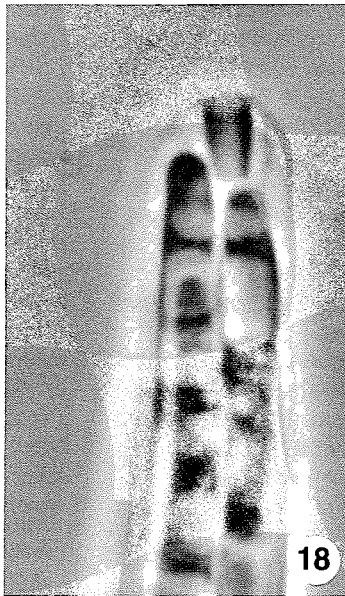
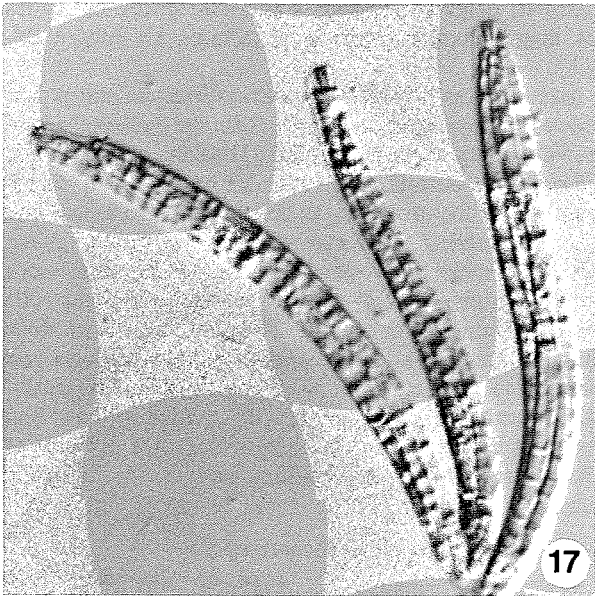
FIGS. 1–6. *Pseudohalonectria lignicola*. Fig. 1. Developing and mature asci and paraphyses. $\times 410$. Fig. 2. Attachment of asci and a paraphysis to ascogenous hyphae. $\times 775$. Fig. 3. Ascus mounted in water showing thimble-shaped ascus apparatus. $\times 450$. Fig. 4. Ascus fixed and stained in lactic acid with azure A and showing free basal end. $\times 480$. Fig. 5. Immature ascus with partially ruptured wall mounted in water and showing thimble-shaped ascus apparatus and frill of ascogenous hyphae attached to basal end. $\times 633$. Fig. 6. Ascospores mounted in water showing cells filled with lipid droplets. $\times 595$. FIGS. 7 and 8. *P. adversaria*. Fig. 7. Ascospores mounted in water showing cells filled with lipid droplets. $\times 997$. Fig. 8. Developing asci and paraphyses attached to ascogenous hyphal system. $\times 550$.

FIG. 9. *Pseudohalonectria adversaria*. Ascus showing thimble-shaped ascus apparatus and free basal end. $\times 590$. FIGS. 10–15. *P. falcata*. Fig. 10. Mature ascus mounted in water. $\times 574$. Fig. 11. Falcate ascospore mounted in water showing cells filled with lipid droplets. $\times 608$. Fig. 12. Ascus mounted in lactic acid with azure A showing flattened ascus apical apparatus. $\times 737$. Fig. 13. System of ascogenous hyphae and developing tortuous paraphyses. $\times 440$. Fig. 14. Paraphysis mounted in water showing the large, pseudoparenchymatic cells of which it is comprised. $\times 827$. Fig. 15. Asci and paraphyses attached to the system of ascogenous hyphae. $\times 737$. FIG. 16. *P. longirostrum*. Filiform and sigmoidal ascospores. $\times 800$.

FIGS. 17 and 18. *Pseudohalonectria longirostrum*. Fig. 17. Asci mounted in lactic acid and azure A showing sigmoidal shape and the thimble-shaped apical apparatus. $\times 780$. Fig. 18. Thimble-shaped apical apparatus. $\times 2960$. FIGS. 19–22. *P. lutea*. Fig. 19. Ascospore mounted in lactic acid with azure A showing prominent septa. $\times 1033$. Fig. 20. Ascospore mounted in water showing cells filled with lipid droplets and different consistency of cell contents of the end cells. $\times 944$. Fig. 21. Ascus with free basal end. $\times 605$. Fig. 22. Ascus apex with thimble-shaped ascus apparatus. $\times 990$. FIGS. 23–26. *P. phialidica*. Fig. 23. Ascus with free basal end. $\times 878$. Fig. 24. Asci and paraphyses attached to the system of ascogenous hyphae. $\times 428$. Fig. 25. Filiform ascospore with cells filled with lipid droplets and characteristic regions devoid of lipid droplets. $\times 600$. Fig. 26. Phialides with developing falcate conidia. $\times 2000$.







discharge, and lying free in venter cavity. Paraphyses long, acuminate, 1- to 4-septate, attached to ascogenous hyphae, $144-240 \times 7.2-9.6 \mu\text{m}$. Ascospores (Figs. 16, 33X) hyaline to pale yellow, yellow to orange in mass, 4- to 8-septate, filiform, or sigmoid, cells filled with lipid, $84-105.6 (\bar{x} = 91.3) \times 3.0-4.0 (\bar{x} = 3.6) \mu\text{m}$.

HOLOTYPE: A dried culture grown on alfalfa stems, isolated from a twig from Shannon Creek, Barro Colorado Island, Panama, March 1981. CS-656-1, NY.

TYPE CULTURE: ATCC 52674.

ETYMOLOGY: From Latin *longus* meaning long and *rostrum* meaning beak, referring to the long beaks.

Pseudohalonectria longirostrum formed soft-rot cavities on balsa and beechwood in this study, and on ash and cottonwood in another study (Zare-Maivan and Shearer 1988). In paired culture with other aquatic Ascomycetes and Fungi Imperfecti, *P. longirostrum* inhibited the growth of all species at a distance, (Shearer and Zare-Maivan 1988) and culture filtrates inhibited the growth of several yeasts (A. Asthana and C. A. Shearer, unpublished).

***Pseudohalonectria lutea* Shearer sp. nov.**

Figs. 19-22, 33R-33T

Ascomata in ligno immersa, luteo-brunnea, membranacea, rostrata; venter globosus vel subglobosus, $290-540 \mu\text{m}$ in diam.; rostrum cylindricum, periphysatum, $300-600 \times 160-200 \mu\text{m}$. Asci hyalini, cylindrici, $122-192 \times 14.4-18 \mu\text{m}$, apparatu apicali digitaliformi, a hyphis ascogenis separata et in ventre liberi jacentes. Ascospores hyalinae vel pallide aurantiacae, in cumulo aurantiacae, ellipsoidales, 5-septate, $99-68 (\bar{x} = 56.6) \times 4.8-8.4 (\bar{x} = 6.7) \mu\text{m}$.

HOLOTYPE: NY.

Colonies on CMA floccose, yellow, becoming greyish yellow with age, dull yellow in reverse, yellow pigment diffuses from colony into the agar; hyphae immersed and superficial. Ascomata (Fig. 33R) on wood immersed, yellowish brown, membranous, rostrate, venter globose to subglobose, $290-540 \mu\text{m}$ diam.; peridium of tissue textura angularis in face view; beak cylindrical, periphysate $300-600 \times 160-200 \mu\text{m}$. Asci (Figs. 21, 22, 33T) hyaline, cylindrical, $122-192 \times 14.4-18 \mu\text{m}$, with thimble-shaped ascus apparatus, asci separating from ascogenous hyphae and lying free in venter cavity. Ascospores (Figs. 19-20, 33S) hyaline to pale orange, end cells less pigmented than inner cells, orange in mass, ellipsoidal, 5-septate, $48-68 (\bar{x} = 56.5) \times 4.8-8.4 (\bar{x} = 6.7) \mu\text{m}$.

HOLOTYPE: Slide of a specimen from submerged wood collected from a small stream at Esteros Llanquehue, Region X, Chile. 26 June 1984, CS-744-1A, NY.

ISOTYPE: Additional slides of the holotype collection, CS-744-1B-D.

TYPE CULTURE: ATCC.

ADDITIONAL MATERIAL EXAMINED: On submerged wood

from a stream originating from a thermal spring at Aguas Calientes near Puyehue, Region X, Chile. 13 July 1984, CS-744-2.

Little material was available in both collections and it was impossible to remove intact ascomata from the extremely hard wood in which they were embedded. Thus far cultures have produced only one ascoma. With such limited material, therefore, a number of features such as perithecial dimensions and structure and staining characteristics of the ascus apical apparatus could not be determined. The ascospores of *P. lutea* are distinctive in that the end cells differ from the inner cells in consistency and pigmentation. The end cells lose their cytoplasmic contents while the inner cells retain theirs and ascospores with empty end cells were encountered frequently. *Pseudohalonectria lutea* caused soft-rot cavities in balsa and beech wood in this study and is antagonistic at a distance to other filamentous fungi (A. Asthana and C. A. Shearer, unpublished).

***Pseudohalonectria phialidica* Shearer sp. nov.**

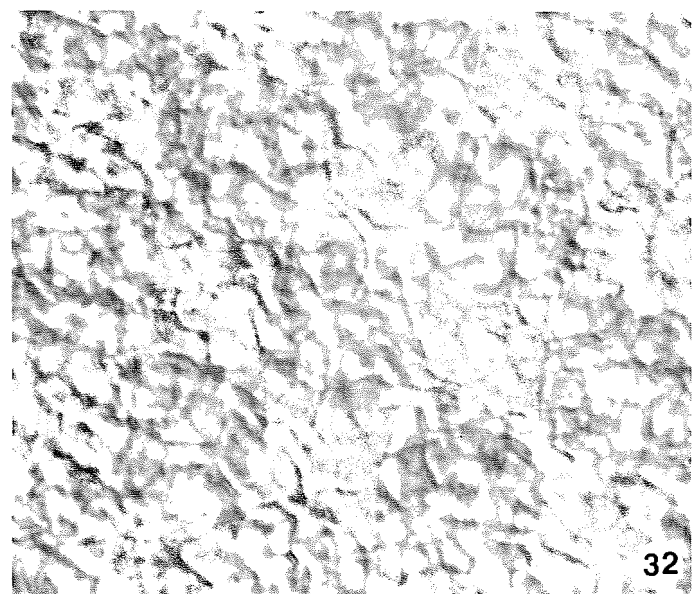
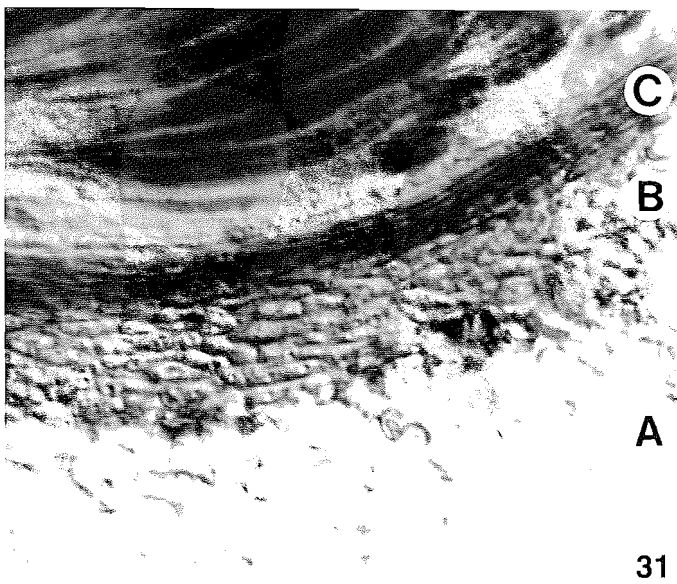
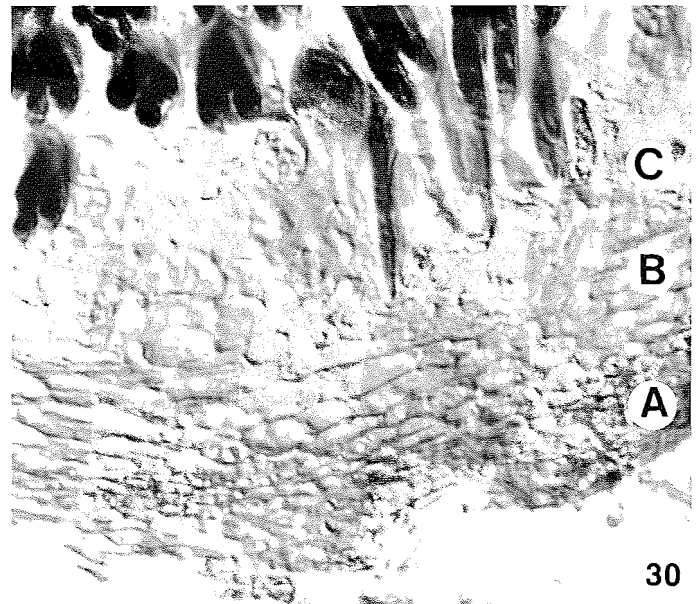
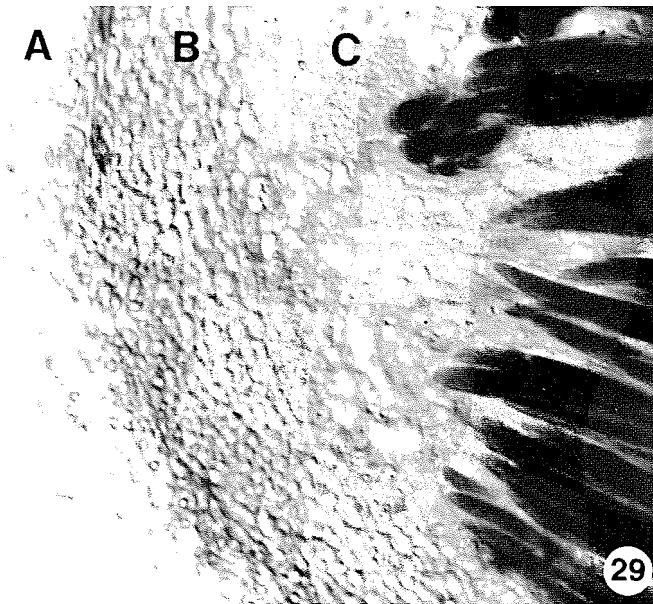
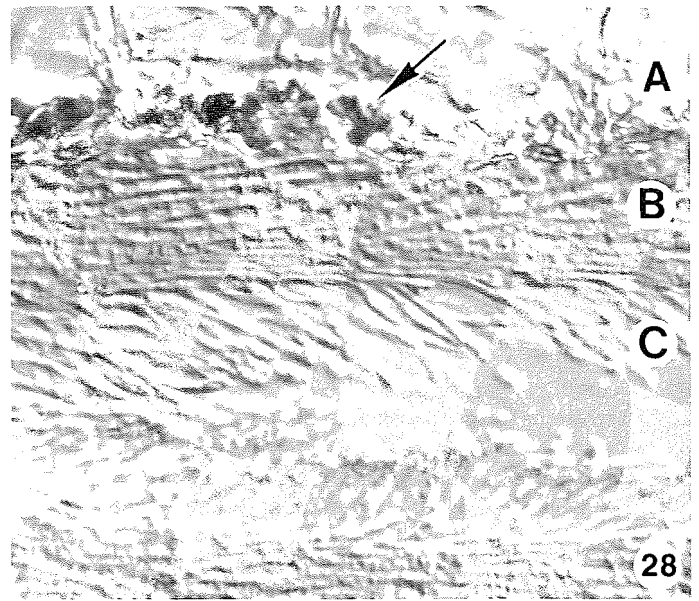
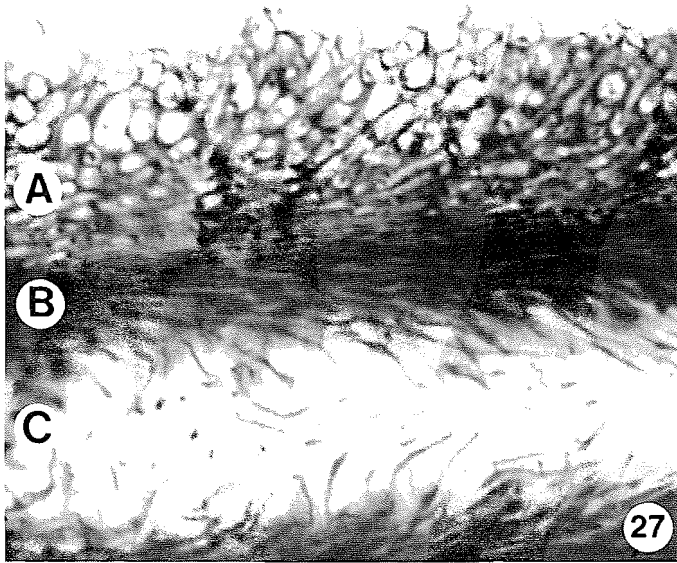
Figs. 23-26, 33M-33Q

Ascomata in ligno disserta vel aggregata, immersa vel partim immersa rostrata, lutea vel brunnea; venter applanato-globosus, $129-366 \times 257-426 \mu\text{m}$, peridio membranaceo, a textura angulari compositum; rostra cylindrica, longa, $614-1940 \times 89-129 \mu\text{m}$, periphysata, e hyphis parallelis composita eorum cellulae terminales parum inflatae, rotundatae. Asci pallide lutei, cylindrici, recti vel sigmoidales, $82-99 \times 5-7.9 \mu\text{m}$, apparatu apicali digitaliformi, in liquore iodato haud tincto, $3.2-5.0 \times 2-3.5 \mu\text{m}$, ad septum basale a hyphis ascogenis separati et in ventre liberi jacentes. Paraphyses 2- ad 4-septatae, $132-168 \times 4.8-9.6 \mu\text{m}$ ad basim attenuatae, ad apicem leniter rotundatae. Ascospores hyalinae, in cumulo luteae, demum aurantiacae, filiformes, subcurvatae vel sigmoidales, $64.5-79 (\bar{x} = 72.4) \times 2 \mu\text{m}$, 0- ad 4-septatae. Anamorphia hyphomycetum instar, phialidica. Phialides micronematae, cylindricae, ad apicem attenuatae, $9.9-26.7 \times 2-4 \mu\text{m}$, collarulo $2-3 \mu\text{m}$ lato. Conidia allantoidea, haud septata, $2-4 \times 0.5-1 \mu\text{m}$.

HOLOTYPE: NY.

Colonies on cornmeal agar floccose, appressed, cream colored, becoming tinged with grey, colony reverse yellow. Hyphae septate, smooth walled, branched, hyaline to pale yellow, $1-3.5 \mu\text{m}$ diam. Ascomata (Fig. 33M) on wood solitary to somewhat aggregated, immersed, partially immersed, or superficial; venter flattened globose, yellow becoming greyish yellow $129-366 \times 257-426 \mu\text{m}$, peridium membranous, of tissue textura angularis in face view, brownish yellow, inner layer of laterally compressed hyphae, yellow, paler towards venter cavity, cells of outer layer less compressed and surrounded on the outside by loosely interwoven hyphae bearing orange-brown amorphous material (Fig. 31); beaks cylindrical, long, $614-1946 \times 89-129 \mu\text{m}$, periphysate, comprising

FIG. 27. *Pseudohalonectria lignicola*. Longitudinal section through the beak showing (A) the outer layer of outwardly directed hyphae of enlarged cells; (B) the inner layer of compressed, parallel hyphae of narrow, elongate cells; and (C) periphyses. $\times 910$. FIG. 28. *P. phialidica*. Longitudinal section through the beak showing (A) the outer layer of loosely woven hyphae (arrow indicates orange, amorphous material deposited on the surface of ascomata); (B) the inner layer of parallel hyphae comprising narrow, elongate cells; and (C) periphyses. $\times 950$. FIG. 29. *P. falcata*. Longitudinal section through the venter showing (A) the outer layer of loosely woven hyphae; (B) the middle layer of pseudoparenchyma; and (C) the hymenium. $\times 625$. FIG. 30. *P. lignicola*. Longitudinal section through the venter showing (A) the outer layer of compressed pseudoparenchyma; (B) the middle layer of pseudoparenchyma; and (C) the hymenium. $\times 780$. FIG. 31. *P. phialidica*. Longitudinal section through the venter showing (A) the outer layer of loosely woven hyphae; (B) the middle layer of pseudoparenchyma with slightly elongate and laterally compressed cells; and (C) the inner layer of tightly compressed pseudoparenchyma. $\times 935$. FIG. 32. *P. lignicola*. Face view of peridium tissue. $\times 1190$.



an inner layer of parallel hyphae with elongated cells, middle layer of outwardly divergent hyphae, and an outer layer of loosely interwoven hyphae on which orange-brown amorphous material is deposited (Fig. 28). Asci (Figs. 23, 24, 33N) pale yellow, cylindrical, straight or sigmoid, $82-99 \times 5-7.9 \mu\text{m}$, with IKI-negative thimble-shaped apical apparatus (Fig. 33P) that stains bright blue in slightly acidic aqueous cotton blue, $3.2-5.0 \times 2-3.5 \mu\text{m}$, detaching from ascogenous hyphae at the basal septum and lying free in venter cavity. Paraphyses (Fig. 24) longer than asci, broad at base and tapering to a gently rounded point, attached to ascogenous hyphae, $132-168 \times 4.8-9.6 \mu\text{m}$ at base. Ascospores (Figs. 25, 33O) hyaline, yellow in mass, becoming orange, filiform, slightly curved or sigmoid, $64.5-79$ ($\bar{x} = 72.4$) $\times 2 \mu\text{m}$, 0- to 4-septate, with oil droplets interrupted at regular intervals by nonrefractile regions. Anamorph (Figs. 26, 33Q) hyphomycetous, phialidic. Phialides hyaline, micronematous, flask shaped, $9.9-26.7 \times 2-4 \mu\text{m}$, collarette $2-3 \mu\text{m}$ wide. Conidia allantoid, hyaline, nonseptate, $2-4 \times 0.5-1 \mu\text{m}$.

HOLOTYPE: A dried culture grown on balsa wood, CS-719-1, derived from a single ascospore from an ascoma on submerged woody debris from the Salt Fork of the Vermilion River, Vermilion County, IL. NY.

TYPE CULTURE: ATCC 56674.

ETYMOLOGY: From Latin *phiala* meaning a small closed vessel, referring to conidiogenous cell of the anamorph.

This species was the only one in which an ascus apical refractile body, characteristic of the Sordariales was observed. The refractile body was quite small and regular and disappeared after fixation in lactic acid. *Pseudohalonectria phialidica* is the only species that formed an anamorph state in culture. The phialidically produced conidia did not germinate on water agar or CMA and may be spermatia rather than conidia. Although ascospores germinated by producing germ tubes, phialides were found on some ascospores. *Pseudohalonectria phialidica* formed soft-rot cavities on balsa and beechwood.

Discussion

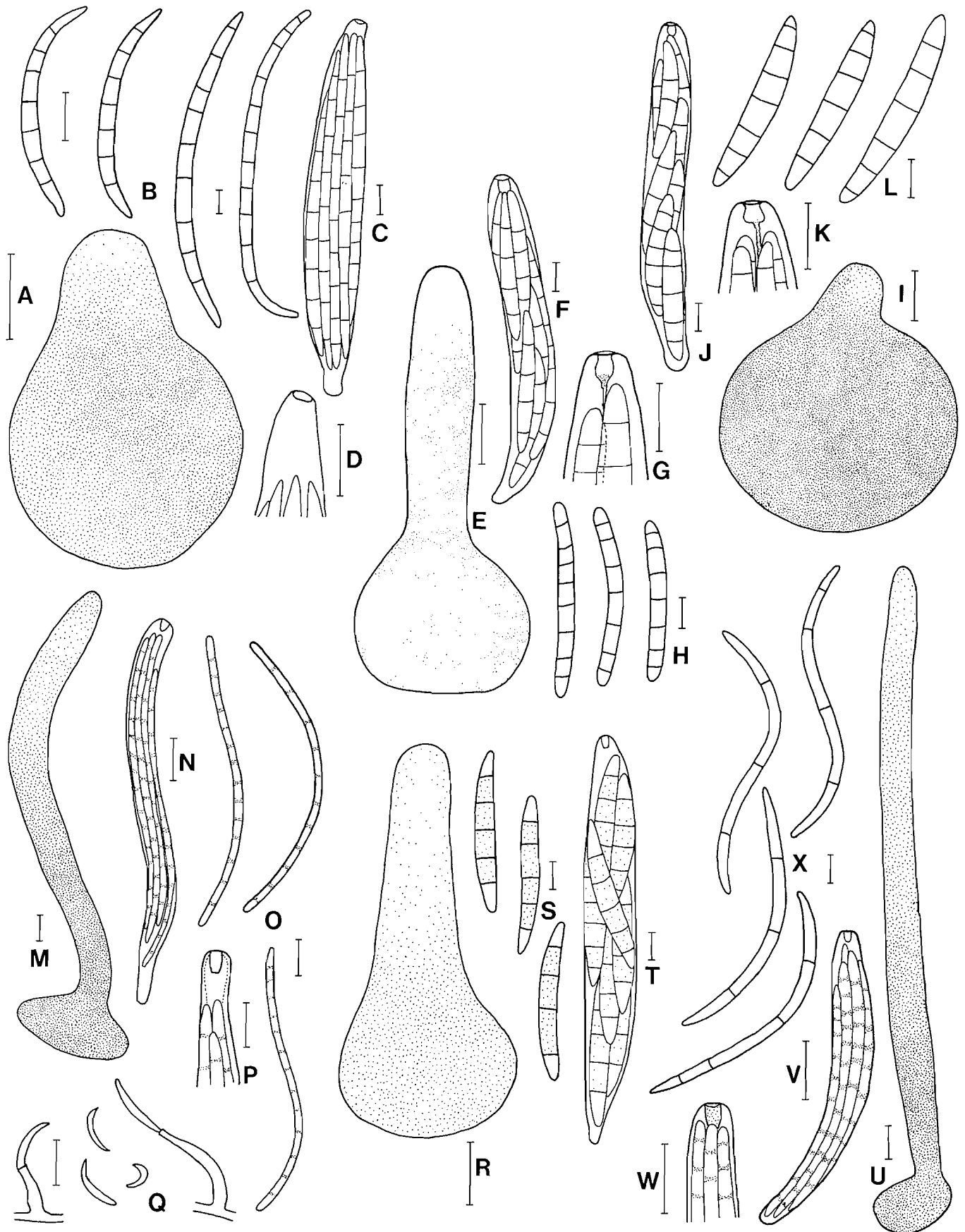
When the genus *Pseudohalonectria* was established by Minoura and Muroi (1978), they did not mention the placement of this taxon at the class or family level. The authors note its similarity to *Halonectria milfordensis* Jones, a monotypic genus which Jones (1965) placed near *Trailia* and *Orcadia* in the Hypocreales. *Pseudohalonectria* does not belong in the Hypocreales because it has paraphyses that originate at the base, grow upwards and are persistent, while paraphyses in the Hypocreales originate from the perithecial apex just below the paraphyses, grow downwards, and often are not evident at maturity (Hanlin 1961, 1964, 1965, 1971).

Although a developmental study of *Pseudohalonectria* was not made, examination of thin sections and squash mounts of ascomata revealed a Diaporthe-type centrum sensu Luttrell (1951) as modified by Jensen (1983). The most distinctive features of this centrum type are (i) asci that are separate from ascogenous hyphae and lie free in the venter cavity, (ii) promi-

nent pseudoparenchyma, (iii) paraphyses, and (iv) a hemispherical basal hymenium. All of these features, except presence of a prominent central pseudoparenchyma, were seen in mature ascomata. Pseudoparenchyma is present subtending the hymenial layer and forming the peridium; the amount of pseudoparenchyma varies among species. Developmental studies are necessary to determine whether a central pseudoparenchyma exists in early developmental stages in *Pseudohalonectria*.

The Diaporthe centrum is characteristic of both the Diaporthales and Sordariales (Luttrell 1951), thus placement of *Pseudohalonectria* at the ordinal level is problematic. In many aspects, such as membranous ascomata composed of tissue textura angularis, cylindrical ascus shape, thimble-shaped apical apparatus, asci that separate from ascogenous hyphae, septate paraphyses, phragmoseptate scolecosporous ascospores, and habitat, *Pseudohalonectria* is very similar to *Ophioceras dolichostomum* (Berk. & Curt.) Sacc. and *O. leptosporum* (Iqbal) Walker. Conway and Barr (1977) in a redescription of *O. dolichostomum* were faced with a similar dilemma in taxonomic assignment to order. They placed *Ophioceras* in the Sordariales sensu Barr (1976) and Huang (1976) rather than the Diaporthales because of the peridium structure and hypersaprobic habit. In the Sordariales, peridium tissue is of the textura angularis type and comprises large, pseudoparenchyma cells, while in the Diaporthales, peridium tissue is of textura epidermoidea or angularis (Jensen 1985). Peridia of all six species of *Pseudohalonectria* comprise tissue textura angularis when examined in face view, but there is considerable variation among species in the size of the pseudoparenchyma cells. The presence of a phialidic anamorphic state and ascospores that form phialides, as in *P. phialidica*, supports placement in the Sordariales, especially in the Lasiosphaeriaceae where species commonly form a phialidic state in culture (Lundqvist 1972; Kendrick and DiCosmo 1979). In contrast, imperfect states in the Diaporthales are more variable and include mostly acervular, pycnidial forms, although phialidic states have also been noted (Barr 1978; Kendrick and DiCosmo 1979). That all six species were found on rotting wood is more consistent with the general habitat of the Sordariales than that of the Diaporthales. Whether *Pseudohalonectria* is truly hypersaprobic has not been demonstrated and all species, through the formation of soft-rot cavities, are able to degrade wood that has not been invaded by other fungal species. In addition, some Diaporthalean fungi such as *Gnomonia* are common as woody saprophytes. Thus the case for habitat does not seem all that strong. The long necks characteristic of some *Pseudohalonectria* species and *Ophioceras*, the chitinous ascus apical apparatuses, and asci which separate from ascogenous hyphae are characters more strongly related to the Diaporthales than the Sordariales. *Pseudohalonectria* and *Ophioceras* clearly link the Diaporthales and Sordariales and lend further support to relationships established through perithecial developmental studies. Jensen (1983), in agreement with comments by Uecker (1976), noted that "... developmental patterns in the taxa represented by the *Xylaria*, *Sordaria*, and *Diaporthe* centrum types are variations on the same basic theme."

FIG. 33. *Pseudohalonectria* species. Scale bars for all figures except A, E, I, M, R, and U = $10 \mu\text{m}$; scale bars for A, E, I, M, R, and U = $100 \mu\text{m}$. (A-D) *P. falcata*. A, ascoma; B, ascospores; C, ascus; D, ascus apex. (E-H) *P. lignicola*. E, ascoma; F, ascus; G, ascus apex; H, ascospores. (I-L) *P. adversaria*. I, ascoma; J, ascus; K, ascus apex; L, ascospores. (M-Q) *P. phialidica*. M, ascoma; N, ascus; O, ascospores; P, ascus apex; Q, phialidic state. (R-T) *P. lutea*. R, ascoma; S, ascospores; T, ascus. (U-X) *P. longirostrum*. U, ascoma; V, ascus; W, ascus apex; X, ascospores.



Pseudohalonectria seems most closely related to lignicolous taxa in the Sordariales (as Sordariaceae in Carroll and Munk 1964) and to *Ophioceras*. I thus follow Conway and Barr (1977) in their placement of *Ophioceras* by placing *Pseudohalonectria* in the Lasiosphaeriaceae of the Sordariales.

Pseudohalonectria spp. can be distinguished from most genera in the Lasiosphaeriaceae by their bright yellow, membranous ascomata. Ascomata of most taxa in the Lasiosphaeriaceae are dark brown to black. One exception is *Mycomedusispora flavida* (Rick) Carroll and Munk. This species has membranous, yellow perithecia and long vermiform, 1-septate ascospores which fragment into small sections of irregular length. *Pseudohalonectria* differs from this monotypic genus in having ascospores that are multiseptate, but which do not fragment at maturity, and in asci which are not ventricose and which, except for *P. phialidica*, lack a subapical refractive globule. *Mycomedusispora flavida* was collected from a very humid cloud forest and Carroll and Munk (1964) considered that its outstanding characters give it the appearance of an extremely hygrophytic representative of the Lasiosphaeria group.

Pseudohalonectria is also closely related to *Ophioceras*, in habitat, in all aspects of morphology except pigmentation and firmness of ascomata, and in method of ascospore discharge, which will be discussed later. The ascospores of *Pseudohalonectria* spp. are similar to those of *Lasio-sphaeria lapaziana* Carroll & Munk and *L. raciborski* (Penz. & Sacc.) Carroll & Munk, species found on rotting wood in a moist cloud forest and a lowland tropical rain forest, respectively. The ascomata of *L. lapaziana* are black and carbonaceous while those of *L. raciborski* are black with thick-walled black hairs, characters not found in *Pseudohalonectria*. It appears that within the Lasiosphaeriaceae, taxa in several related genera, *Lasio-sphaeria*, *Mycomedusispora*, *Ophioceras*, and *Pseudohalonectria*, are found in water-soaked or submerged rotting wood and have long, cylindrical, or filiform ascospores. The Lasiosphaeriaceae is a family much in need of re-evaluation (Carroll and Munk 1964; Lundqvist 1972), especially the lignicolous taxa. An intriguing question is whether the coprophilous and lignicolous taxa represent two distinct evolutionary lines within the Lasiosphaeriaceae.

Although asci become detached from ascogenous hyphae at the ascus basal septum and lie free in ascomatal cavities in all *Pseudohalonectria* species, method of ascospore discharge varies within and among *Pseudohalonectria* species. All species, when examined on natural substrata in moist chambers, discharged ascospores through their beaks and ascospores accumulated in a mass at the beak apices. The oozing of spores to form globules is thought to indicate less than favorable

environmental conditions (Luttrell 1957; Conway and Kimbrough 1978). Three of the four species that fruited abundantly in culture, *P. adversaria*, *P. lignicola*, and *P. phialidica*, ejected ascospores forcibly from perithecia on incubated balsa or beechwood strips on agar in tubes and Petri dishes. A fourth species, *P. falcata*, did not forcibly discharge ascospores, rather, they accumulated in a mass at the beak apex. When perithecia of the above four species were placed in water, both ascospores and asci were discharged from the beak, with asci and ascospores frequently assuming a sigmoid shape. In *P. adversaria*, *P. falcata*, and *P. lignicola*, ascospores in asci discharged into the water were released from the posterior end of the ascus by the breakdown of the ascus wall. This type of ascospore release was particularly evident in *P. falcata* and germ tubes emanating from ascospores contained in asci also were frequently observed. In *P. phialidica*, in asci released into water, ascospores were discharged through the apical pore. Different environmental conditions may effect different types of discharge mechanisms in the same fungus. It is likely that all mechanisms of discharge come into play in nature, where the woody substrata of *Pseudohalonectria* species may be submerged, partially submerged, or washed onto floodplains where they are exposed. Conway and Kimbrough (1978) observed the discharge of intact asci by *Ophioceras dolichostomum* (Berk. & Curt.) Sacc., a species closely related to *Pseudohalonectria* species and found on wood in water. Fazzani and Jones (1977) also reported the discharge of asci and ascospores from a long-necked aquatic pyrenomycete from water-cooling towers which they identified as *Sillia ferruginea* (Pers. ex Fries) Karsten, but which is most likely a species of *Ophioceras*. Whether the discharge of intact asci confers an advantage to species in aquatic habitats and is a unique aquatic adaptation as suggested by Conway and Kimbrough (1978) remains to be determined.

One of the most intriguing aspects of *Pseudohalonectria* species is their ability to inhibit the hyphal growth of other fungi by the production of diffusible substances. *Pseudohalonectria longirostrum*, (as *Pseudohalonectria* sp. 1), *P. adversaria* (as *Lasio-sphaeria* sp. 1), *P. falcata* (as *Pseudohalonectria* sp. 2), and *P. lignicola* were among the five most inhibitory species of 25 aquatic Ascomycetes and Fungi Imperfecti grown in paired culture (Shearer and Zare-Maivan 1988). Present studies (A. Asthana and C. A. Shearer, unpublished) show that *P. lutea* is also inhibitory at a distance to other fungi. Studies are underway to characterize the range of fungi susceptible to inhibition by these fungi and to isolate and characterize the antagonistic compounds they produce.

Key to species

1. Ascospores 2 μ m or less in width *P. phialidica*
1. Ascospores wider than 2 μ m 2
 2. Ascospores mostly longer than 100 μ m *P. falcata*
 2. Ascospores mostly shorter than 100 μ m 3
3. Necks short (250 μ m or less) *P. adversaria*
3. Necks long (250 μ m or greater) 4
 4. Ascospores filiform (80 μ m or longer) *P. longirostrum*
 4. Ascospores cylindrical (shorter than 80 μ m) 5
5. Ascospores consistently 5-septate, end cells lighter than inner cells *P. lutea*
5. Ascospores 4- to 11-septate, hyaline or evenly pigmented throughout *P. lignicola*

Acknowledgements

Appreciation is expressed to Prof. D. P. Rogers for preparing the Latin descriptions and for his helpful comments on the manuscript. Appreciation is also expressed to Lisa Cerligione for technical assistance and to Dr. Carol Augspurger for collecting samples in Panama.

- BARR, M. E. 1976. Perspectives in the Ascomycotina. Mem. N.Y. Bot. Gard. **28**: 1–8.
- . 1978. The Diaporthales in North America with emphasis on *Gnomonia* and its segregates. J. Cramer, Lehre, Germany.
- CARROLL, G. C., and MUNK, A. 1964. Studies on lignicolous Sordariaceae. Mycologia, **56**: 77–98.
- CONWAY, K. E., and BARR, M. E. 1977. Classification of *Ophioceras dolichostomum*. Mycotaxon, **5**: 376–380.
- CONWAY, K. E., and KIMBROUGH, J. W. 1978. Release of ascospores and asci from the perithecia of *Ophioceras dolichostomum*. Mycologia, **70**: 895–898.
- EMERSON, R. 1958. Mycological organization. Mycologia, **50**: 589–621.
- FAZZANI, K., and JONES, E. B. G. 1977. Spore release and dispersal in marine and brackish water fungi. Mater. Org. (Berl.), **12**: 235–248.
- HANLIN, R. T. 1961. Studies in the genus *Nectria*. II. Morphology of *N. gliocladioides*. Am. J. Bot. **48**: 900–908.
- . 1964. Morphology of *Hypomyces trichothecoides*. Am. J. Bot. **51**: 201–208.
- . 1965. Morphology of *Hypocrea schweinitzii*. Am. J. Bot. **52**: 570–579.
- . 1971. Morphology of *Nectria haematococca*. Am. J. Bot. **58**: 105–116.
- HUANG, L. H. 1976. Developmental morphology of *Triangularia backusii* (Sordariaceae). Can. J. Bot. **54**: 250–267.
- JENSEN, J. D. 1983. The development of *Diaporthe phaseolorum* variety *sojae* in culture. Mycologia, **75**: 1074–1091.
- . 1985. Peridial anatomy and pyrenomyces taxonomy. Mycologia, **77**: 688–701.
- JONES, E. B. G. 1965. *Halonectria milfordensis* Gen. et sp. nov., a marine pyrenomyces on submerged wood. Trans. Br. Mycol. Soc. **48**: 287–290.
- KENDRICK, B., and DiCosmo, F. 1979. Teleomorph-anamorph connections in Ascomycetes. In The whole fungus. Vol. 1. Edited by B. Kendrick. National Museum of Canada, Ottawa, Canada.
- LUNDQVIST, N. 1972. Nordic Sordariaceae s. lat. Symb. Bot. Upsal. **20**: 1–374.
- LUTTRELL, E. S. 1951. Taxonomy of the Pyrenomyces. Univ. MO. Stud. **24**, No. 3, Columbia, MO.
- . 1957. Ascospore ejaculation in *Gaeumannomyces graminis*. Phytopathology, **47**: 242.
- MINOURA, K., and MUROI, T. 1978. Some freshwater Ascomycetes from Japan. Trans. Mycol. Soc. Jpn. **19**: 129–134.
- SHEARER, C. A., and ZARE-MAIVAN, H. 1988. *In vitro* hyphal interactions among wood- and leaf-inhabiting Ascomycetes and Fungi Imperfecti from freshwater habitats. Mycologia, **80**: 31–37.
- UECKER, F. A. 1976. Development and cytology of *Sordaria humana*. Mycologia, **68**: 30–46.
- ZARE-MAIVAN, H., and SHEARER, C. A. 1988. Extracellular enzyme production and cell wall degradation by freshwater lignicolous fungi. Mycologia, **80**: 365–375.