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.

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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.


L’auteur décrit et illustre le Pseudohalonectria lignicola et cinq nouvelles espèces de Pseudohalonectria recoltées à partir de bois submergé en eau douce. Les espèces sont dans l’ensemble morphologiquement semblables et produisent des pètiphiètes de jaune-brillant à brun, des asques munis 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 filamentueux et levures en cultures de confrontation; elles forment de plus des cavités de pourriture molle dans le bois de balsa et hêtre.

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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 sterile distilled water and the resulting ascospore suspension was plated on CMA agar (30 mL/dish) in plastic Petri dishes. Wood strips were inoculated 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 globoso to flattened globoso, 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, 1–5-septate. Asc (Figs. 3–5, 33F) uniloculate, 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 (x = 62.6) × 3.6–6.6 (x = 5.1) μm, 5- to 11-septate, not constricted at the septa, discharged forcibly or accumulating in a mass at the beak opening.


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 following 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, 331–33L

Ascomata superficialia vel partim immersa, brunneoaurantica, membranacea, rostrata; venter globosus vel subglobosus, 445–614 μm diametro; peridium e textura angulari constitituum; rostrum breve, cylindricum, 79–248 × 109–158 μm periphysatum. Ascii hyalini vel pallide aurantiaci, cylindrici vel clavati, 120–150 × 13–20 μm, apparatus apiculatus digitaliformis, liquore iodo haud tincto, 2.5–5 × 2.5–3 μm, apice ad porum apicale ab lacuna signato, apophysis ascogenis separati et in ventre libri facientes. Paraphyses septatae, 133–230 μm longae, 4.0–7.8 μm latae ad basim, ad acumen rotundatum attenuatae. Ascosporeae hyalinae, in cumulo aurantiacae vel pallide aurantiacae, ellipsoideae, 5-septatae vel nonnumquam 6-7-septatae, 33.5–49 (x = 41.9) × 4.5–7 (x = 5.9) μm.

Holotypus: NY.

Colonies on cornmeal agar floccose, with regular margin, aerial hyphae white, immersed hyphae creamy yellow, reverse dull yellow. Ascomata (Fig. 331) solitary to clustered, superficial or immersed, orange brown, membranous, rostrate; venter globosus, 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 × 109–158 μm, periphysate. Asci (Figs. 9, 33L) unitunicate, containing eight ascospores in two overlapping fascicles of four, hyaline to pale orange, cylindrical or clavate, 120–150 × 13–20 μm, with IKI-negative, thimble-shaped apical apparatus (Fig. 33K) 2.5–5 × 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 (x = 41.9) × 4.5–7 (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 ligne immersa, luteo-brunnea, membranacea, a hyphis stricte textis, luteis obducta, rostrata; venter globosus vel subglobosus, 277–535 μm diametro; peridium e textura angulari constitituum; rostrum cylindricum, apice rotundato, pallide luteum vel brunneo-luteum, periphysatum, 81–162 × 81–108 μm. Ascii hyalini, cylindrici vel cymbiformes, 106–244 × 14.4–21.6 μm, apparatus apicali placulitico, liquore iodo haud tincto. Paraphyses 2–5–septatae, ad septa nunc constrictae,.nunc non, 94–209 × 4.8–12 μm. Ascosporeae hyalinae, falcatae 6–ad 16-septatae, 97–166 (x = 135) × 4.2–7.2 (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. Ascospores (Figs. 10, 33C) hyaline, falcate, sometimes slightly sigmoid, 6- to 16-septate, 97 - 166 μm. Asci (Figs. 10, 33C) hyaline, cylindrical to cymbiform, sometimes sigmoidal, 106 - 244 × 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 × 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 (x = 135) × 4.2 - 7.2 (x = 5.8) μm.


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 a 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 ascospores, 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 balsa 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.

Fig. 16 - 18, 33U - 33X

Ascomata in ligno partim immersa, vivide lutea, in brunneo-lutea furcescentes, rostrata; venter globoso vel apllanato-globosus, 178 - 396 × 267 - 396 μm; rostrum praelongum, 1683 - 3712 × 118 - 168 μm, cylindricum, periphysatum, a capillus brevis in cellulam nodulem terminatis atque ab areolis capillarium longarum instar hypharum rectum. Ascì cylindrici, recti vel sigmoidales, apparatu apicali digitaliformi in liquore iodato haud tincto, brevi-stipitati, 94 - 120 × 8.4 - 12 μm, ad septum basale a hyphis ascogenis separati et in ventre liberi jacentes. Paraphyses acuminatae, 1-ad 4-septatae, 144 - 240 × 7.2 - 9.6 μm. Ascosporea hyalinae vel pallide luteae, in cumulo luteae vel aurantiacea, 4-ad 8-septatae, filiformes, rectae vel sigmoideae, 84 - 105.6 (x = 91.3) × 3.8 - 4.0 (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 globoso to flattened globose, 178 - 396 × 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 × 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 × 2 μm (Figs. 18, 33W), short stalked, 94 - 120 × 8.4 - 12 μm, separating from ascogenous hyphae at basal septum of ascus prior to ascospore discharge.
discharge, and lying free in venter cavity. Paraphyses long, acuminate, 1- to 4-septate, attached to ascogenous hyphae, 144-240 × 7.2-9.6 μm. 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 (x = 91.3) × 3.0-4.0 (x = 3.6) μm.

**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 μm in diam.; rostrom cylindricum, peripheratum, 300-600 × 160-200 μm. Ascii hyalini, cylindrici, 122-192 × 14.4-18 μm, apparatu apicali digitaliformi, a hyphus ascogeni separata et in venter liberi jacentes. Ascosporeae hyalinae vel pallide aurantiacae, in cumulo aurantiacae, ellipsoidales, 5-septatae, 99-68 (x = 56.6) × 4.8-8.4 (x = 6.7) μm.

**HOLOTYPUS:** 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 μm diam.; peridium of tissue textura angularis in face view; beak cylindrical, peripheratum 300-600 × 160-200 μm. Asci (Figs. 21, 22, 33T) hyaline, cylindrical, 122-192 × 14.4-18 μm, with thimble-shaped ascus apparatus, ascii 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, ellipsoidare, 5-septate, 48-68 (x = 56.5) × 4.8-8.4 (x = 6.7) μm.

**HOLOTYPUS:** Slides 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 Puheheu, 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 beechwood in this study and is antagonistic to 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 disseorta vel aggregata, immersa vel partim immersa rostrata, lutea vel brunnea; venter planato-globosus, 129-366 × 257-426 μm, peridio membranaceo, a textura angulari compositum; rostra cylindrica, longa, 614-1940 × 89-129 μm, peripherata, e hyphus parallelius composita eorum cellulae terminales parum inflatae, rotundatae. Asci pallide lutei, cylindrici, recti vel sigmoideae, 82-99 × 5-7.9 μm, apparatu apicali digitaliformi, in liquore iodato hauv tincto, 3.2-5.0 × 2-3.5 μm, ad septum basale a hyphis ascogenis separati et in venter liberi jacentes. Paraphyses 2-4-septatae, 132-168 × 4.8-9.6 μm ad basim attenuatae, ad apicem leniter rotundatae. Ascosporeae hyalinae, in cumulo luteae, demum aurantiacae, filiformes, subcurvatae vel sigmoideae, 64.5-79 (x = 72.4) × 2 μm, o-4 sepetatae. Anamorpha hyphomycetum instar, phialidia. Phialides micronematae, cylindraceae, ad apicem attenuatae, 9.9-26.7 × 2-4 μm, collari 2-3 μm lato. Conidia allantoidae, hau septata, 2-4 × 0.5-1 μm.

**HOLOTYPUS:** 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 μm diam. Ascomata (Fig. 33M) on wood solitary to somewhat aggregated, immersed, partially immersed, or superficial; venter flattened globose, yellow becoming greyish yellow 129-366 × 257-426 μm, 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 × 89-129 μm, peripherata, comprising...
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 x 5-7.9 µm, with IKI-negative thimble-shaped apical apparatus (Fig. 33P) that stains bright blue in slightly acidic aqueous cotton blue, 3.2-5.0 x 2-3.5 µm, 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, 64.5-79 (x = 72.4) x 2 µm, 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 x 2-4 µm, collarette 2-3 µm wide. Conidia allantoid, hyaline, nonseptate, 2-4 x 0.5-1 µm.

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 ascocarps revealed a Diaporthaceae-type centrum sensu Luttrell (1951) as modified by Jensen (1983). The most distinctive features of this centrum type are (i) ascii that are separate from ascogenous hyphae and lie free in the venter cavity, (ii) prominent 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 Diaporthaceae 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 scleosporous ascospores, and habitat, Pseudohalonectria is very similar to Ophioceras dolichostomum (Berk. & Curt.) Sacc. and O. leptosorum (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 Diaporthaceae fungi such as Gnomonia are common as saprophytes. Thus the case for habitat does not seem all that strong. The long necks characteristic of some Pseudohalonectria species and Ophioceras, the chitinoïd 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 "... development patterns in the taxa represented by the Xylaria, Sordaria, and Diaporthaceae centrum types are variations on the same basic theme."

**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 multisepate, 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 *Lasiosphaeria 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, *Lasiosphaeria*, *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 ascii become detached from ascogenous hyphae at the ascus basal septum and lie free in ascocatal 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 (Luttrel 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 beechnwood 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. Fazzanti 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 Lasiosphaeria 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.

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**Key to species**

1. Ascospores 2 μm or less in width .................................................. *P. phialidica* .......................... 2

2. Ascospores wider than 2 μm .................................................. *P. falcata* .......................... 3

3. Asci mostly longer than 100 μm .................................................. *P. adversaria* .......................... 4

4. Asci mostly shorter than 100 μm .................................................. *P. longirostrum* .......................... 5

5. Ascospores consistently 5-septate, end cells lighter than inner cells .................................................. *P. lutea* .......................... 5

6. Ascospores 4- to 11-septate, hyaline or evenly pigmented throughout .................................................. *P. lignicola*
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