New records and new taxa of fungi from the mycorrhizae of terrestrial orchids of Alberta

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Pure cultures of endophytic fungi were obtained from the mycorrhizae of some native Alberta orchids (*Amerorchis rotundifolia, Calypso bulbosa, Coeloglossum viride, Corallorhiza maculata, Platanthera dilatata, P. hyperborea, P. obtusata*). Isolates of *Rhizoctonia* constituted the largest group of endophytes, but few could be identified to species. One of these strains was identified as *Rhizoctonia repens*, a species reported to be a ubiquitous and common mycorrhizal fungus of orchids. Another strain produced the teleomorph stage and was identified as *Ceratobasidium obscurum*. ***Rhizoctonia anaticula*** Currah, sp.nov. is described based on five isolates bearing monilioid cells linked by prominent, narrow connections. A second group of isolates consisted of sterile, greenish black to grey fungi. After prolonged incubation, eight of these isolates formed conidia. Two isolates produced phialoconidia and were identified as *Phialocephala fortinii*. Five isolates, demonstrating sympodial development of conidia in the apical region of swollen or unswollen hyaline conidiogenous cells, are disposed in ***Leptodontidium orchidicola*** Sigler & Currah, sp.nov. The remaining conidial isolate was identified as *Trichocladium opacum*. Two isolates, resembling *Rhizoctonia* in cultural features, sporulated and are described as ***Trichosporiella multisporum*** Sigler & Currah, sp.nov.

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Les auteurs ont obtenu des cultures pures de champignons endophytes à partir des mycorhizes de quelques orchidées indigènes de l'Alberta (*Amerorchis rotundifolia, Calypso bulbosa, Coeloglossum viride, Corallorhiza maculata, Platanthera dilatata, P. hyperborea, P. obtusata*). Des isolats appartenant aux *Rhizoctonia* spp. constituent la majeure partie des endophytes mais très peu peuvent être identifiés à l'espèce. Un de ces isolats a été identifié comme *Rhizoctonia repens*, une espèce rapportée comme uniquiste ainsi que comme endophyte mycorhizien courrant chez les orchidées. Une autre souche produit un stade téléomorphique et a pu être identifié comme *Ceratobasidium obscurum*. Les auteurs décrivent le ***Rhizoctonia anaticula*** Currah, sp.nov. en se basant sur cinq isolats qui produisent des cellules monilioïdes réunies par de minces connections proéminentes. Un deuxième groupe d'isolats est constitué par des souches stériles gris à noir-verdâtre. Après incubation prolongée, huit de ces isolats ont formé des conidies. Deux isolats ont produit des phialoconidies et ont été identifés comme *Phialocephala fortinii*. Cinq isolats montrant un développement sympodial des conidies dans la région apical de cellules conidiogènes hyalines, enflées ou non enflées, sont attribués à ***Leptodontidium orchidicola*** Sigler & Currah, sp.nov. Les autres isolats ont été identifiés comme *Trichocladium opacum*. Deux isolats ressemblant à des *Rhizoctonia* ont sporulé et sont décrits comme ***Trichosporiella multisporum*** Sigler & Currah, sp.nov.

[Traduit par la revue]

Introduction

Most studies dealing with the isolation and (or) classification of orchid endophytic fungi have emphasized the recovery and at least partial characterization of strains assignable to *Rhizoctonia* (Alexander and Hadley 1983; Bernard 1909; Burgeff 1936; Curtis 1939; Gaümann *et al.* 1960; Harvais 1973; Harvais and Hadley 1967; Nishikawa and Ui 1976). The most detailed taxonomic studies of orchid endophytic fungi have been conducted by Warcup and Talbot (1967, 1971, 1980), who have worked primarily with isolates from Australian terrestrial orchids. Virtually no equivalent taxonomic work has been done with isolates from north temperate orchids. Furthermore, routine isolation procedures involving orchid roots and associated structures (such as tuberoids, rhizomes, etc.) usually yield a variety of fungi belonging to Hyphomycetes and Mycelia sterilia. No work has been done on the taxonomy of these fungal endophytes of orchids.

In 1985, we initiated an investigation into the mycorrhizal relationships of terrestrial orchids of Alberta. We found that the fungal endophytes in the subterranean organs (roots, rhizomes, tubers), when isolated in pure culture, initially could be divided among three groups: *Rhizoctonia, Mycelium radicis atrovirens* (MRA) (Melin 1922), and Hyphomycetes.

Rhizoctonia was the most common taxon isolated, but numerous culturally and morphologically distinct strains were recovered. One of these strains was identified as *Rhizoctonia repens* Bernard, a species routinely referred to in the literature as a ubiquitous orchid endophyte (Hadley 1982). Only a few brief descriptions of the taxon's cultural and morphological features are available (Bernard 1909; Burgeff 1936; Curtis 1939; Saksena and Vaartaja 1961). Therefore a full description is given here based on this new isolate from Alberta. *Rhizoctonia anaticula* Currah, sp.nov., represented by five different isolates, is described as new based primarily on the unique morphology of its monilioid cells. In another *Rhizoctonia* isolate, the teleomorph formed and was identified as *Ceratobasidium obscurum*. The cultural and morphological characteristics of this little-known species are described in detail.

A number of our isolates initially grew in the form of sterile, dark mycelium, but different growth rates and minor cultural differences among strains indicated that this group was a heterogeneous assemblage of taxa. These nonsporulating, dematiaceous isolates were initially identified as *Mycelium radicis atrovirens* (MRA) (Melin 1922). The name MRA has been applied to nonsporulating, dematiaceous fungi associated with the roots of conifers. In 1985, Wang and Wilcox identified some isolates of MRA as the hyphomycetes *Phialocephala fortinii, Phialophora finlandia*, and *Chloridium paucisporum*. After prolonged incubation under a variety of

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conditions, eight of our isolates sporulated. Two isolates could be identified as *Phialocephala fortinii*; one isolate is *Trichocladium opacum* (Corda) Hughes. Five other isolates represent a new species, *Leptodontidium orchidicola* Sigler & Currah, sp.nov.

One hyaline hyphomycetous endophyte was recovered from two different orchids. It is described as *Trichosporiella multisporum* Sigler & Currah, sp.nov.

Descriptions of these new records and new taxa of orchid endophytic fungi are provided here along with some discussion of their taxonomy and ecology.

Materials and methods

Healthy root–rhizome–tuber segments were obtained from collections, made during the summer of 1985, of *Amerorchis rotundifolia* (Banks) Hulten, *Calypso bulbosa* (L.) Oakes, *Coeloglossum viride* (L.) Hartm., *Corallorhiza maculata* (Raf.) Raf., *Platanthera dilatata* (Pursh) Lindl., *P. hyperborea* (L.) Lindl., and *P. obtusata* (Banks ex Pursh) Lindl. (Orchid nomenclature follows Luer (1975).)

Segments were surface sterilized in a 20% solution of household bleach for 1 min, rinsed twice in sterile distilled water, and decorticated with a sterile scalpel. Clumps of cells were removed from the inner cortex, macerated in a drop of sterile water, and plated in molten modified Melin–Norkran's agar (MMN, Marx 1969) cooled to 55°C. Plates were allowed to solidify and were incubated in the dark at 18°C until hyphae were visible growing from the cortical cells into the medium. Hyphal tips were transferred to potato dextrose agar (PDA, Difco) and serially transferred until pure cultures were obtained. Growth rates were determined by transferring a small fragment of mycelium (approximately 1 mm^2) to the periphery of a PDA plate. Radial increase was measured in two directions every 48 h over a 2-week period, using plates incubated at 18°C in the dark. Growth rates represent averages based on three replications. *Rhizoctonia* strains were also grown on cornmeal agar (CMA, McGinnis 1980) and tap water agar (TWA, 20 g agar/1000 mL tap water), and examined after 3 weeks for formation of sclerotia, and other cultural characteristics. To induce sporulation in strains of MRA, cultures on PDA and cereal agar (CER, Padhye *et al.* 1973) were sealed with Parafilm, incubated at 22°C for 6 weeks, and then transferred to a 4°C incubator. Plates were examined for the presence of conidia at 4 and 6 months. For microscopic examinations, fertile hyphae were stained with lactofuchsin (Carmichael 1955) and mounted in glycerin jelly.

Type specimens and ex-type cultures are maintained in the University of Alberta Microfungus Collection and Herbarium (UAMH). Subcultures have been deposited in the Commonwealth Agricultural Bureaux International Mycological Institute.

Results and discussion

Rhizoctonia repens Bernard 1909, Ann. Sci. Nat. Bot. 9: 26–31 Figs. 1–3

On PDA colonies 5–7 cm diam. after 28 days (hourly growth rate at 18°C approximately 0.06 mm/h). Margin submerged, entire, glabrous, white to cream. Vegetative mycelium mostly growing beneath the surface of agar media. Aerial mycelium sparse, forming in irregular, low, cream to yellowish patches. On CMA, mycelium nearly completely submerged, white to cream. Sclerotia minute, submerged, scattered, undifferentiated, loosely arranged clusters of monilioid cells (Figs. 1–3). Vegetative hyphae septate, hyaline, constricted at branch points; the first septum of lateral branches arising 2–6 μm beyond their point of origin; main hyphae 2.5–3.5 μm diam. Monilioid cells thin-walled, hyaline, ellipsoidal to nearly spherical, 13–18 × 8–17 μm in short, branched or unbranched chains (Figs. 1, 2), occasionally forming larger clusters (Fig. 3). On TWA, mycelium very sparse, submerged with short chains of monilioid cells forming at points along the main hyphae.

MATERIAL EXAMINED: UAMH 5430 ex root of *Platanthera obtusata*, Grassi Lakes, Alberta.

Bernard (1909) provided an illustration of the monilioid cells and pelotons formed in culture of the fungus he named *R. repens*. The main distinguishing features of the taxon were the creamy white colour of the mycelium and the presence of monilioid cells in short chains. Curtis (1939) took up Bernard's name for some orchid isolates from Wisconsin but noted that in his strains aerial mycelium was lacking. Curtis placed great taxonomic emphasis on monilioid cell morphology and presumably he identified his isolates as *R. repens* based on the similarities between the monilioid cells of his fungi and those illustrated by Bernard. Saksena and Vaartaja (1961) applied the name to isolates from *Pinus* mycorrhizae and damped off seedlings of *Pinus sylvestris*. Their isolates were light coloured, demonstrated very little aerial mycelium in culture, and had globose monilioid cells in short chains.

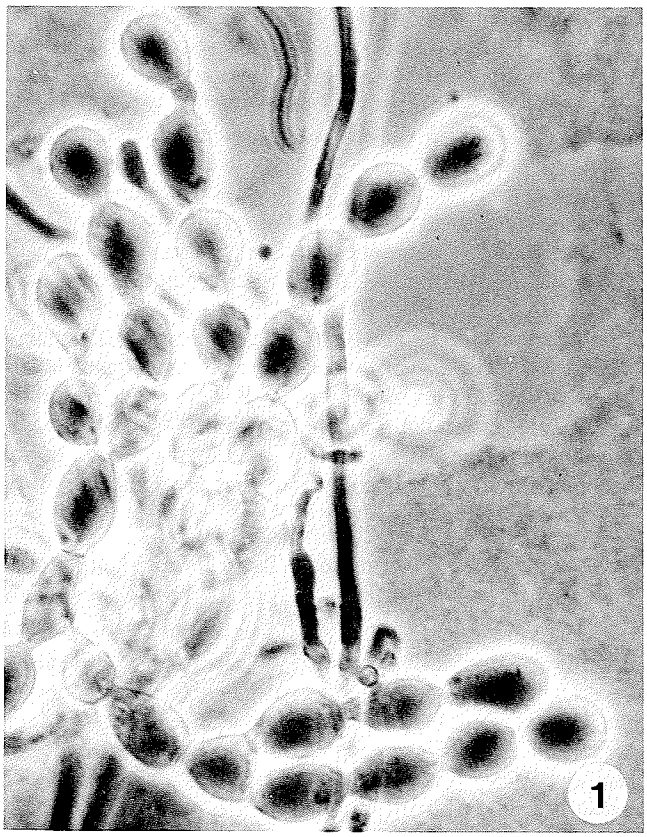
Warcup and Talbot (1967), using a soil on agar casing method, obtained the teleomorph, *Tulasnella calospora* (Boudier) Juel, of isolates identified as *Rhizoctonia repens*. They described the vegetative state in culture as being "white and creeping" and the monilioid cells as "catenate, in groups, hyaline, thin-walled, subspherical to broad ellipsoid." The size of the monilioid cells of their strains was not provided. In most respects their strains could be considered similar to ours. We have attempted to induce the formation of the teleomorph by using various types of media, and the "soil on agar casing method." So far our application of these techniques has been unsuccessful.

Only the strain described above (UAMH 5430) could clearly be identified as *R. repens*, even though a number of our *Rhizoctonia* strains bear a superficial cultural resemblance to *R. repens*. Among these unidentified strains significant variation exists in the size, shape, and organization of the monilioid cells. We suspect that a number of hitherto unpublished species are represented in this group.

Rhizoctonia anaticula Currah, sp.nov. Figs. 4–7

Coloniis in agaro lentissime crescentibus. In agaro PDA mycelium obscurum, densum, cremeum, margo nitidus, submersus. In agaro CMA mycelium album, submersus. Hyphae septatae, constrictus ad basim ramorum, sclerotia submersi, lenticulares, eburnei, aggregatae. Cellulae monilioide late elliptici vel clavatae, 14–18 × 7–10 μm in catenis, cum angustis contractis connectivus, 3–4 μm late. TYPUS: colonia

FIGS. 1–3. *Rhizoctonia repens*, UAMH 5430 from *Platanthera obtusata*. Fig. 1. Chains of smooth, thin-walled, ellipsoidal to nearly spherical monilioid cells. ×500. Fig. 2. Small cluster of branched chains of ellipsoid to spherical monilioid cells. ×310. Fig. 3. Sclerotium showing arrangement of monilioid cells in chains. ×125. FIGS. 4–6. *Rhizoctonia anaticula*, UAMH 5434 from *P. obtusata*. Fig. 4. Chains of monilioid cells from the margin of a submerged sclerotium. ×220. Fig. 5. Thin-walled, broadly elliptical to clavate monilioid cells, appearing spatulate in profile. ×890. Fig. 6. Terminal monilioid cells with beak-like projections of various lengths. ×890. FIG. 7. *R. anaticula*, UAMH 5431 from *P. dilatata*. Loose sclerotium of characteristic monilioid cells. Note the beak-like projections on the terminal monilioid cells. ×400.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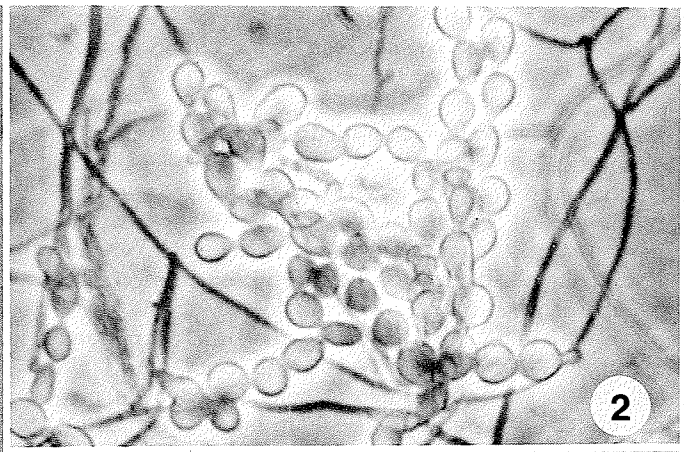
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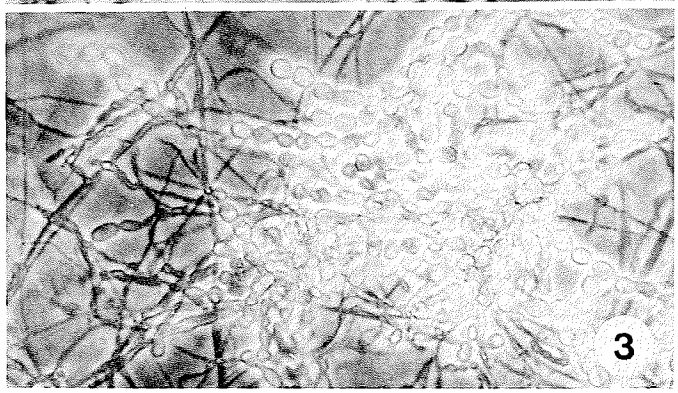
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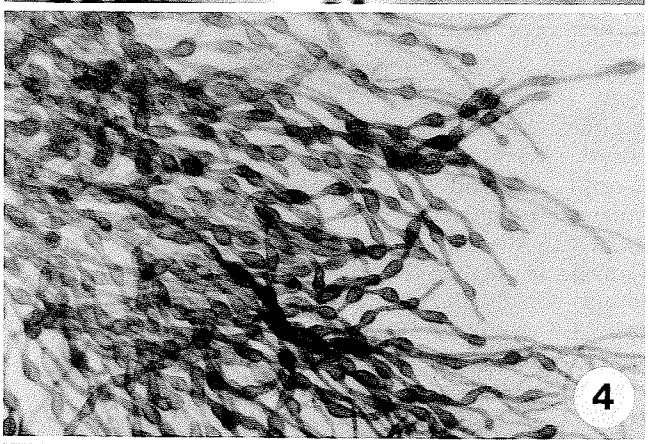
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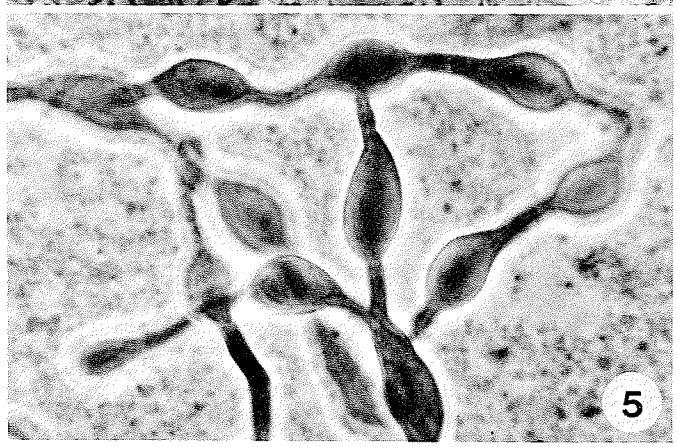
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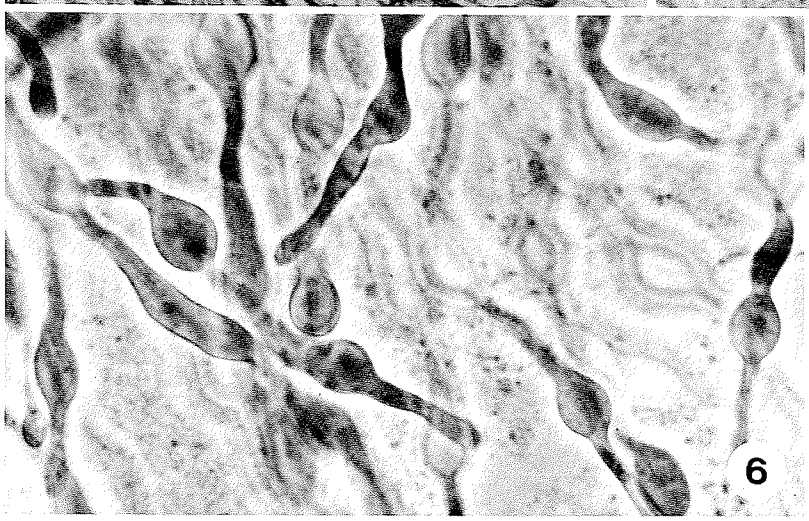
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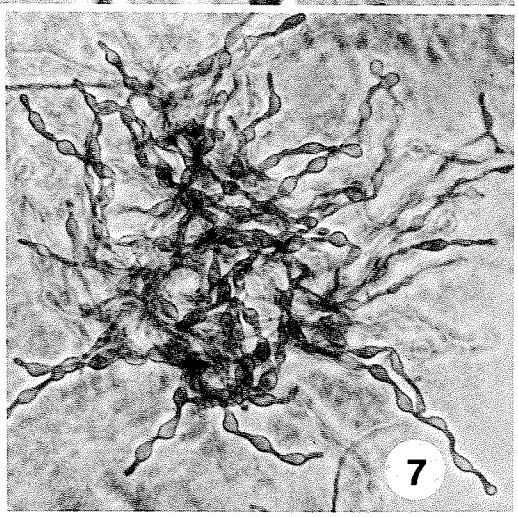
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exsiccata "UAMH 5434," ex radicis Platanthera obtusata, Albertiensis.

ETYMOLOGY: After the resemblance of the terminal monilioid cells to the profile of the head of a duckling in the genus *Anas*.

On PDA, aerial mycelium thin, dense, cream, margin submerged, appearing glabrous, growth rate 0.06–0.1 mm/h. On CMA, mycelium white, submerged. Vegetative hyphae simple septate, constricted at the branch points, runner hyphae 2.5–3.0 μm diam. Sclerotia submerged, lenticular, cream, forming in scattered clusters consisting of loose arrangements of simple or branched chains of monilioid cells (Figs. 4, 7). Monilioid cells thin-walled, broadly elliptical to clavate, appearing spatulate in profile, 14–18 μm in length, 7–10 μm broad at the widest point, adjacent cells linked by a pronounced isthmus or connection, 3–4 μm broad (Figs. 5, 6); the terminal cell in a chain proliferating blastically (Fig. 7) and usually bearing a short or long, beak-like projection.

MATERIAL EXAMINED: UAMH 5428 ex root of *Calypso bulbosa*, Swan Hills, Alberta. UAMH 5431 ex root of *Platanthera dilatata*, Castle River, Alberta. UAMH 5433 ex root of *P. obtusata* Grassi Lakes, Alberta. UAMH 5434, TYPE, ex root of *P. obtusata*, Imperial Mills, Alberta. UAMH 5435 ex tuber of *P. obtusata*, Imperial Mills, Alberta.

There have been over 30 species of orchid endophytic rhizoctonias described to date (Bernard 1909; Burgeff 1936; Curtis 1939). None of these has been reported to have monilioid cells similar to *R. anaticula*. With respect to the pathogenic species of *Rhizoctonia*, only the monilioid cells of *R. oryzae-sativae* (Sawada) Mordue (Mordue 1974), a pathogen of rice, resemble those of *R. anaticula* in having a pronounced isthmus or connective between the cells. However, the hyphae of *R. oryzae-sativae* are yellowish brown and the monilioid cells are spherical rather than ellipsoidal. Furthermore, *Rhizoctonia oryzae-sativae* forms densely organized sclerotia.

Rhizoctonia anaticula is culturally very similar to *R. repens* in having cream-coloured, submerged mycelium and scattered submerged sclerotia. The prominent connective between adjacent monilioid cells and the characteristic "beak" on the terminal monilioid cells of *R. anaticula* distinguish these two taxa.

Further collection and isolation work involving fungal endophytes of orchids of north temperate regions should provide additional records of the distribution and occurrence of *R. anaticula*. Attempts to induce the formation of the teleomorph, and genetic studies concerning this species, are now underway. Results of these investigations will be reported elsewhere.

Ceratobasidium obscurum Rogers 1935, Iowa Stud. Nat. Hist. 6–7 Figs. 8–10

On PDA, growth rate approximately 0.2 mm/h. Mycelial mat low, plane, pale cream yellow becoming pale orange yellow and densely cottony after several months. Hyphae 4–6 μm diam., thin-walled with dolipore septa, lacking clamp connections, binucleate. Hymenium forming only on CMA at room temperature in diffuse daylight after 1 month, composed of white columnar tufts (200–500 μm tall and 200–300 μm wide) of aerial hyphae and appearing granular as a result of the development of numerous short branches, basidia, and basidiospores. Basidia thin-walled, 10–14 × 9–11 μm, obovate to clavate (Fig. 8), often with a protuberance or bulge on one side (Fig. 9, arrowhead). Sterigmata four, 15–23 μm long, 3 μm at the base tapering to 1 μm at the tip, occasionally septate and branched (Fig. 9, arrow). Basidiospores 7.2–9.0 × 5.8–6.5 μm, smooth, thin-walled, broadly ellipsoid, germinating by repetition (Fig. 10, arrow).

MATERIAL EXAMINED: UAMH 5443 ex root *Amerorchis rotundifolia*, River Valley Road, Edmonton, Alberta.

Warcup and Talbot (1967) described a fertile culture of *Ceratobasidium obscurum* that they had obtained from the Australian terrestrial orchid *Acianthus reniformis* (R. Br.) Schlecter. Our strain matches their description reasonably well except that the basidia in UAMH 5443 are noticeably swollen or papillate on one side. In this respect, our isolate resembles *C. papillatum* Warcup and Talbot (1980). However, the basidiospores of *C. papillatum* are much larger than those of *C. obscurum*. On examination of Rogers' type (Herb. K, D.P.R. 291), it was not possible to obtain a clear impression of the shape of the basidia. The basidiospores of the type resembled in shape and size those discussed in Warcup and Talbot's description and those of UAMH 5443.

Phialocephala fortinii Wang & Wilcox 1985, Mycologia, 77: 954 Figs. 11–16

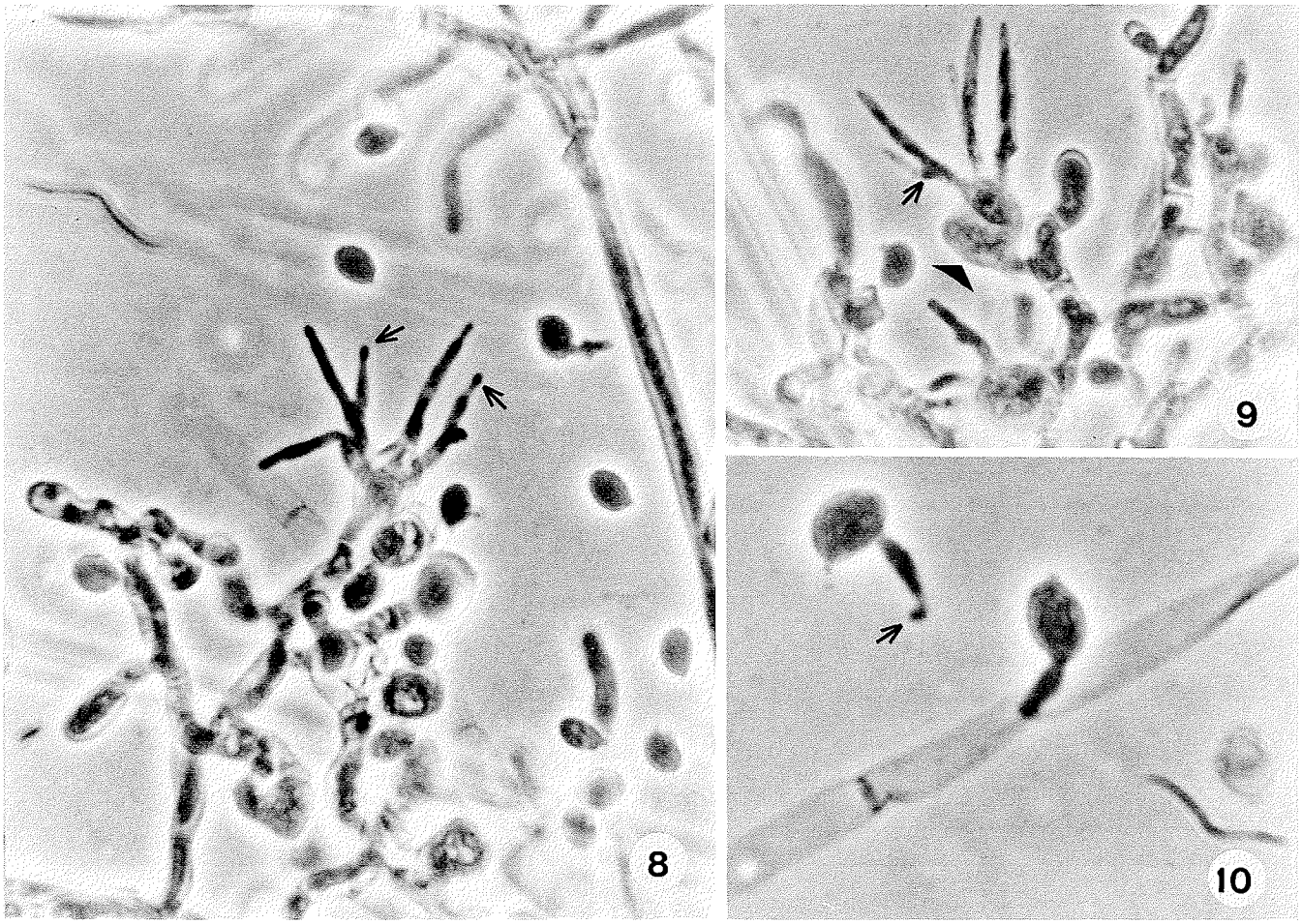
On PDA colonies 80–85 mm diam. after 23 days (hourly growth rate approximately 0.065 mm/h). Colony mouse gray to dark gray, felt-like, plane to sulcate near point of inoculation. Margin submerged, entire, appearing glabrous, greenish black. Reverse black to greenish black. Hyphae septate, submerged hyphae olive brown to greenish, forming a dense plaque-like layer of closely packed, toruloid hyphae up to 10 μm diam. Aerial hyphae smooth-walled to asperulate, or smooth and bearing pigment deposits on the outer walls (Fig. 16), 3.5–4(–5) μm diam. Coils or loops 20–40 μm diam. present (Fig. 14). The conidial apparatus sessile on undifferentiated hyphae, or borne on thick-walled, olive brown, smooth to verrucose conidiophores, 10–40 μm long; narrowly to broadly fan-shaped, to 22 μm across, of 3 or 4 series of verticillate branches. Conidiogenous cells phialides, 6–8 × 2–4 μm wide, paler than the subtending cells. First-formed conidium ellipsoidal 2–3 × 1–1.5 μm, subsequently formed conidia globose 1.5–2 μm diam. Conidiophores and conidia forming on both CER and PDA between 4 and 6 months when incubated in the dark at 4°C.

MATERIAL EXAMINED: UAMH 5424 ex corm of *Calypso bulbosa*, Grassi Lakes, Alberta. UAMH 5425 ex root of *Amerorchis rotundifolia*, Wagner Natural Area, Alberta.

Wang and Wilcox (1985) described *P. fortinii* from the pseudomycorrhizae of *Pinus resinosa* and *P. sylvestris*. It is one of at least four known fungal taxa that belong to Melin's *Mycelium radicis atrovirens*, a group of gray, greenish black, or black, normally sterile fungi present in or on the roots of conifers. Our isolates of *P. fortinii* differ from those of Wang and Wilcox by having a faster growth rate and by having a smaller conidial apparatus. In all other respects, our isolates agree closely with the original description of *P. fortinii*.

Leptodontidium orchidicola Sigler & Currah, sp.nov. Figs. 17–22

In coloniis PDA post dies 23 ad 9 cm diam. attingentes (fere 0.078 mm in horas crescentes). Coloniae pallidae ad fusco-oliveo-glaucae vel fusco-brunneae, coactae, planae, sed prope locum inoculationis exigue sulcatae, margine lata, submersa, in PDA alba. Facies reversa pallida ad fusco-oliveo-glauca. Hyphae septatae, hyphae submersae oliveo-brunneae,



FIGS. 8–10. *Ceratobasidium obscurum*, UAMH 5443 from *Amerorchis rotundifolia*. Fig. 8. Young basidium. Note developing basidiospores at the tips of the sterigmata (arrows) and the free, apiculate basidiospores. ×670. Fig. 9. Portion of hymenium showing thin-walled basidium with protuberance or bulge on one side (arrowhead). Note branched sterigma (arrow). ×670. Fig. 10. Germinating basidiospores. Note basidiospore germinating by repetition (arrow). ×1290.

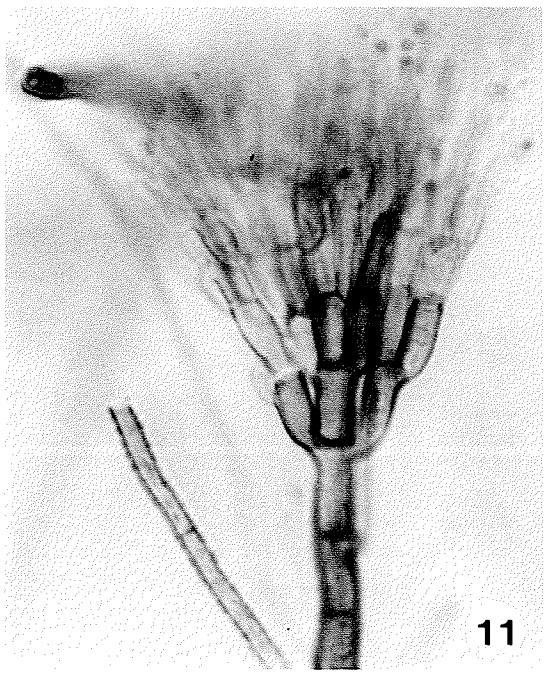
tunicam densam texturae scleroticae a catenis cellularum brevium, globosarum, irregulariter, 5–8 μm diam. Hyphae aeriae maximam partem leves vel exigue asperatae, 2–3.5 μm diam., aliquando sarcinas vel laqueos hypharum formantes. Hyphae fertiles hyalinae ad pallide brunneae, aegre variae, leves vel tenuiter asperatae, 1.5–2 μm latae, sustinent conidia singularia, sessilia, lateralia, vel conidia ex cellulis tumentibus vel non tumentibus in hypha eadem vel in ramis brevibus et lateralibus. Conidia extrema sympodialiter in nodis apicatis 2–4 conidia continentibus crescentia, sulci non inventi. Conidia hyalina, levia, pyriforma, 3–5(7) × 1–3 μm, raro disiuncta. TYPUS: colonia exsiccata "UAMH 5422," ex Platanthera hyperborea, Albertiensis.

ETYMOLOGY: From orchids.

On PDA colonies attaining 9 cm diam. after 23 days (hourly growth rate approximately 0.078 mm/h). Colonies pale to dark olivaceous gray or gray brown, felted, plane but slightly sulcate near the inoculation point, margin broad, submerged, white on PDA. Reverse pale to dark olivaceous gray. On CMA, mycelium mostly submerged, greenish or grayish brown with a few sparse tufts of whitish aerial mycelium. Hyphae septate, submerged hyphae olive brown, forming a dense layer of sclerotic tissue composed of chains of short, swollen, globose to teardrop to dumbbell-shaped cells, 5–8 μm diam. Aerial hyphae mostly smooth or slightly asperulate, 2–3.5 μm diam., occasionally forming hyphal strands and loops. Fertile hyphae hyaline to pale brown, scarcely differentiated, smooth or asperulate, 1.5–2 μm wide, bearing solitary, sessile, lateral conidia (Figs. 17, 22) or terminal conidia arising from unswollen or slightly swollen conidiogenous cells occurring on the same hypha or on short lateral branches (Figs. 18, 19, 21). Terminal conidia produced sympodially, arising in a small apical cluster of 2–4 conidia (Figs. 19, 21), scars not apparent. Conidia hyaline, smooth, pyriform, 3–5(7) × 1–3 μm, not readily detached (Fig. 20).

MATERIAL EXAMINED: UAMH 5420 ex root of *Coeloglossum viride*, Devonian Botanic Garden, Alberta. UAMH 5421, 5423 ex rhizome of *Corallorhiza maculata*, Castle River, Alberta. UAMH 5422, TYPE, ex root of *Platanthera hyperborea*, Devonian Botanic Garden. UAMH 5441 ex root of *Calypso bulbosa*, Swan Hills, Alberta.

de Hoog erected the genus *Leptodontium* in 1977 but renamed it *Leptodontidium* in 1979 since the former name was a generic homonym (de Hoog 1977, 1979). *Leptodontidium* accommodates a group of dematiaceous fungi in which conidia are produced sympodially in the apical region of a narrow, straight, hyaline to pale brown conidiogenous axis. In contrast to some similar genera in which conidia are borne on denticles (*Acrodontium* de Hoog, *Ramichloridium* Stahel ex de Hoog, *Rhinocladiella* Nannf. *sensu stricto*), the conidia in *Lepto-*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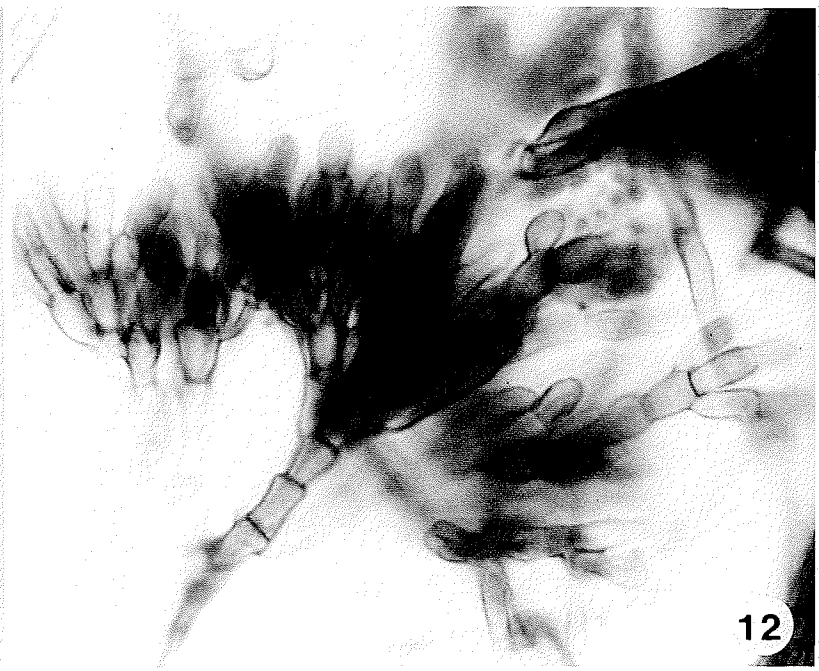
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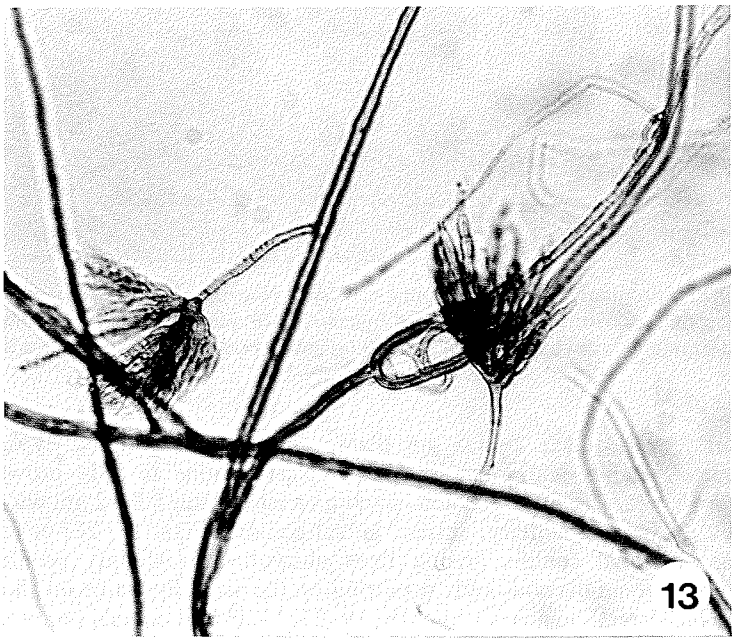
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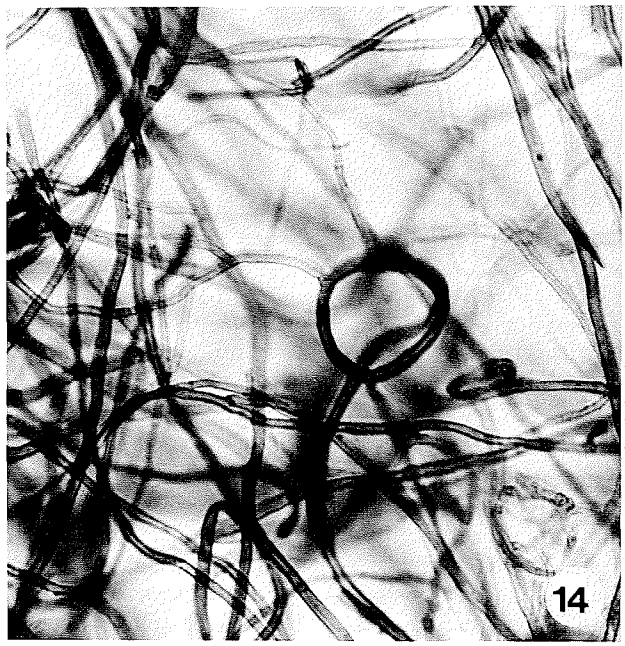
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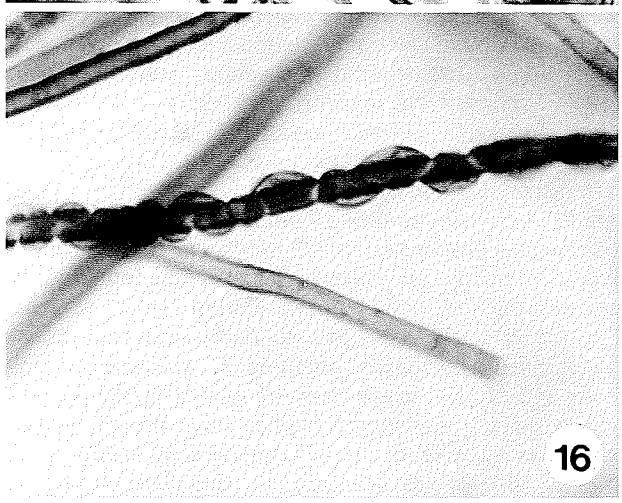
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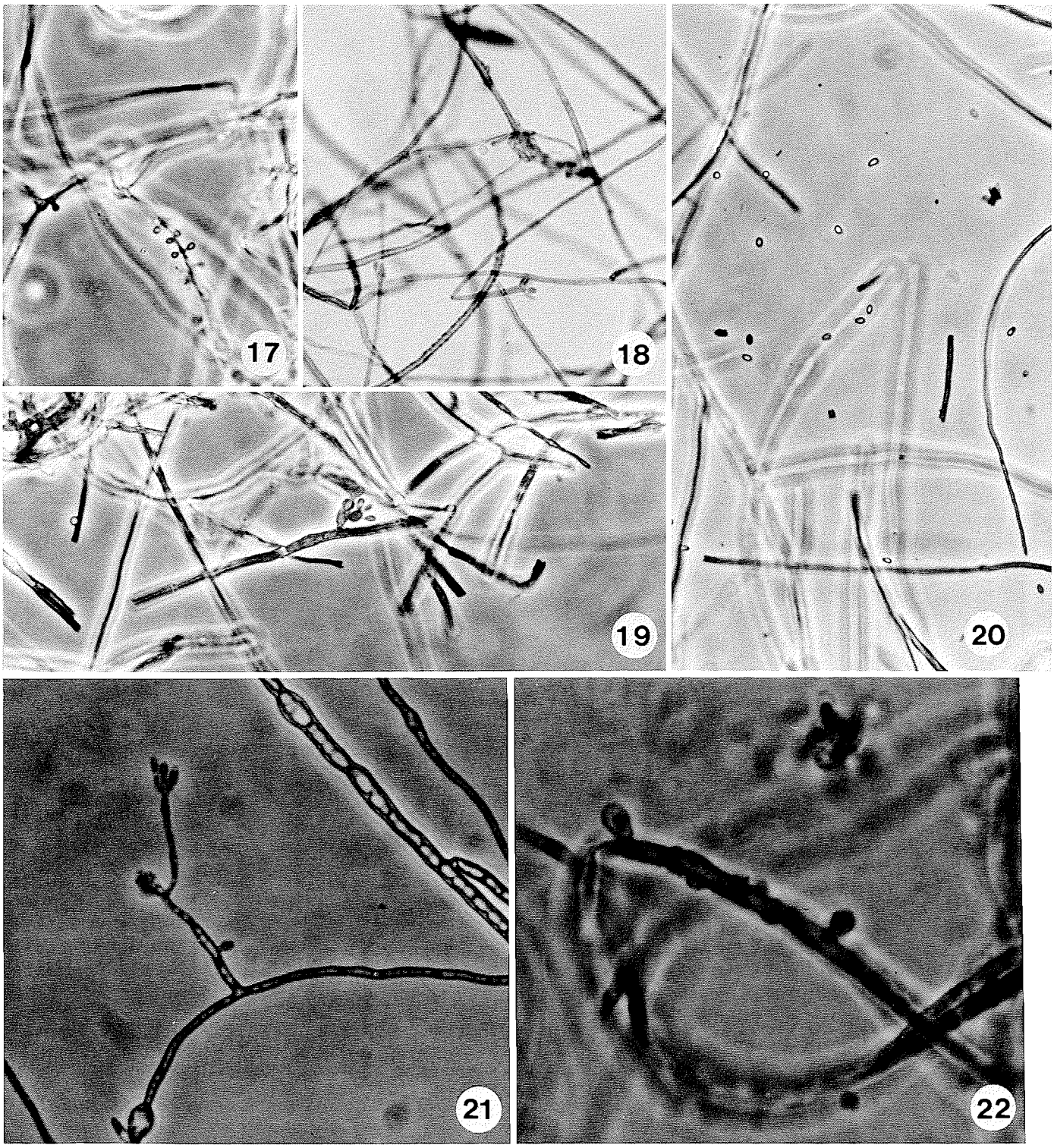
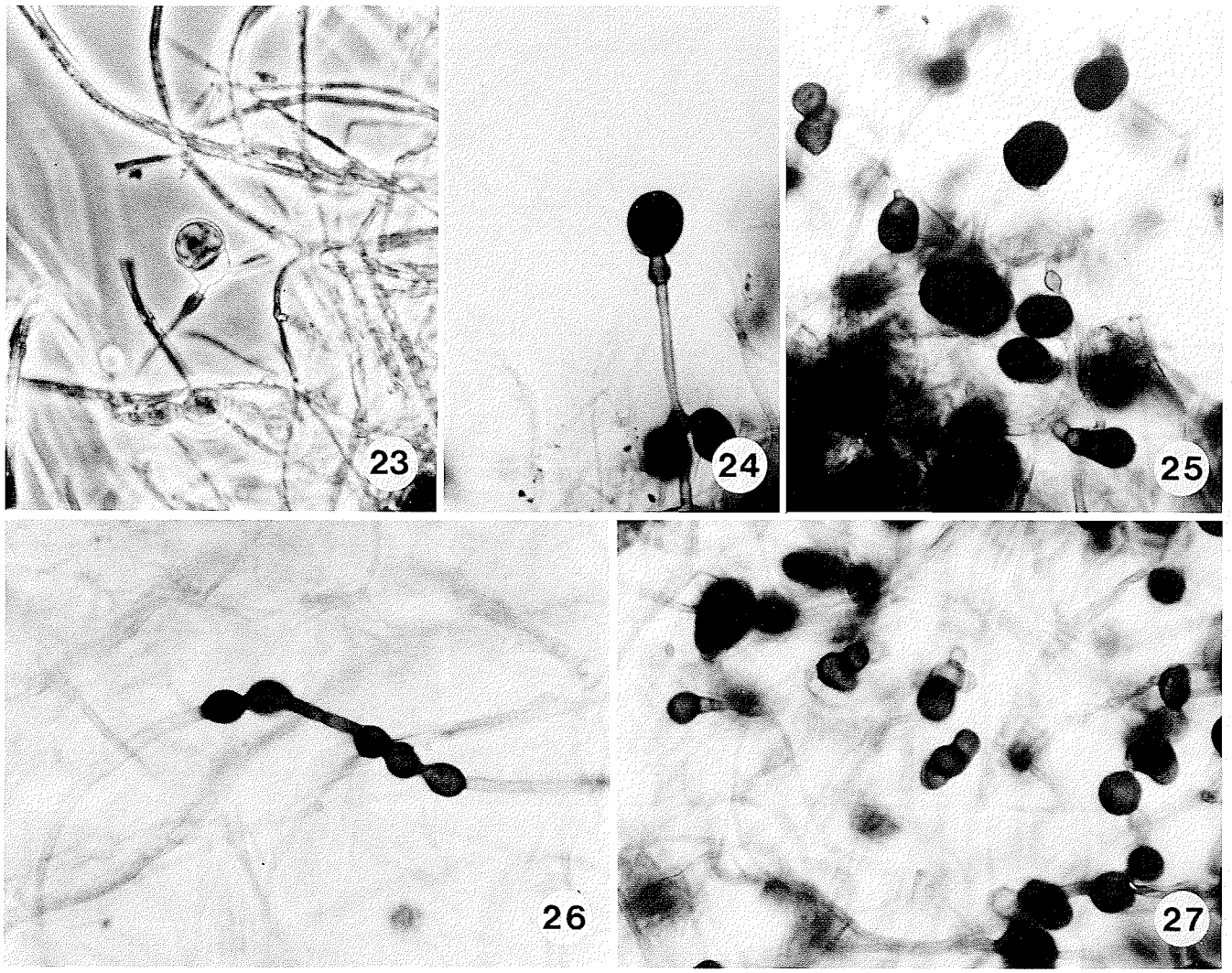


FIGS. 17–22. *Leptodontidium orchidicola* sp.nov. Fig. 17. Lateral, sessile, pyriform conidia arising from undifferentiated fertile hypha. UAMH 5422 from *Platanthera hyperborea*. ×590. Fig. 18. Terminal conidia arising sympodially from undifferentiated fertile hypha. UAMH 5421 from *Corallorhiza maculata*. ×590. Fig. 19. Conidia arising sympodially from a short swollen conidiogenous cell. UAMH 5420 from *Coeloglossum viride*. ×590. Fig. 20. Detached pyriform conidia. UAMH 5423 from *Corallorhiza maculata*. ×590. Fig. 21. Terminal conidia produced sympodially arising in an apical cluster of three conidia. Note lateral conidium on the subtending hypha. ×770. Fig. 22. Asperulate hypha bearing sessile lateral conidia. ×1500.

FIGS. 11–16. *Phialocephala fortinii*. Fig. 11. Stalked, narrowly fan-shaped conidial apparatus with four series of verticillate branches. UAMH 5424 from *Calypso bulbosa*. ×1000. Fig. 12. Broadly fan-shaped conidial apparatus. Note thick-walled supporting cells. UAMH 5425 from *Amerorchis rotundifolia*. ×1000. Fig. 13. Conidial apparatus borne on conidiophores with thick, asperulate walls. UAMH 5424. ×270. Fig. 14. Thick-walled, aerial vegetative hyphae with a loop. UAMH 5424. ×400. Fig. 15. Hyphal bundle of thick-walled vegetative hyphae. UAMH 5424. ×375. Fig. 16. Vegetative hypha with pigment deposits on outer walls. UAMH 5424. ×800.



FIGS. 23–27. *Trichocladium opacum*. UAMH 5426 from *Platanthera hyperborea*. Fig. 23. Conidium borne on nonspecialized intercalary conidiogenous cell. ×470. Fig. 24. Terminal conidium. ×450. Fig. 25. Aerial hyphae showing one- and two-septate conidia. ×450. Fig. 26. Dark, olivaceous chlamydospores in an intercalary chain in submerged hypha. ×500. Fig. 27. Aerial hyphae showing one- and two-septate, straight and curved conidia. ×295.

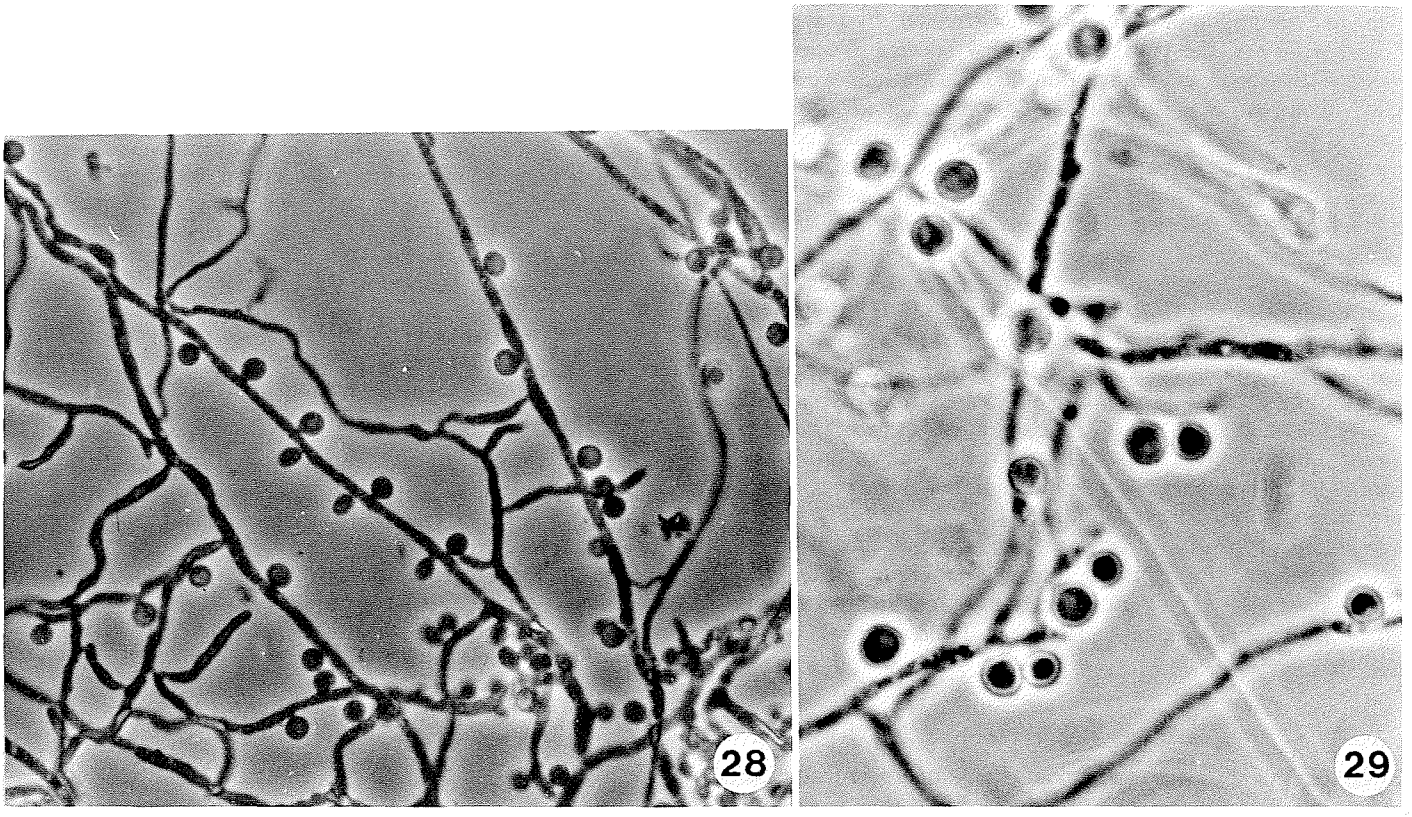
dontidium are sessile and, when detached, leave indistinct scars. A species recently added to *Leptodontidium* (Rao and de Hoog 1986) differs from the seven originally described species in producing conidia on distinct denticles.

Our orchid endophyte is assigned to *Leptodontidium* with some hesitation, based on the sympodial development of conidia in the apical region of swollen or unswollen hyaline conidiogenous cells. Two key differences separate our fungus from the other species of *Leptodontidium*: (*i*) solitary, sessile, lateral conidia are produced on fertile hyphae and (*ii*) conidia are rarely found detached. Our isolates appear most similar to *L. boreale* and *L. obscurum*, two species in which de Hoog noted that the mechanism of conidium development was difficult to determine.

Although black fungi are commonly found associated with the roots of herbaceous plants, their mycorrhizal significance is not yet understood (Haselwandter and Reid 1982; Currah and Van Dyk 1986). Several workers have referred to isolates of "sterile," nonrhizoctonia fungi from orchids as "*Rhacodium* species" (Harvais and Hadley 1967; Harvais 1973) or have given them new names within the genus *Rhizoctonia* (e.g., *Rhizoctonia subtilis* var. *nigra* (Curtis 1939)). Based on the descriptions of their isolates we consider it possible that these authors were dealing with strains of *Phialocephala* or *Leptodontidium*.

Trichocladium opacum (Corda) Hughes 1952, Trans. Br. Mycol. Soc. 35: 154–156 Figs. 23–27

On PDA, after 22 days, colony diam. 6.6 cm (hourly growth rate approximately 0.063 mm/h). Margin abrupt, entire, not submerged. Colony olive gray, sulcate, cracking medium, cottony, some zones of fluffy white overgrowth. Reverse olive black in centre, greenish to cream at margin. Hyphae septate, submerged hyphae hyaline to pale brown, smooth, often with dark olivaceous chlamydospores in intercalary chains (Fig. 26). Aerial hyphae hyaline to pale brown. Conidiogenous cells nonspecialized, terminal (Fig. 24) and intercalary (Fig. 23), hyaline to faintly pigmented. Conidia solitary, dry, dark brown and thick-walled when mature, 1–2(–3) septate, straight or curved, or sinuate, smooth to slightly asperulate, 18–36 × 14–21(–23) μm long (Figs. 25, 27), sometimes with a germ pore, not readily detached.



FIGS. 28 and 29. *Trichosporiella multisporum* sp.nov. UAMH 5179 from *Coeloglossum viride*. Fertile hyphae bearing smooth-walled, sessile to short-pedicellate, globose to ellipsoid conidia. Fig. 28. ×770. Fig. 29. ×1375.

MATERIAL EXAMINED: UAMH 5426 ex tuber of *Platanthera hyperborea*, Wagner Natural Area, Alberta.

This soil fungus is probably world wide in distribution, but it is rarely reported (Domsch *et al.* 1980). *Trichocladium opacum* has been isolated from wood and other plant materials and from the rhizosphere of a number of crop plants. This is the first record of its isolation from the roots of orchids. It seems unlikely that this fungus would be mycorrhizal and its presence in the roots of *Platanthera hyperborea* is probably incidental. Our observations agree with those of Kendrick and Bhatt (1966), who described the propensity of this fungus to form chains of chlamydospores where the hyphae grow submerged in agar media (Fig. 26). The "conidia" formed on the aerial hyphae lack a mechanism for dehiscence and could also be termed chlamydospores. They differ from the submerged intercalary conidia in being morphologically more differentiated.

Trichosporiella multisporum Sigler & Currah, sp.nov.
Figs. 28–29

In coloniis PDA post dies 28 ad 4 cm diam. attingentes (fere 0.03 mm in horas crescentes). Coloniae planae, sulcatae, glabrae, flavida. Facies reversa aurea ad aurantia. Hyphae fertiles hyalinae, septatae, 2.5–3 μm diam., sustinent conidia solitaria, sessilia, lateralia, raro in protrusionibus brevibus. Conidia hyalina, levia, globosa vel ellipsoidea, abundans, 3–5 × 3.5–4.5 μm. Chlamydosporae absunt. Status teleomorphosis ignota est. TYPUS: colonia exsiccata "UAMH 5179" ex Coeloglossum viride, Albertiensis.

On PDA after 28 days, colony 4 cm diam. (hourly growth rate approximately 0.03 mm/h). Colony initially flat, sulcate, glabrous and yellow, developing sparse tufts of white aerial mycelium toward the centre of the colony, margin entire, reverse golden yellow to pale orange. Fertile hyphae septate, 2.5–3 μm diam., bearing abundant lateral conidia, sessile, rarely short-pedicellate; conidia smooth-walled, globose to ellipsoidal, 3–5 × 3.5–4.5 μm. Conidia seceding by schizolysis.

MATERIAL EXAMINED: Of *Trichosporiella multisporum*: UAMH 5179, ex tuber of *Coeloglossum viride*, Cardinal River Divide, Alberta; UAMH 5382, ex tuber of *Platanthera hyperborea*, Bickerdike, Alberta. Of *Trichosporiella cerebriformis*: UAMH 3461 (= CBS 243.68 = ATCC 22553); UAMH 3462, wheat-field soil, Germany, W. Gams (= CBS 244.68 = ATCC 22551); UAMH 3463 T, *T. hyalina* (= CBS 135.68 = ATCC 22552). Of *Trichosporon sporotrichoides*: UAMH 5670 T, *Trichosporiella sporotrichoides*, soil, Surinam, J. H. van Emden (= CBS 671.74).

The anamorph (form-)genus *Trichosporiella* Kamyschko ex Gams & Domsch is characterized by solitary, sessile, blastic conidia, borne scattered on more or less undifferentiated fertile hyphae. van Oorschot (1980) included the genus in her monograph of *Chrysosporium* Cda. and allied genera based on the apparent similarity in conidium development, but the key difference separating *Trichosporiella* from these genera is the method of conidium dehiscence. In *Chrysosporium* and allied genera, the conidia secede by rhexolytic (aleuric) dehiscence. The conidia are released by autolysis of the hyphal walls of the cell subtending the conidium or by disintegration of the entire fertile hypha. In *Trichosporiella*, the fertile hypha does not undergo autolysis; indeed, the conidia do not secede readily and it is difficult to find many liberated conidia even in tease mounts.

In her monograph, van Oorschot treated two species, *T. cerebriformis*, the type, and the new species *T. sporotrichoides*. Based on the description and illustration, we originally identified our isolates as *T. sporotrichoides*, distinguished by its faster growth rate, and by its smaller conidia arising 2 or 3 or 4 per conidiogenous cell, in contrast to *T. cerebriformis* in which the conidia are produced sparingly on hyphae with few septa. *Trichosporiella sporotrichoides* was further distinguished by the propensity of the fertile hyphae to disarticulate, a characteristic not shared by our isolates.

Subsequently, van Oorschot and de Hoog (1981) transferred *T. sporotrichides* to the genus *Trichosporon* of the Blastomycetes based on its yeast-like habit of growth and its apparent basidiomycetous affinities. Our isolates are strictly hyphal and clearly distinct from the yeast-like *Trichosporon sporotrichoides*. Since our isolates differ from the type in producing smaller and more abundant lateral conidia, we have proposed a new species to accommodate them.

Williams (1985) illustrated an "orchidaceous rhizoctonia" bearing chlamydospores isolated from a pot culture of *Glomus fasciculatum*. Based on our examination of his illustrations, his isolate (DAR 29830) appears similar to *Trichosporiella multisporum*.

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