University of Alberta

Mycorrhizas of the Ericaceae: Diversity and Systematics of the Mycobionts

by

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ABSTRACT

This research comprises two approaches to the study of the ericoid mycorrhizal association in natural environments in Alberta, Canada. The first is ecological in focus; the objective was to isolate and identify the mycorrhizal endophytes of plant species in the Ericaceae native to Alberta. The second focus is on the evolution and systematics of the mycobionts; the objective was to examine the taxonomic and phylogenetic relationships among the taxa recovered.

Fungal endophytes were isolated from surface-sterilized roots of 19 plant species in the Ericaceae collected from three distinct habitats: alpine heathland, stable sand dune and acidic peatland. Fungi were identified using morphological characters; molecular analysis with restriction endonucleases was used to clarify the delimitation of sterile taxa and the affiliation of strains with atypical morphology. A total of 269 plant collections was processed and all had the typical morphology of ericoid mycorrhizal complexes in their roots.

Four fungal taxa were considered common root endophytes: *Scytalidium vaccinii*; *Oidiodendron maius*; an unidentified sterile fungus, named Variable White Taxon (VWT) and characterized with molecular markers; and *Phialocephala fortinii*. RFLP markers revealed two genotypes within *S. vaccinii*. The genetic difference was significant at the sub-specific level and correlated with habitat; Type I was from the alpine heathland and sand dune while Type II was from the bog. One Type II strain of *S. vaccinii* produced apothecia of *Hymenoscyphus ericae* (Leotiales) in culture, confirming a previous suggestion that the two species are conspecific.

Using nuclear ribosomal DNA sequences (internal transcribed spacer region), species delimitation in the asexual genus *Oidiodendron* was clarified and its putative phylogenetic placement in the Myxotrichaceae (Onygenales) was examined. Three monophyletic groups, each one a pair of species, were resolved within *Oidiodendron*. A

low level of sequence divergence within each pair suggested conspecificity. Conidiophore length and pigment production were too variable for species identification and several historically important ericoid *Oidiodendron* strains were re-identified as *O. maius*. All species were resolved as part of a monophyletic group within the Myxotrichaceae. Parsimony analysis of small subunit rDNA sequences confirmed the hypothesis that the four ascomycetous fungi isolated from roots of the Ericaceae share a common ancestor with inoperculate discomycetes. Thus the inferred phylogenetic placement for the Myxotrichaceae is within the Leotiales and the Onygenales is revealed as polyphyletic.

Dedicated to

Denis, Alanna, Emily and Caitlin

for sharing the journey

Signal Hill to Outpost Lake

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Introduction

Plants in the Ericales are integral and sometimes dominant components of bogs, heathlands, alpine regions and boreal forest ecosystems. They are able to grow in low nutrient, acid and often phenol-toxic soils where other plant families do not succeed. This ability is partly attributable to the development of a symbiotic relationship with fungal taxa which proliferate in actively growing lateral root cells.

Extensive studies of fungal strains identified as *Hymenoscyphus ericae* (Read) Korf & Kernan have shown that the fungal partner or mycobiont can degrade complex organic substrates providing an adequate source of nitrogen, phosphorus and other nutrients for plant growth in soils where nutrients are bound in unavailable forms (Read and Bajwa, 1985). As nutrients are released by the enzymatic activity of the fungus, they become available to the host plant and are accessed across the fungus/host interface in the root epidermal cells. Evidence suggests that the fungus receives a portion of its carbon budget as photosynthates in return (Stribley and Read, 1974).

Ericoid mycorrhizal isolates have also been shown to detoxify the soil environment of phenolic materials in the humus layer (Leake, 1987) and to sequester metallic ions, whether naturally occurring or from acid precipitation, which become increasingly soluble at low pH (Bradley et al., 1982). The simple presence and activity of fungal mycelium in the soil creates a favorable microenvironment for the germination and early growth of ericaceous seedlings before the mycorrhizal relationship is established (Leake et al., 1989).

It is clear that these fungi play an important role in marginal habitats with their wide-ranging enzymatic capabilities, and ability to sequester heavy metals, tolerating elevated concentrations of potentially toxic compounds. Unfortunately, information about the diversity and distribution of the fungal taxa involved in this specialized ecological relationship is lacking, and yet critical to future research initiatives, especially those in more applied areas such as bioremediation or habitat rehabilitation. Comprehensive biodiversity studies are hampered by the difficulty of obtaining fungal specimens (fruiting bodies of ericoid mycorrhizal fungi have not yet been found in the field); extensive manipulation in the lab of field-collected material is required.

Ericoid Mycorrhizas

The Ericales has a worldwide distribution, and four of the eight families recognized by Cronquist (1988) are found in the northern hemisphere. The Empetraceae and Ericaceae are primarily ericoid mycorrhizal while the Monotropaceae and Pyrolaceae and some genera in the Ericaceae are ectendomycorrhizal. The Ericaceae is the largest family and contains the plant species that have formed the cornerstone of ericoid mycorrhizal research, though several recent studies from the southern hemisphere have focused on the Epacridaceae. Molecular phylogenetic analyses of plant taxa suggest that the four northern families and the Epacridaceae form a monophyletic group within the Ericales and they are now considered members of one large family, the Ericaceae (Kron, 1996). Furthermore, analyses of chloroplast and ribosomal DNA sequences indicate that within this expanded family, ericoid mycorrhiza-forming taxa have a single phylogenetic origin (Cullings, 1996). Several key characters are common to these heath plants which thrive in some of the most inhospitable habitats in the world.

Ericaceous plants are, in general, dwarf shrubs with simple leathery leaves adapted to harsh conditions. The root systems of taxa that form ericoid mycorrhizas have a uniform morphology, developing as a dense system of woody rhizomes and fine lateral roots which form a mat in the surface layers of accumulated organic matter (Read, 1991). Unlike the actively growing roots of most plants, the fine "hair" roots have no root hairs, and are reduced to a narrow stele surrounded by only two to three layers of cells comprising the epidermis and endodermis, with or without a subepidermis, though these layers are alternatively referred to as a cortex with no epidermis.

The ericoid mycorrhizal association is one of several endomycorrhizal types in which the fungal partner proliferates within the root cells, with no external evidence of mycorrhizal development (unlike ectomycorrhizas and ectendomycorrhizas). In this particular type, penetration of the root by the fungus takes place enzymatically through the outer wall of the individual epidermal cells, behind the apical meristem, and these cells become almost completely filled with fine and lightly pigmented hyphae, more or less densely intertwined (hyphal complexes). The normal role of the root hair as the site of the bulk of nutrient and water uptake is assumed by the extra-radical portion of the fungal thallus that colonizes organic fragments near the root system (Read, 1991). The plasma membrane of the host cell invaginates to accommodate the hyphal proliferation and over a period of about five weeks it is thought that an active exchange of ions takes place across this interface (Dudderidge and Read, 1982), before both fungus and plant cytoplasm within

the cell degenerates.

The determination of mycorrhizal status is not a straightforward matter. An assortment of fungi can be found growing within plant roots and their life strategies can range from parasitic to commensalistic. In theoretical terms mycorrhizal associations are generally characterized as mutualistic with the type and degree of benefit to each partner varying depending on mycorrhizal type. Mycorrhizal type and function has been correlated with the specific climatic and edaphic conditions that define the major terrestrial biomes (Read, 1991). In practical terms, mycorrhizas are identified based on the morphology observed in and on roots, which, in the case of endomycorrhizas, requires a microscopic examination. Mycorrhizal status for isolated fungi is inferred, especially if the morphology in roots is re-established in resynthesis trials. The experimental step of determining the kind of physiological interaction between individual isolates and their plant associates is seldom taken.

Fungal Symbionts: historical perspective

Since the first half of this century, researchers recognized the importance of mycorrhizal fungi for the growth and success of the Ericales. Doak (1928), Bain (1937), Gordon (1937), and McNabb (1961) isolated fungi from ericaceous plant roots and then observed the formation of the typical ericoid mycorrhizal morphology in root cells of the host plant grown in axenic resynthesis trials using those isolates. The fungi were sterile in culture and remained unidentified though descriptions of isolates indicate that they share a number of morphological and cultural similarities such as narrow hyphal width, slow growth and dematiaceous pigmentation in culture.

It was not until the 1970s that one of these strains, isolated from *Calluna vulgaris* in Great Britain (Pearson and Read, 1973), formed inconspicuous stalked cup-shaped fruiting bodies when grown in pot culture with the host plant. The fungus was named *Pezizella ericae* Read (Leotiales, Ascomycotina) and described by Read (1974), though it was later renamed *Hymenoscyphus ericae* (Kernan and Finocchio, 1983), the name in current use. Although the fungus seems to be widespread in Europe associated with ericaceous plants, as yet there are no reports of these apothecia found in the field either in Europe or North America. For some time after 1973, research focused on the physiological capabilities of *H. ericae*, highlighting its important role in the breakdown of organic sources of nitrogen and phosphorus and in the increased tolerance conferred on mycorrhizal plants to toxicity from heavy metals (Read, 1983). Other research explored the mechanisms of mycorrhizal

formation and the transfer of nutrients at the ultrastructural level (Bonfante-Fasolo and Gianinazzi-Pearson, 1979; Dudderidge and Read, 1982).

Studies assessing the fungal presence in root cells established that the "ericoid" morphology was widespread among and restricted to plants in the Ericales (Haselwandter, 1979; Largent et al., 1980; Haselwandter and Read, 1980; Koske et al., 1990). Limited numbers of other researchers also isolated fungi from roots and, while strains appeared morphologically similar to *H. ericae*, it was not possible to make positive identifications because most isolates remained vegetative in culture (Reed, 1989; Hutton et al., 1994; Steinke et al., 1996).

Since 1983, reports in the literature of single isolations of other species of fungi from plants collected in disparate geographic locations have led to questions about the number of fungal species that may be involved in ericoid mycorrhizas. As well, in recent years, a variety of molecular approaches aimed at characterizing the range of sterile taxa recovered from ericaceous roots indicates that the diversity of fungal associates may be greater than was previously thought (Straker, 1996).

There are very few supporting records for some of the other species cited: *Oidiodendron griseum* Robak (Couture et al., 1983, Stoyke and Currah, 1991; Xiao and Berch, 1992); *O. maius* Barron (Douglas et al., 1989; Perotto et al., 1994); *Scytalidium vaccinii* Dalpe, Sigler & Litten (Dalpé et al., 1989). In each case, the fungus was first isolated from roots in the laboratory and the association was re-established in axenic culture to confirm mycorrhizal status. All have affinities to the ascomycetes. Unlike *Hymenoscyphus ericae*, these other species are known only in the anamorph or asexual state, making it difficult to confidently place them in the fungal taxonomic hierarchy which is based primarily on the characters associated with the sexual reproductive phase or teleomorph. Their affiliation with teleomorphic taxa can only be inferred. Molecular characters present the best option for testing hypotheses concerning anamorph-teleomorph relationships. In the case of *Scytalidium vaccinii*, sequence analysis of ribosomal DNA has been used to support the hypothesis that this taxon in fact represents the anamorph state of *H. ericae* (Egger and Sigler, 1993) and that the two are conspecific.

Mention should be made of the reports of a basiodomycetous fungus, *Clavaria* sp., which has been observed growing with field grown nursery stock of *Rhododendron* and other ericaceous genera. There is ultrastructural evidence of basidiomycetous hyphae in the roots (Bonfante-Fasolo, 1980), and evidence of transfer of materials between plant and

fungus has been obtained using radioisotope translocation and fluorescent antibody studies (Englander and Hull, 1980; Seviour et al., 1973). The fungus has never been isolated from the roots and grown in culture, though, so its mycorrhizal status has not been properly assessed.

Molecular Analysis: use in systematics

The study of evolutionary relationships in fungi has accelerated in recent years with the refinement of accessible methodologies for analyzing molecular characters. The most notable events were the development of the Polymerase Chain Reaction (PCR) (Mullis et al., 1986) for amplifying quantities of target DNA and the isolation of a thermostable DNA polymerase that allowed automation of PCR reactions (Saiki et al., 1988). The proliferation of universal and fungal-specific primers for various genes, the growing international databases of nucleotide sequences and the reduction in the financial commitment required for such research have made molecular systematic studies increasingly possible and useful.

Ribosomal DNA has proved to be the gene of choice for phylogenetic reconstruction in fungi for several reasons (Hibbett, 1992), though systematists are currently turning to other genes, such as those coding for proteins like β-tubulin and cytochrome oxidase, in an effort to refine the resolution achieved. Nuclear encoded ribosomal DNA (rDNA) comprises both coding and non-coding regions as well as spacer regions. By choosing appropriately from the mosaic of conserved and variable regions, analysis of rDNA sequences can be used to address questions about relationships among taxa at various taxonomic levels. The presence of universally conserved regions that serve as primer sites, allows for comparisons of very distant taxa as well as the amplification of more rapidly evolving regions (Bruns et al., 1991). Also, in spite of the fact that rDNA is present in the nuclear genome as a multicopy tandem repeat which could lead to the comparison of paralogous copies, it appears to evolve as a single copy gene due to homogenization of the copies through the process of concerted evolution (Hillis et al., 1996). More recently, two divergent paralogous or xenologous copies have been reported from within the ITS region of the rDNA of *Gibberella fujikuroi* complex of *Fusarium* (O'Donnell and Cigelnik, 1997) but it is not known how frequently such paralogous copies occur in fungal rDNA in general or what impact their occurrence will have on the use of this gene for phylogenetic reconstructions.

Research Rationale and Objectives

To address the lack of comprehensive information on the range and distribution of fungi mycorrhizal with the Ericaceae, the first objective of the research undertaken for this dissertation was to determine the range of fungal taxa involved in natural habitats in Alberta, Canada. To sample as many plant species as possible, fungi were isolated from the roots of taxa collected from three different habitats where the Ericaceae are the dominant understory plants.

The key issues to be addressed within this objective included: *i)* the discrimination between the recovery of fungi that were isolated from inside root cells (endophytic) as opposed to rhizosphere fungi growing on the surface of the roots, *ii)* the identification of isolates, especially in view of the expectation that a proportion would remain sterile in culture, *iii)* an evaluation of the potential ecological role of the species recovered, i.e., are the species recovered mycorrhizal taxa, or endophytic but with a different relationship vis-a-vis the host, and *iv)* the distribution of fungal taxa according to habitat and plant host.

The second research objective focused on the phylogenetic relationships of the fungi that are implicated in the ericoid mycorrhizal association. Based on reports in the literature, species in two genera were already cited as mycobionts: *Hymenoscyphus ericae*, for which the mycorrhizal status has been clearly demonstrated in numerous experiments as discussed above; and the two species of *Oidiodendron*, *O. griseum* and *O. maius*, which have been considered mycorrhizal by virtue of the observation that they form hyphal complexes in root cells similar to those formed by *H. ericae*.

The question is whether these two genera of fungi might be more closely related evolutionarily than is implied by their taxonomic affinities as currently understood. The genera *Hymenoscyphus* and *Oidiodendron* are classified in or have putative affinities in disparate Ascomycete groups, the Leotiales and the Onygenales, which are traditionally deemed to be taxonomically distant. Though *Oidiodendron* is a form-genus and lacks ordinal status, it is hypothesized to belong to the Myxotrichaceae (Onygenales) since the only teleomorphic species with *Oidiodendron* as the anamorph state belong to this family (Currah, 1985; Hawksworth et al., 1995, p.320). The Onygenales form cleistothecial fruiting bodies (though in some taxa the peridium is reduced to a network of loosely woven or mesh-like thick-walled hyphae) while the Leotiales form open, cup-shaped apothecia. Formerly these orders were classified in the descriptive classes Plectomycetes and Discomycetes respectively, but formal taxonomic structure above the order level is in a state

of flux and these terms are now considered to represent artificial assemblages. Emphasis was formerly placed on sexual state characters for classification purposes in fungi but recent molecular phylogenetic studies have provided evidence that these characters can be subject to convergence (Saentz, 1994; Landvik et al., 1996; Hibbett et al., 1997).

On the other hand, there are several features which link these taxa closely. They share an ability to live saprobically and also of forming a specialized mycorrhizal association. Vegetative characters of the mycelium in culture are similar and both produce arthroconidia, a form of asexual propagule not widespread among ascomycetous fungi. Finally, the morphology of the fungal complexes in root cells appears to be uniform regardless of the mycobiont involved suggesting that the taxa share the same unique ecological niche.

The second research objective, therefore, was to investigate the hypothesis that fungi that are apparently involved in such a highly specialized and restricted ecological relationship, with a monophyletic group of plant species, would themselves share a recent common ancestor. This objective is addressed using molecular characters, specifically nuclear ribosomal DNA sequence data. As further justification for the hypothesis, there have been previous suggestions that some members of the Onygenaceae (sensu Malloch, 1981) and more specifically those of the Myxotrichaceae (Currah, 1994), would find their phylogenetic placement with the discomycetes.

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Chapter 2

Fungal Endophytes from the Roots of Alpine and Boreal Ericaceae [1]

Introduction

The Ericales is primarily ericoid endomycorrhizal and ericoid hyphal complexes or infection units have been reported in a range of host species from Europe (Haselwandter and Read, 1980), western U.S.A. (Largent et al., 1980), Hawaii (Koske et al., 1990), Australia (Hutton et al., 1994; Steinke et al., 1996) and New Zealand (McNabb, 1960). *Hymenoscyphus ericae* (Read) Korf & Kernan (Leotiales) (Read, 1974; Kernan and Finocchio, 1983) has been considered the major mycobiont and studies have elucidated the ecological role of this fungus (Read, 1983, 1991). Molecular and morphological evidence indicates that *Scytalidium vaccinii* Dalpé, Litten & Sigler (Dalpé et al., 1989), is the anamorph of *H. ericae* (Egger and Sigler, 1993), thus extending the taxon's known distribution to eastern North America.

In most studies where the mycobionts were isolated, numerous strains of dark sterile ascomycetous fungi have been recovered and divided into cultural groups that match variants of *Hymenoscyphus ericae* (McNabb, 1960; Singh, 1974; Reed, 1989; Steinke et al., 1996). In others, isolates have been identified as *H. ericae* based on cultural, morphological or biochemical characters (Perotto et al., 1990; Johansson, 1994) in the absence of ascomata or conidia. Straker (1996) summarizes recent serological data, suggesting that some South African strains of *H. ericae*-like endophytes may differ from European strains at the genus level. Similarly, Hutton et al. (1994) were unable to identify culturally similar sterile isolates from Australian Epacridaceae as *H. ericae*, because pectic zymograms varied among their isolates and with an authentic one.

Species of a second genus, *Oidiodendron*, are also endophytic in ericoid mycorrhizas. *O. griseum* Robak (Couture et al., 1983) and *O. maius* Barron (Douglas et al., 1989) were isolated from cultivars of *Vaccinium corymbosum* L. and *Rhododendron* L. respectively, but supporting records are few. *O. griseum* has been recovered from ericaceous plants in natural habitats in western Canada (Stoyke and Currah, 1991; Xiao and

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Berch, 1992) and Denmark (Johansson, 1994) while *O. maius* was reported from Italy (Perotto et al., 1994, 1996).

Neither Stoyke and Currah (1991) nor Xiao and Berch (1992) reported strains of *Hymenoscyphus ericae* from either Alberta or British Columbia in Canada, though the number of plant species sampled overall was low. These results imply that *H. ericae* is not mycorrhizal with the Ericaceae in these regions, which is unexpected in view of its widespread occurrence elsewhere.

It has become increasingly clear that ericoid mycorrhizas are not simply an association between *Hymenoscyphus ericae* and one plant order (Straker, 1996), but the range and relative importance of the fungal partners is unclear. The presence of basidiomycetous endophytes has been documented using ultrastructural and serological evidence (Englander and Hull, 1980; Peterson et al., 1980; Bonfante-Fasolo, 1980) with a *Clavaria* sp. being definitively cited (Mueller et al., 1986). The ultrastructural work as well as more recent molecular analysis (Perotto et al., 1994, 1996) reveals that more than one endophyte can be present in the same root system. There are also records of multiple types of mycorrhizas occurring at low frequencies with some ericoid-formers in the Ericales (Largent et al., 1980; Koske et al., 1990; Dighton and Coleman, 1992; Smith et al., 1995).

More comprehensive sampling of plant species from a range of habitats along with more precise methods of characterizing isolates are needed to answer questions about the identity and distribution of fungi associated with ericaceous roots. The objective of this study was to assess the mycorrhizal status of the Ericaceae s.l. (Kron, 1996) native to Alberta, Canada by: (1) isolating and identifying the endophytic fungi from a broad range of hosts collected in three distinct habitats, i.e., alpine heath, stable sand dune, and ombrotrophic wetland (bog), (2) evaluating whether the distribution of these fungi varies depending on habitat and time of collection in the growing season and (3) examining root samples for the presence of multiple mycorrhizal types. The identification of isolates was assisted by the use of restriction fragment length polymorphism (RFLP) analysis.

Materials and Methods

Plants were obtained from three distinct habitats. Site 1, alpine heathland, is in the region of Outpost Lake and Arrowhead Lake near Jasper, Alberta in the Canadian Rocky Mountains. To maximize the number of different plant species, samples were collected from several sub-sites: a dwarf shrub heath dominated by *Cassiope mertensiana* (Bong.) D. Don, *Phyllodoce glanduliflora* (Hook.) Coville, *P. empetriformis* (Smith) D. Don, and

Abies lasiocarpa (Hook.) Nutt.; an open meadow dominated by *Cassiope mertensiana* (Bong.) D. Don, *Gaultheria humifusa* (Graham) Rydb., and *Abies lasiocarpa*; and a dry ridge dominated by *Loiseleuria procumbens* (L.) Desv. and *Vaccinium uliginosum* L. Site 2, stable sand dune, approximately 2 km east of the townsite of Slave Lake, Alberta, is an open *Pinus banksiana* Lamb. forest with a ground cover dominated by *Vaccinium myrtilloides* Michx. and *Arctostaphylos uva-ursi* (L.) Spreng. Site 3, ombrotrophic bog, located southeast of Athabasca, Alberta is dominated by *Picea mariana* (Mill.) BSP., *Ledum groenlandicum* Oeder, and *Sphagnum* spp. Plant nomenclature follows Packer (1983).

Nineteen species from thirteen genera of Ericaceae (Table 2.1) were sampled: 62 collections were made during the summers of 1993 and 1994; an additional 207 collections were made during 1995 from three collecting times per site corresponding to spring, summer and fall. *Arctostaphylos uva-ursi* (L.) Spreng., though found at Site 2, was excluded since it typically forms ectendo-mycorrhizal associations (Zak, 1976).

Plants were dug with a portion of the root system and surrounding soil intact. Samples were examined for the presence of potential teleomorphs. Roots were cleaned of debris and foreign roots, and scanned for mycorrhizas. Segments of healthy fine hair roots were used to isolate endophytic fungi. Voucher specimens of plant collections are held at ALTA (Herbarium, Biological Sciences Department, University of Alberta, Edmonton, Alberta, Canada).

Root segments two cm long were sterilized in 20% household bleach (approx. 1% sodium hypochlorite) for three minutes and rinsed three times in sterile distilled water. Initial trials determined the optimum sterilization time, which reduced the recovery of rhizosphere fungi to <1%. One half of the segment was finely diced, and the fragments streaked on corn meal agar (CMA: Difco-Bacto, Detroit, USA) with 0.01% oxytetracycline. Unless otherwise specified, plates were incubated in the dark at room temperature. Cleared and stained squashes of the other half of the root segment (Brundrett et al., 1984) and mounts of unprocessed stained fresh roots (Hutton et al., 1994) were examined microscopically for endophytic fungi. One or two root segments were processed per collection.

Transfers of hyphal tips or conidia were made to CMA. Strains were maintained and characterized primarily on CMA, potato dextrose agar (PDA: Difco-Bacto) and malt agar (MEA: 2% malt extract, Difco-Bacto). Cereal (CER) slide cultures (Sigler, 1993) were

used for the identification of *Oidiodendron* species. Conidiophore length was measured on 24- to 30-day-old slide culture mounts. Ten strains of *Scytalidium vaccinii* were grown on Melin's agar under a black light (Philips, BL 20W)/"grolight" (Sylvania gro-lux wide spectrum 20W) regime (Webster, 1976) for the induction of apothecia (12 h illumination cycle). Strains suspected of being *Phialocephala fortinii* were grown on CMA and PDA, and incubated at 4°C for 6 months or longer to induce conidiogenesis (Wang and Wilcox, 1985).

For molecular analysis, strains were grown on thin plates of E-Strain agar (ESA) (Egger and Fortin, 1990) for three weeks. Eight plugs of mycelium, 0.5 cm in diameter with a minimum of agar attached, were used for DNA extraction following the chloroform/isopropanol protocol of Gardes and Bruns (1993) with minor modifications. (The precipitated DNA was pelleted by a 3 min centifugation at full speed and the pellets were resuspended in 50 µl sterile, filtered milli-Q water after washing.) Primers ITS1 (White et al., 1990) with NL6Amun (Egger, 1995) were used to amplify a portion of the nuclear ribosomal gene (rDNA). Nucleotide sequences for the primers used are given in Appendix 1a with a diagram illustrating their relative priming locations. PCR reaction volumes of 100 µL contained PCR buffer, 25 mM $MgCl_2$, 50 µM each of dGTP, dATP, dTTP, and dCTP (Boehringer Mannheim Biochemica), 0.4 µM of each primer and 2 units of *Taq* DNA polymerase. One µL of diluted DNA was used as the template for each reaction except for a DNA-free, negative control. Diluted samples were amplified on a DNA Thermal Cycler (Gene E, Techne Ltd., Princeton, USA) using the following cycle parameters: 96°C denaturation for 1 min, 55°C annealing for 1 min, 72°C extension for 2 min. The total number of cycles was 30 with an initial denaturation step of 2 min and a posttreatment of 72°C for 7 min.

Amplified products were digested with restriction endonucleases *Rsa*I, *Alu*I, *Hha*I, and *Hin*fI (Pharmacia Biotech Inc., Baie D'Urfe, Québec, Canada) for 5 h at 37°C using the buffer provided by the manufacturer. After gel electrophoresis in a 1% Tris-borate-EDTA buffer using a 2% NuSieve (FMC BioProducts, Rockland, USA) /1% agarose (ICN Biomedicals, Aurora, USA) gel, restriction fragment length polymorphism (RFLP) data were recorded on a digital imager (The Imager, Appligene Inc., Gaithersburg, USA) and banding patterns were compared.

The basepair (bp) lengths of individual fragments were determined using the software RFLPscan (Scanalytics, CSPI, Billerica, USA) standardized with a 1Kb DNA ladder (Gibco BRL, Life Technologies, Toronto, Canada). Where RFLP profiles were

visually identical, slight differences in bp values from the computer analysis were averaged out. Strains analysed are listed in Tables 2.2 and 2.3, and have been deposited in the University of Alberta Microfungus Collection and Herbarium (UAMH) (see Appendix 2 for UAMH deposition numbers). Reference cultures for DNA analysis and morphological/cultural comparisons were obtained from UAMH.

Results

Cleared and stained root samples revealed typical ericoid mycorrhizas in all collections. Varying percentages of the outer epidermal cells of the youngest fine roots were filled with hyphal complexes or "coils" of hyaline narrow hyphae. Hyphae of dematiaceous fungi were also present in some cells. Neither ecto- nor ectendo-mycorrhizal associations were found. *Clavaria vermicularis* Fries, fruiting within clumps of *Empetrum nigrum* and *Phyllodoce* spp., was the only potential teleomorph collected in the field.

Three fungal taxa were obtained repeatedly from all three sites: *Oidiodendron maius*, *Scytalidium vaccinii* (Figs. 2.1-2.7) (compare with authentic strains UAMH 6735 and 5828, Figs. 2.8, 2.9), and "Variable White Taxon" (VWT), a non-sporulating group delimited using RFLP markers (Figs. 2.10-2.12), which was culturally similar to *S. vaccinii* but often lacked pigmentation. In 30% of the collections, both *O. maius* and *S. vaccinii* were isolated from the same root segment. *O. griseum* was isolated rarely, from only four of the 269 plant collections (1.5%). Strains assignable to the complex *Mycelium radicis atrovirens* (MRA) (Wang and Wilcox, 1985) were isolated in quantity from the alpine heath and sand dune sites but rarely from the bog. Sixty percent of the strains isolated during 1993 and 1994 sporulated after incubation at 4°C and they were identified as *Phialocephala fortinii* Wang & Wilcox. Based on vegetative morphology (Currah and Tsuneda, 1993), the remaining sterile isolates are also considered to be *P. fortinii*. The number of collections yielding each endophyte for each host is compared in Table 2.1 (see Appendix 3 for a summary of collection and isolation information for each collection). There was no correlation between fungal species recovered and the time of collection in the growing season.

Phialocephala fortinii grew quickly on the initial isolation plates and tended to mask other fungi. Plates were kept for up to six weeks to allow slower growing colonies to appear. Sporulating fungi were easily separated from the sterile mycelium of *P. fortinii* in subsequent transfers. The endophytes were all distinctive culturally on the initial isolation

plates and the early sporulation of *Oidiodendron* spp., and to a lesser extent *S. vaccinii*, allowed for ease of identification and transfer to pure culture.

Percentages of total collections yielding each major endophyte for each site are presented in Fig. 2.13. There was a low percentage recovery of *Phialocephala fortinii* from the bog and higher percentages from the other two sites. Inversely correlated with this are the results for both *Scytalidium vaccinii* and the VWT. Recovery of *Oidiodendron maius* was high from the sand dune and bog, but lower from the alpine heath.

Scytalidium vaccinii was isolated from all species, and identified by characteristic narrow, hyaline arthroconidia (1.5-2.5 μm wide) produced in zig-zag chains, with the development of dematiaceous strands and toruloid cells in older colonies (Fig. 2.14). Conidia were not produced on PDA, and rarely on MEA. Sporulation was best on CMA but varied, with many strains readily producing conidia within 7 days, some after lengthy incubation periods up to five months and others only in cereal slide culture preparations. One group of initially-sterile isolates formed submerged, inflated, non-disarticulating conidia in curled chains at the outer edges of older colonies (Fig. 2.15). When these areas were transferred to fresh media, typical arthroconidia formed. One strain of the 10 tested produced fruiting bodies after 4 weeks on Melin's agar under the black light/grolight regime. The apothecia of this strain, S-14Bb (UAMH 8680), (Figs. 2.6, 2.7), isolated from *Ledum groenlandicum* (bog site), match the description of *Hymenoscyphus ericae*. Consistent characters on PDA among the isolates and including authentic strains of *H. ericae* (UAMH 6735) and *S. vaccinii* (UAMH 5828) were the restricted colony size with submerged lighter-coloured margin and the deep radial sulcae that split open with age or were obscured by aerial hyphae (Figs. 2.1-2.6, 2.8, 2.9). Though the fungi shown in Figs. 2.11 and 2.12 exhibit similar characteristics, they were identified as VWT primarily using RFLP analysis (see following discussion).

Scytalidium vaccinii comprised two cultural and genetic variants that correlated with habitat. Type I, from the alpine and sand dune sites, was highly variable on PDA in pigmentation and degree of aerial hyphal growth. Colour varied from steel grey to dark or light brown for variable portions of the centre of the colony (Figs. 2.1-2.4). On CMA growth was mostly submerged with stiff, hyaline or light brown aerial strands at the centre of the colony. Type II, from the bog and rarely from wet areas of the other sites, was consistently distinctive on both PDA (Figs. 2.5, 2.6) and CMA (Fig. 2.7). On CMA, abundant conidia were produced from tufts of white aerial fertile hyphae; on PDA, colonies were white in the centre grading to grey-brown at the edges, with a smooth velvety surface.

Type II rDNA lacked the restriction site of Type I with the restriction enzyme *Rsa*I. Restriction digests with the other three enzymes were identical for all strains tested (for more detailed molecular results, see following paragraphs).

Strains of *Oidiodendron* spp. were isolated from all species except *Kalmia polifolia*, *Menziesia ferruginea*, and *Vaccinium membranaceum*. The number of collections for *K. polifolia* and *M. ferruginea* was also low. Sporulation occurred readily on CMA within seven days of inoculation. Most of the strains isolated here were identified as *O. maius*. Erect dematiaceous conidiophores were tall, the majority ranging from 200 to 500 μm, with undulate, branching fertile hyphae producing subhyaline arthroconidia of variable shape (long-cylindric to subglobose to branched) and size (2-2.5 X 2.5-4 μm). Conidia were essentially smooth but sometimes appeared slightly roughened at 1000X magnification. Conidiophore length of some isolates (from 100 to 250 μm) overlapped the ranges for *O. griseum* and *O. maius*, while conidial size and shape corresponded more closely with those of *O. maius*. Only four strains were identified as *O. griseum*. In this species, conidiophores are generally shorter, < 150 μm (though S-31b ranged as tall as 225 μm), with conidia hyaline to pale brown, subglobose to short-cylindric (1.6-2 X 2-3.6 μm), and slightly roughened at 1000x magnification. All four strains of *O. griseum* produced a dark amber diffusing pigment (absent from strains of *O. maius*) on PDA, MEA and CER, with eventual formation of submerged orange crystals on CER.

VWT was characterized by creamy white colonies on PDA (Fig. 2.10), though some strains were grey to black (Figs. 2.11, 2.12). After prolonged incubation, white colonies gradually turned black in some cases. In other respects such as growth rate, radial furrows, the presence of fasciculate strands in the centre of the colony and hyphal width, these isolates were similar to *Scytalidium vaccinii*. On CMA, growth was very restricted, completely submerged and no conidia were formed, only short swollen, lobed hyphae (Fig. 2.16). Isolates were recovered from all plant species except *Gaultheria humifusa*, *Menziesia ferruginea*, *Phyllodoce glanduliflora*, and *Vaccinium uliginosum*.

For all taxa processed, the fragment of rDNA amplified by the universal primer ITS1 and the fungal specific primer NL6Amun was 945 basepairs (bp) in length except for the VWT which was slightly longer at 960 to 980 bp. It includes both internal transcribed spacers, ITS1 and ITS2, and a portion of the 5' end of the large subunit. Egger (1995) provides a discussion of the rationale for using the ITS region for species level delimitation and a diagrammatic view of the nuclear ribosomal gene with relevant primers. Photographs

of representative ethydium bromide stained agarose gels that were analysed with RFLPscan are given in Appendix 4.

The restriction digest profiles of some isolates of *Scytalidium vaccinii* were compared with authentic strains of *Hymenoscyphus ericae* (UAMH 6735) and *S. vaccinii* (UAMH 5828) (Table 2.2). All profiles were the same for three of the four enzymes tested; *Alu*I, *Hha*I and *Hin*fI. For *Rsa*I, Type II isolates lacked the restriction site observed with Type I strains and the amplified DNA remained uncut. Restriction digests of the reference cultures of *H. ericae* and *S. vaccinii* matched those of Type I. The restriction digest profiles of selected isolates placed in VWT were consistent amongst themselves but different from both genotypes of *S. vaccinii* for *Alu*I, *Hha*I and *Rsa*I (Table 2.2). With *Hin*fI, the profiles are effectively the same for the two taxa. Though two fragments are each 15 bp longer for VWT (455/308/170) than for *S. vaccinii* (440/293/170), this is more likely due to the length difference of the uncut amplified DNA than to restriction site differences.

The restriction digest profiles of selected *Oidiodendron* strains were compared to an authentic culture of *O. griseum* (UAMH 1403) and an ex-type culture of *O. maius* (UAMH 1540) (Table 2.3). The digest profiles of *O. maius* are distinct from *O. griseum* for all four enzymes. Strains with morphological character states intermediate between *O. maius* and *O. griseum* (S-27b and S-81Bc) were confirmed as *O. maius* based on comigration of restriction fragments.

Discussion

The study of ericoid mycorrhizal fungi has presented some unique problems, not least of which is that, regardless of the fungal partner used in resysnthesis trials with the host, the morphology of the hyphal complexes in the epidermal cells of the fine "hair roots" is uniform (Xiao and Berch, 1995). Thus, identification of mycobionts without isolation into pure culture is hampered. This uniformity may also have led to an assumption by early researchers that only one fungal taxon was responsible for the mycorrhizal association. Certainly, the consistent isolation of strains of slow-growing, dark, sterile fungi culturally similar to *Hymenoscyphus ericae* by different researchers from different geographical locations would have promoted this perception. Both *H. ericae* (and its anamorph state, *Scytalidium vaccinii*) and species of *Oidiodendron* have been cited as mycobionts of the Ericales though the acceptance of the latter as mycorrhizal candidates has been hindered by the few records of its isolation.

Four distinct endophytic taxa were isolated here from boreal and alpine Ericaceae: *Scytalidium vaccinii*, *Oidiodendron maius*, the unidentified "Variable White Taxon" (VWT), and *Phialocephala fortinii*. The sampling approach was to process a small portion of many collections of each plant species collected throughout each site rather than to thoroughly sample the root system of fewer plants. The dense mat of fine hair roots and the rhizomatous habit made the latter approach unfeasible. The percentage of collections yielding each of the four endophytes was taken as an indication of the taxon's relative frequency of occurrence as an endophyte. The results show that both *S. vaccinii* and *O. maius* were ubiquitous in the roots of the five bog species while the VWT was isolated from a substantial percentage of the collections. The same trend is reflected in the results for the other two sites, although the percentages are reduced because of the vigorous initial growth of *P. fortinii* on the primary isolation plates which accentuated factors associated with the isolation process such as the small root sample, growth rate and cultural morphology.

Phialocephala fortinii was isolated from almost all of the sand dune collections (93%) and from a high percentage of alpine collections (61%). Its early, profuse, aerial growth had a major impact on the ease of recovery of slow-growing and submerged taxa. Those latter fungi were recovered from within colonies of *P. fortinii* but often only after prolonged incubation. Their high recovery from the bog, where the incidence of *P. fortinii* was low (3%), further indicates that their frequency in roots from the alpine and sand dune sites may be underestimated by the percentages of their isolation. Schild et al. (1988) reported a comparable disparity in the distribution of MRA (*Mycelium radicis atrovirens*) strains on sites reclaimed with Sitka Spruce. High recovery of MRA and relatively low incidence of *Oidiodendron* spp. for a well-drained site contrasted with high numbers of *Oidiodendron* spp. and low MRA from recently reclaimed wet peaty soils.

Phialocephala fortinii is already known as a common root associate of the Ericaceae and other plants of alpine habitats (Stoyke and Currah, 1991). It is now confirmed as a significant component in stable sand dunes. The results of this study also indicate that the distribution of *P. fortinii* depends on edaphic factors rather than host availability. The high moisture levels of the acidic wetland habitat may account for its low incidence as an endophyte even in *Vaccinium vitis-idaea*, the one plant species found in all three habitats. The ecological role of this fungus is not well understood. In resynthesis studies with a variety of hosts, the effect of *P. fortinii* may be parasitic, amensal, or neutral but results vary depending on the experimental conditions and strains used (Wilcox and Wang, 1987; Currah et al., 1993b; Fernando and Currah, 1996). Though not normally considered a

mycorrhizal endophyte, ectomycorrhizas formed when seedlings of *Salix glauca* L. were inoculated with a strain originally isolated from that host (Fernando and Currah, 1996). With ericaceous plants, *P. fortinii* forms dematiaceous intracortical sclerotial structures unlike ericoid hyphal complexes (Stoyke and Currah, 1991) and has been shown to have a neutral to slightly negative effect on plant growth in axenic resynthesis experiments (Stoyke and Currah, 1993; Currah et al., 1993b).

The status of *Hymenoscyphus ericae* as an important mycobiont of the Ericales is well-founded (Read, 1983), but reliable identification of the taxon has been difficult because reproductive characters are not readily produced in culture. The teleomorph is rare under laboratory conditions (Perotto et al., 1995) and has never been reported from the field. Much of the physiological research has focussed on strains obtained from heathland sites in England which were often referred to as "the ericoid endophytes" and identified by an observed initial growth from root epidermal cells combined with a check for an ability to form the ericoid morphology in resynthesis with the host (for example, Stribley and Read, 1980).

The arthroconidial anamorph state was not well-described initially (Read, 1974), though a reference to the segmenting nature of the hyphae had also been made earlier by McNabb (1960). The original isolation and description of *Scytalidium vaccinii* from the roots of *Vaccinium angustifolium* (Dalpé et al., 1989) is the only distributional record of this taxon. Numerous strains of *S. vaccinii* have now been isolated from a wide range of ericaceous plants, indicating its importance as a mycorrhizal fungus in natural habitats in Alberta.

The common use of MEA and PDA as media (Pearson and Read, 1973a; Reed, 1989; Leake and Read, 1991; Perotto et al., 1990, 1994, 1996; Hutton et al., 1994) is unfortunate because these do not stimulate the formation of conidia. Isolates of *Scytalidium vaccinii* recovered in this study were grown on CMA to promote conidiogenesis and were positively identified. New RFLP data plus the production of apothecia by a single Type II strain (S-14Bb) confirms the anamorph-teleomorph connection to *H. ericae* proposed by Egger and Sigler (1993). In addition, Type II isolates differ by lacking one restriction site with only one of the four enzymes tested, *Rsa*I, suggesting a very low level of sequence divergence between Type I and II.

Cultural groupings similar to ours have been reported by other researchers characterizing dark sterile isolates. In some cases the cultural variant may reflect an

adaptation to the abiotic factors in the different ecosystems where ericaceous plants thrive. Identification of genetic variants based on cultural characters alone would be difficult but the unique absence of a restriction site in the rDNA of the bog isolates provides a marker for this wetland morphotype. In other cases, variants such as the VWT may represent a different taxon. In tandem with *Hymenoscyphus ericae*, another isolate used to study protease activity of endophytes from ericaceous plants and shown to form ericoid mycorrhizas with the host, was described as being creamy-white (Leake and Read, 1990). Xiao and Berch (1992) also report an unidentified white strain that formed ericoid mycorrhizas in vitro with *Gaultheria shallon* Pursh.

Strains of VWT were isolated frequently enough to indicate the taxon is a common endophyte. Resynthesis trials to determine whether typical ericoid complexes are formed in the roots are underway. Recovery was affected by the vigorous growth of *Phialocephala fortinii* which concealed early growth on the isolation medium. For the sand dune, where 93% of the collections yielded *P. fortinii*, the sterile white fungus was recovered from only 9% of the collections. Even for the bog isolations, from which *P. fortinii* was rarely recovered, submerged colonies were sometimes concealed by the other taxa.

Some uniformly grey-black strains (for example, S-77Ac in Fig. 2.11) were originally thought to be *Scytalidium vaccinii* in spite of being sterile because of the similarities in colonial pigmentation, growth rate and texture as well as the narrow hyphal width. An identical restriction digest profile for all four enzymes clearly links these strains to VWT, primarily characterized by the production of creamy white colonies on PDA. It is our opinion that VWT may be a separate taxon based on the molecular data. For three of the four enzymes, the RFLP patterns are different from both Type I and II of *S. vaccinii*. Gardes and Bruns (1996), using restriction endonucleases to characterize the ITS region of DNA extracted from ectomycorrhizal root tips, used the criterion of perfect matches with two enzymes for species level identification. This criterion is met by *Hymenoscyphus ericae* and all strains of *S. vaccinii* but VWT is mismatched for those same enzymes, *Alu*I and *Hha*I.

Strains of *Oidiodendron* were recovered from the roots of all but three species (*Kalmia polifolia*, *Menziesia ferruginea*, and *Vaccinium membranaceum*). Since two of those were under-represented in the numbers of collections and only a small proportion of each root system was sampled per collection, it is possible that increased sampling would yield strains from all ericaceous species in these habitats. The delimitation of species is based on conidiophore length, shape and size of the conidia, and colony pigmentation

(Barron, 1962; Domsch et al., 1980). All of these characters can be extremely variable within and among strains, and identification may be problematic except for species with additional distinctive characters.

Most strains were identified as *Oidiodendron maius* and restriction digest data confirmed the identification of isolates in which conidiophore ranges were intermediate between *O. griseum* and *O. maius*. *O. griseum*, characterized by the production of a dark amber diffusing pigment on PDA, MEA and ESA, was isolated at the same low frequency as rhizosphere taxa which were disregarded. Identification was based on conidium size and shape, and RFLP data. Pigment was noted as lacking in the original description of *O. griseum* (Melin and Nannfeldt, 1934, p. 440), but was observed for this species by Tokumasu (1973) and is found in several strains from other substrates (UAMH 1693, from Douglas fir timber; UAMH 5847, contaminant on UAMH 5794; UAMH 7719, from indoor air, honeybee overwintering facility).

The first records of both *Oidiodendron maius* and *O. griseum* as endophytes of ericaceous plants were from cultivated plants: *O. griseum* from *Vaccinium corymbosum* cultivars (Couture et al., 1983) and *O. maius* from *Rhododendron* cultivar "Pink Pearl" (Douglas et al., 1989). Mycorrhizal status was assumed when typical ericoid mycorrhizas developed with the host plant in monoxenic culture. Though a report for *O. griseum* by Burgeff (1961) predates those records, the objective of that study was to isolate all types of peatland fungi and no attempts were made to sterilize the roots or otherwise focus on endophytic taxa. Other isolations from the Ericaceae in natural habitats are limited to *O. griseum* from *Loiseleuria procumbens* (Stoyke and Currah, 1991), *Gaultheria shallon* (Xiao and Berch, 1992) and *Calluna vulgaris* (L.) Hull. (Johansson, 1994), and *O. maius* also from *Calluna vulgaris* (Perotto et al., 1994, 1996). The paucity of isolates of *O. griseum* recovered in this study indicates that the species does not play a significant endophytic role in the habitats sampled. By contrast, our results show that *O. maius* is strongly endophytic with the Ericaceae in Alberta but that, due to the variability in key conidial characteristics, researchers may experience difficulties identifying some strains.

Resynthesis trials have tested the ability of numerous *Oidiodendron* species to form ericoid mycorrhizas with *Vaccinium angustifolium* Ait. (Dalpé, 1986, 1989, 1991) and *Gaultheria shallon* (Xiao and Berch, 1995). Results show that many freely do so in axenic culture although only two species, *O. griseum* and *O. maius*, have been isolated from plants in natural or agricultural soils. In this study, sites were chosen where ericaceous plants were dominant; it is possible that other species of *Oidiodendron* cannot tolerate the

extreme abiotic factors associated with heathland habitats (Read, 1991). Ericoid mycorrhizas from more moderate soils with a more diverse flora may yield different species of *Oidiodendron.*

Although six species of fungi were shown to have the ability to establish ericoid hyphal complexes in the roots of *Gaultheria shallon* in the laboratory, only one of those six, *Oidiodendron griseum*, was also isolated from the field site roots (Xiao and Berch, 1995). Currah et al. (1993a) isolated *O. periconioides* from roots of *Rhododendron* grown in pot-cultures in which unsterilized milled horticultural peat moss and sterilized beech sawdust was used as a rooting medium. Although this species and others can form ericoid mycorrhizas in monoxenic culture, it is not known if they do so in nature.

Endophytes may be involved in a variety of interactive associations with host roots, whether beneficial or otherwise to either partner. There are multiple benefits of mycorrhizal association for ericaceous plants with *Hymenoscyphus ericae* (Read, 1983, 1991) and there is also evidence that the mycobiont receives a portion of its carbon budget from its host (Pearson and Read, 1973b). *Oidiodendron maius* and *O. griseum* are generally accepted as mycorrhizal endophytes (Straker, 1996) though limited experiments have been done to determine whether the host plant benefits in similar ways from the association (Leake and Read, 1991). The presence of these species in stressed habitats would seem to imply that they are enzymatically versatile in nutrient capture. Their repeated isolation from vigorous plants indicates that they are not pathogenic and supports the speculation that they are either increasing the fitness of the host or at least are commensalists. Most species in the genus are saprotrophs on cellulosic materials so that the possible benefits of mycorrhizal association for the fungus are not obvious.

There has been strong though indirect evidence in the literature that more than one species of fungus could be present in the same root system. Ultrastructural analyses have revealed the presence of ascomycete and basidiomycete hyphae in adjacent root cells (Bonfante-Fasolo, 1980; Peterson et al., 1980). Perotto et al. (1994) have used PCR-RAPD (random amplified polymorphic DNA) techniques to show that different unidentified species and different individuals of the same species coexist in the same host root system. In this study, two different mycorrhizal species, *Scytalidium vaccinii* and *Oidiodendron maius*, were isolated from the same root system of 30% of the collections, meaning, in most cases, the same one-centimeter fragment. Since both are ascomycetes with similar hyphal width, ultrastructural data would not identify their simultaneous presence in root cells.

The combined use of direct isolation and genetic analysis will clarify the diversity and distribution of the mycobionts found in association with the Ericaceae in natural habitats. In particular, the use of RFLP patterns to characterize sterile taxa would allow the comparison of isolates from different studies, and potentially to match them with RFLP profiles from DNA amplified directly from mycorrhizas. The use of consistent primer pairs and restriction endonucleases will be needed, as well as published RFLP fragment sizes. The primer pair used in this study utilizes the fungal specific primer NL6Amun and would be suitable for such comparative research. NL6Amun has a greater affinity for ascomycetous fungi but the sister primer NL6Bmun would target basidiomycetes (Egger, 1995). In a recent molecular analysis of the root endophytes isolated from *Calluna vulgaris* in Italy, Perotto et al. (1996) have also reported that cultural groupings of sterile isolates do not always match the groups revealed by RFLP analysis, and that only some isolates match *H. ericae*. Their use of the primer pair ITS1-ITS4 which amplifies a shorter fragment comprising the ITS region only provides fewer polymorphisms. With only one restriction enzyme (*Hin*fI) common to both studies and no size standard on the gel illustrations, it is difficult to make further comparisons.

The choice of isolation techniques and the focus of the researcher can have an effect on the results in research of this kind. In a study aimed at isolating strains of *Phialocephala fortinii* for molecular analysis, roots of ericaceous plants collected from the same alpine site used in this study and cultured for their endophytes resulted in an array of *P. fortinii* variants, one strain of *Oidiodendron griseum* but no isolates of *Scytalidium vaccinii* (Stoyke and Currah, 1991). This study revisits the same alpine site and includes the same ericoid mycorrhizal plant species. Using techniques aimed at isolating mycorrhizal endophytes, different fungi were recovered in spite of the abundant, concealing growth of *P. fortinii*. The techniques used here though, did not favour the growth of basidiomycetes whose recovery could be enhanced by the use of selective media containing benomyl to discourage ascomycetous fungi (Schild et al., 1988). In view of the immunocytochemical evidence that a *Clavaria* sp. is mycorrhizal with *Rhododendron* (Mueller et al., 1986), the discovery of *Clavaria vermicularis* growing among *Empetrum nigrum* and *Phyllodoce* spp. at this site warrants further investigation.

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Table 2.1. Quantity and provenance of collections of 19 ericaceous species and the corresponding number of collections yielding five endophytic fungal taxa.

Species of Ericaceae Sampled	Total Collections	Number of Collections Yielding Endophytes				
		O. maius	*O. griseum*	*S. vaccinii*	VWT	*P. fortinii*
Site #1: Alpine						
Cassiope mertensiana (Bong.) D. Don	13	1		8	3	6
Cassiope tetragona (L.) D. Don	7	1		5	1	3
Empetrum nigrum L.	13	4		12	1	5
Gaultheria humifusa (Graham) Rydb.	5	1		4		2
Kalmia polifolia Wang	7			5	1	6
Loiseleuria procumbens (L.) Desv.	14	2		9	1	13
Menziesia ferruginea J. E. Smith	5			3		3
Phyllodoce empetriformis (Smith) D.Don	11	2		10	2	8
Phyllodoce glanduliflora (Hook.) Coville	8	2	1	6		4
Rhododendron albiflorum Hook.	7	1		3	3	4
Vaccinium membranaceum Dougl. ex Hook	12			6	3	8
Vaccinium scoparium Leiberg	9	2		4	1	6
Vaccinium uliginosum L.	5	1		3		4
Vaccinium vitis-idaea L.	5			4		2
Site #2: Sand Dune						
Vaccinium myrtilloides Michx.	30	8	1	10	4	28
Vaccinium vitis-idaea L.	27	8	2	11	1	25
Site #3: Bog						
Andromeda polifolia L.	18	13		15	7	1
Chamaedaphne calyculata (L.) Moench	18	11		17	7	2
Ledum groenlandicum Oeder	17	16		15	8	
Oxycoccus quadripetalus Gilib.	21	10		20	6	
Vaccinium vitis-idaea L.	17	12		17	8	

Table 2.2. Strains of *Hymenoscyphus ericae*, *Scytalidium vaccinii* and VWT used for RFLP analysis and the corresponding digest patterns with four restriction enzymes. "T" indicates ex-type culture.

Species	Representative Strain Used	Origin	Base pair lengths of bands generated by RFLP *Alu*I	*Hha*I	*Hin*fI	*Rsa*I
Hymenoscyphus ericae	UAMH 6735*	T, ex roots *Calluna vulgaris*	375	549	440	745
	(IMI 182065)	England; D. Read, 1974	243	182	293	179
Scytalidium vaccinii::	UAMH 5828*	T, ex roots *Vaccinium*	109	138	170	
Type I	(DAOM 196925)	*angustifolium*	82	87		
		Maine USA; W. Litten, 1989	69			
	S-109Aa*	Site 2, *V. myrtilloides*				
	S-120a*	Site 2, *V. vitis-idaea*				
	S-87Bb*	Site 1, *E. nigrum*				
	S-85Ac*	Site 1, *V. myrtilloides*				
	S-77Ba	Site 1, *P. empetriformis*				
	S-76Bc	Site 1, *P. empetriformis*				
Hymenoscyphus ericae	S-14Bb*	Site 3, *L. groenlandicum*	375	549	440	917
Scytalidium vaccinii::	S-24Ab*	Site 3, *O. quadripetalus*	243	182	293	
Type II	S-42b	Site 3, *C. calyculata*	109	138	170	
	S-355b	Site 2, *V. vitis-idaea*	82	87		
Variable White Taxon:	S-70Ac*	Site 1, *R. albiflorum*	642	560	455†	560
	S-71Aa	Site 1, *V. membranaceum*	147	333	308†	194
	S-77Ac*	Site 1, *P. empetriformis*	109	87	170	179
	S-86Ae*	Site 1, *E. nigrum*				

* There is a corresponding photograph of the culture on PDA.
† The difference in length as compared to H.e. and S.v. is more likely due to the length difference of the uncut DNA than to restriction site differences.

Table 2.3. Strains of *Oidiodendron* used for RFLP analysis and the corresponding digest patterns with four restriction enzymes. "T" indicates ex-type culture. "A" indicates authentic culture.

Species	Representative Strain Used	Origin	Base pair lengths of bands generated by RFLP			
			*Alu*I	*Hha*I	*Hin*fI	*Rsa*I
Oidiodendron maius	UAMH 1540	T, soil ex cedar bog Ont., Canada G. L. Barron, 1960				
	S-9Ca	Site 3, *C. calyculata*	455	435	435	450
	S-10Aa	Site 3, *O. quadripetalus*	156	182	165†	282
	S-38a	Site 3, *L. groenlandicum*	145	130	135	175
	S-27b*	Site 2, *V. myrtilloides*	108	110		
	S-357Ca	Site 2, *V. vitis-idaea*		85		
	S-74Aa	Site 1, *P. glanduliflora*				
	S-77Aa	Site 1, *P. empetriformis*				
	S-81Bc*	Site 1, *V. scoparium*				
Oidiodendron griseum	UAMH 1403	A, wood pulp	369	525	435	282
	(CBS 249.33)	Sweden	245	182	295	260
		E. Melin, 1933	150	130	170	175†
	S-31b	Site 2, *V. myrtilloides*	108	85		
	S-266b	Site 2, *V. vitis-idaea*				

* Strains with intermediate conidiophore lengths.
† Doublet inferred to balance total fragment size

Figures 2.1 - 2.12.

Colonial morphology of fungal endophytes from ericaceous roots.
All colonies on PDA, except Fig. 7 on CMA, at 25° after 8 weeks, X 0.8. All strains illustrated were analysed with restriction enzymes.

Figs 2.1-2.4. *Scytalidium vaccinii* Type I.

2.1. S-109Aa, ex roots *Vaccinium myrtilloides*, sand dune (Site 2).

2.2. S-120a, ex roots *Vaccinium vitis-idaea*, sand dune (Site 2).

2.3. S-87Bb, ex roots *Empetrum nigrum*, alpine (Site 1).

2.4. S-85Ac, ex roots *Vaccinium myrtilloides*, alpine (Site 1).

Figs. 2.5-2.7. *Scytalidium vaccinii*, Type II.

2.5. S-24Ab, ex roots *Oxycoccus quadripetalus*, bog (Site 3).

2.6 and 2.7. S-14Bb (UAMH 8680), ex roots *Ledum groenlandicum*, bog (Site 3).

Fig. 2.8. *Hymenoscyphus ericae*, UAMH 6735, ex-type culture, ex roots *Calluna vulgaris*.

Fig. 2.9. *Scytalidium vaccinii*, UAMH 5828, ex-type culture, ex roots *Vaccinium angustifolium*.

Figs. 2.10-2.12. Variable White Taxon (VWT).

2.10. S-70Ac, ex roots *Rhododendron albiflorum*, alpine (Site 1).

2.11. S-77Ac, ex roots *Phyllodoce empetriformis*, alpine (Site 1).

2.12. S-86Ae, ex roots *Empetrum nigrum*, alpine (Site 1).

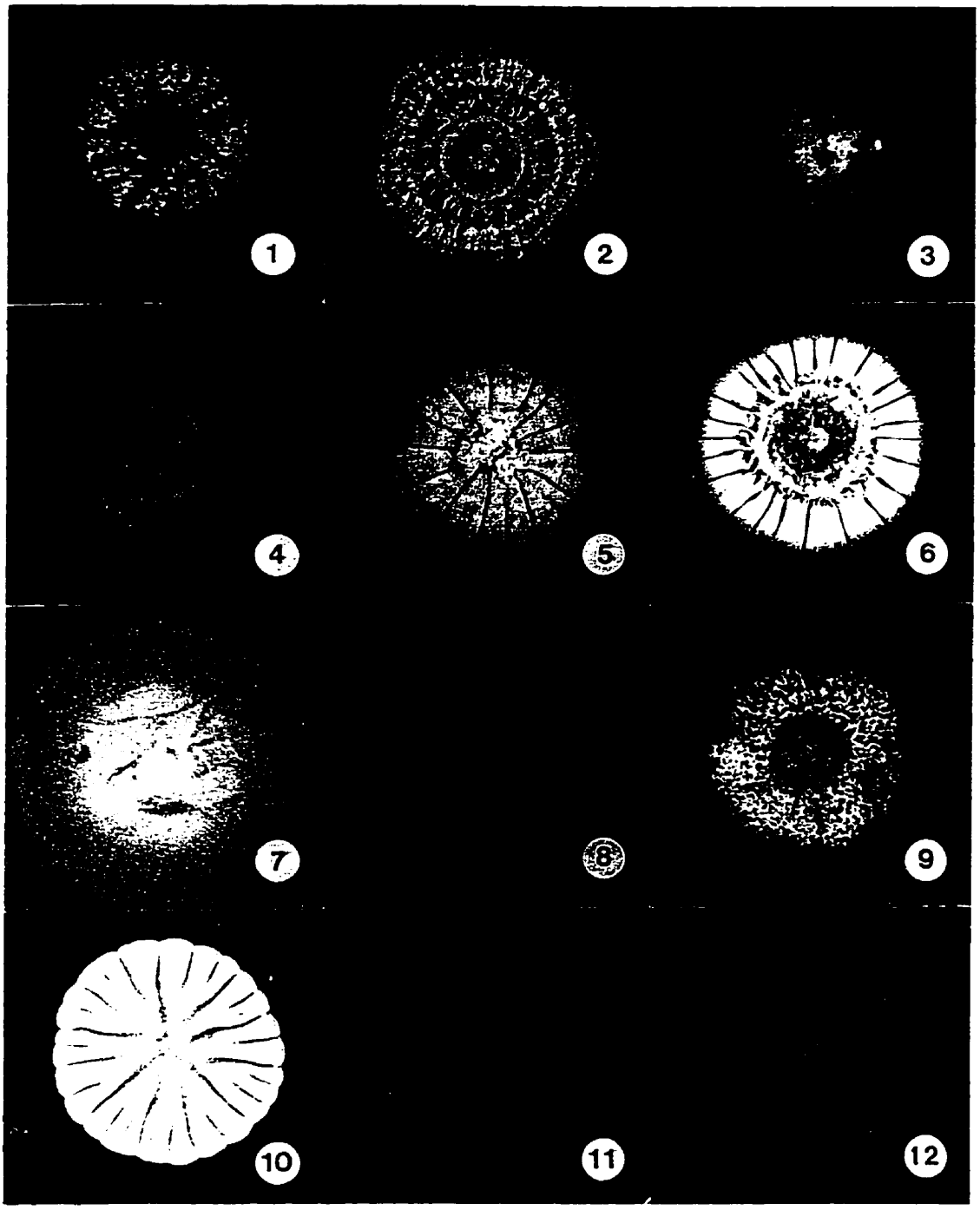
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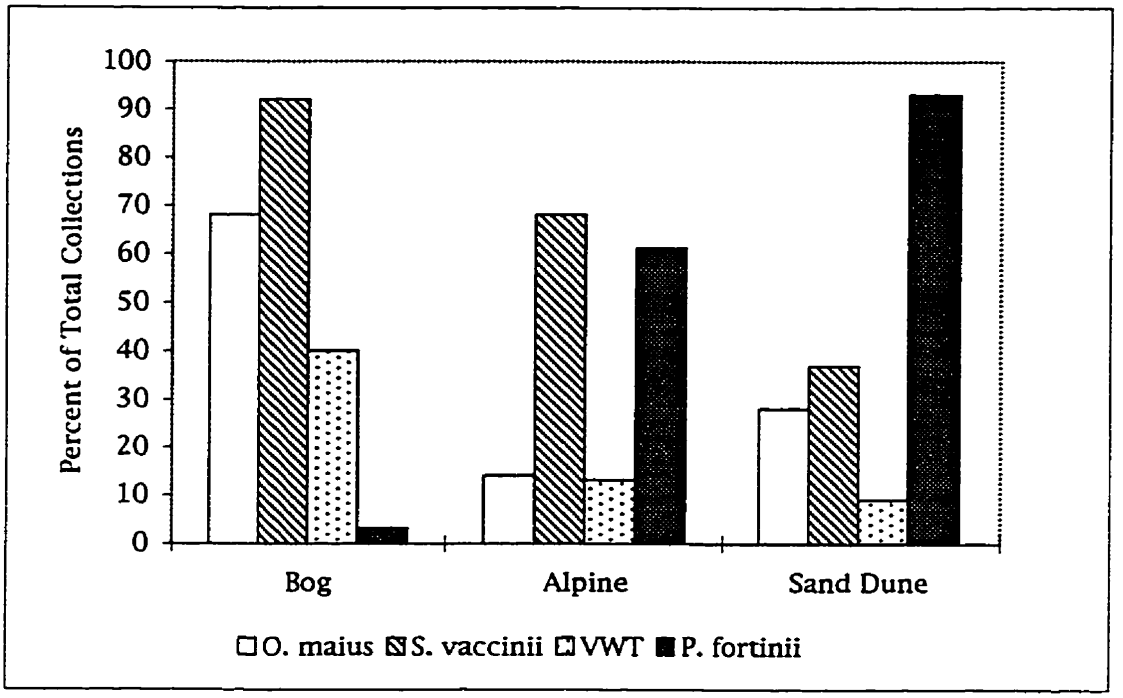
Figure 2.13. Comparison of the recovery of four fungal endophytes from ericaceous roots for each site. The height of the bar indicates the percentage of the total number of collections sampled that yielded each taxon.



Figures 2.14 - 2.16.

Microscopic morphology of fungal endophytes from ericaceous roots.
Scale bars = 10 μm.

Fig. 2.14. Light micrograph of 30d CER slide culture of *Scytalidium vaccinii* , S-355b (ex roots *Vaccinium vitis-idaea*, sand dune, Site 2), showing dematiaceous strands and toruloid cells giving rise to chains of hyaline, cylindrical arthroconidia.

Fig. 2.15. Light micrograph of *Scytalidium vaccinii*, S-243c (ex roots *Vaccinium myrtilloides*, sand dune, Site 2), at 5 mo on CMA showing inflated, non-disarticulating conidia typically produced submerged in the medium near the colony margin in older cultures.

Fig. 2.16. Light micrograph of 5 wk CER slide culture of VWT, S-39a (ex roots *Chamaedaphne calyculata*, bog, Site 3), showing short, swollen hyphal tips but no true conidia.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Chapter 3

Hymenoscyphus ericae: an Expanded Description Based on a New Record from Western Canada [1]

Introduction

Studies involving the isolation of fungal endophytes from ericoid mycorrhizas have dealt with strains of slow-growing, darkly pigmented fungi which often remain sterile on agar media (McNabb, 1960; Pearson and Read, 1973; Singh, 1974; Reed, 1989; Perotto et al., 1990; Hutton et al., 1994; Steinke et al., 1996). Experiments using such strains have elucidated physiological interactions of the mycorrhizal partners and led to an appreciation of the significant role played by ericoid mycorrhizal fungi in the successful colonization of low-nutrient soils by ericaceous plants (Read, 1983). However, little progress has been made in our understanding of the diversity and ecology of these fungal associates because sterile isolates in pure culture are difficult to identify with confidence.

The mycobiont *Hymenoscyphus ericae* (Read) Korf & Kernan (Kernan and Finocchio, 1983) was named when one of these strains, isolated from the roots of *Calluna vulgaris* L. Hull., produced apothecia in pot culture during resynthesis experiments with the host plant (Read, 1974). Dried apothecia from this resynthesis were designated as the type specimen but the teleomorph (originally named *Pezizella ericae* Read; Read, 1974) was described only briefly. Reference was made to hyphae which break up into segments when mature, but an anamorph was not formally described.

Apothecia of most strains of *H. ericae* are not produced readily in culture (Perotto et al., 1995), and, as far as we are aware, have never been collected in the field. The type specimen lacks mature apothecia and there are no other herbarium depositions of teleomorphic material. Consequently, there are good reasons for revisiting the taxonomy of the holomorph of this species.

Since the original description, a taxonomic link to the hyphomycete taxon *Scytalidium vaccinii* Dalpé, Litten & Sigler (1989) has been suggested by molecular

[1] A version of this chapter has been submitted for publication. Hambleton, S., Huhtinen, S., and R. S. Currah 1998. Mycological Research. The description of the teleomorph (p. 43) and Figs. 3.8 and 3.9 are the work of S. Huhtinen.

analysis in which the percent sequence divergence between fungi identified as *Hymenoscyphus ericae* and *Scytalidium vaccinii* was comparable to levels of intraspecific variation reported for other fungi for the same gene (Egger and Sigler, 1993). With restriction fragment length polymorphism (RFLP) analysis, Hambleton and Currah (1997) distinguished two genotypes amongst isolates of *S. vaccinii* isolated from ericaceous roots in Alberta, though microscopically the conidial state was consistent. RFLP data for Type I matched those for the ex-type cultures of *H. ericae* and *S. vaccinii* while Type II differed by the lack of a single four-base restriction site with one of four enzymes tested. These minor differences were correlated with habitat, suggesting that a degree of ecotypic variation exists within the species.

Recently, the production of the teleomorph by a Type II strain of *Scytalidium vaccinii*, identified as *Hymenoscyphus ericae*, provided an opportunity to present a more complete description of the holomorph of this species. This is also the first record of *H. ericae* from western North America.

Materials and Methods

The fungus (UAMH 8680) was isolated from surface sterilized roots of *Ledum groenlandicum* Oeder collected October 16, 1993 (collection #S-14) from an acidic peatland (bog) near Athabasca townsite in central Alberta, Canada (Hambleton and Currah, 1997).

Specimens are deposited at the University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada as UAMH 8680 (S-14Bb), and UAMH 8685, a single-spore isolate from UAMH 8680. Cultures were maintained at room temperature in the dark on CMA (cornmeal agar, Difco-Bacto), MEA (2% malt extract, Difco-Bacto), and PDA (potato dextrose agar, Difco-Bacto). Microscopic observations and measurements of the anamorph were made using cereal (CER) slide culture preparations (Sigler, 1993). Fresh roots were also stained and examined for the presence of ericoid mycorrhizas following the method of Hutton et al. (1994).

Apothecium production was induced on Melin's agar (Melin, 1959), following the procedure outlined by Webster (1976). A subculture from a PDA slant stored at 4°C was incubated on Melin's agar at room temperature under the combined illumination of a black light (Philips, BL 20W) and a "grolight" (Sylvania gro-lux wide spectrum 20W) using a 12 hr illumination cycle. Apothecia appeared after 4 weeks. A single spore isolate, deposited as UAMH 8685, produced more apothecia under the same conditions. The recipe for Melin's agar, per litre of distilled water, is as follows: glucose 20g, KH_2PO_4 1g,

$MgSO_4 7H_2O$ 0.5g, NH_4-tartrate 0.5g (NH_4NO_3 was substituted here), ferric citrate (1% soln.) 0.5 ml, Zn soln. (Zn conc. 1:500) 0.5 ml with 50 µg thiamine added to cooled medium after autoclaving at 121°C for 15min, and pH adjusted to 5.

Procedures for preparing squash mounts and for recording the characters of the apothecia may be found in Huhtinen (1990, 1994) and Baral (1992). Descriptions incorporate CB for Cotton Blue in lactic acid, CR for ammoniacal Congo Red, KOH for 10% potassium hydroxide, MLZ for Melzer's reagent, LUG for Lugol's solution with 1% iodine, and CRB for Cresyl Blue in water. Observations are based on dried material unless otherwise noted. Drawings were made with a drawing tube and measurements include 90% of the variation observed. Specimens were prepared for SEM following the procedure of Currah and Tsuneda (1993) and examined with a JEOL JSM6301FXV scanning electron microscope.

Results and Discussion

Stained roots revealed the presence of typical ericoid hyphal complexes within root cells such as those shown in Fig. 3.1. The production of apothecia by the single spore isolate confirms a previous report that *H. ericae* is homothallic (Webster, 1976). Both UAMH depositions include teleomorphic and anamorphic material as dried herbarium specimens and as living material stored in liquid nitrogen.

Hymenoscyphus ericae (D. J. Read) Korf & Kernan, *Mycologia* 75: 919. 1983

Basionym: *Pezizella ericae* D. J. Read, *Transactions of the British Mycological Society* 63: 381. 1974.

Holotype: Great Britain, Yorkshire, Bolderstone, isolated from the roots of *Calluna vulgaris* L. Hull., July 1970 Read (IMI 182065, isotypes in K and CBS; not studied). (IMI = International Mycological Institute, Egham, United Kingdom; K = Royal Botanic Gardens, Kew, Surrey, United Kingdom; CBS = Centraalbureau voor Schimmelcultures, Baarn, The Netherlands)

Apothecia cupulate (Figs. 3.2-3.4), or rarely strongly convex, up to 1µm in diam when fresh, at first pale greyish, later pale yellowish to orange; flanks smooth appearing minutely hairy when small, margin densely beset with hairlike hyphal ends protruding well over the disc; stipe short, firm, ca. 100-150 x 80-100 µm (Figs. 3.4 and 3.8e). *Ectal excipulum* of hyphoid cells close to the margin (Fig. 3.9c), narrowly prismatic cells on medial flanks and

prismatic to isodiametric cells towards the base (Figs. 3.9d-g); cells at medial flanks 8-15 x 2-3µm ($\overline{Q}$ = 3.0-7.5 in CB), at base 8-15 µm across when fresh in water, 4-9 µm across in CB, when prismatic 8-12 x 3-6 µm; walls 0.2-0.5 µm thick in CB, basally up to 1 µm thick, hyaline, no reaction with MLZ with and without KOH-pretreatment, no reaction with CB and CR, but with CRB deep wine red in places; lacking extraneous crystals, resin and gelatinized parts in all mountants, also when fresh in water. Cells of the ***medullary excipulum*** similar to those of the ectal excipulum but narrower, gradually grading to textura intricata inside. *Margin* of cylindrical hyphal apices (Fig. 3.8a), 1.5-2.0 µm broad when dried, 2.0-2.5 µm wide when fresh in water, apices even to 0.5 µm broad; clear guttules or refractive bodies absent in fresh material mounted in water; walls thin when fresh in water, as well as in CB, CR, MLZ, in places appearing rigid and 0.2-0.5 µm thick in CB. *Lower flanks* irregularly beset with clavate cells measuring 12-20 x 5-7 µm, walls thin. *Asci* (Figs. 3.7, 3.8c, 3.8d, and 3.9a) 63-77 x 8-10 µm when fresh in water, (n= 4); 52-63 x 6.5-8.5 µm in CR ($\overline{x}$= 56.5 x 7.3 µm, n= 10); 50-60 x 6.2-7.0 µm in MLZ ($\overline{x}$= 56.2 x 6.8 µm, n= 10), eight-spored, apex faintly MLZ+ without KOH-pretreatment, clearly MLZ+ with KOH-pretreatment, clearly blue in LUG, the apical ring evenly cylindrical around the porus but flaring outwards at the apex and to a lesser degree at the base (observations in LUG) (Fig. 3.8c); asci simple septate at the base (Figs 3.7, 3.8d, and 3.9a), lacking true croziers but sometimes with a downward protuberance (Fig. 3.8d). *Ascospores* (Figs. 3.6, 3.7, 3.8b, 3.8d, 3.9a, and 3.9b) ellipsoid to slightly subfusoid, 6.2-8.8 x 3-4 µm when fresh in water ($\overline{x}$= 7.4-3.4 µm, $\overline{Q}$= 2.2, n= 20); 5.4-8.8 x 2.3-3.2 µm in MLZ and CB ($\overline{x}$= 6.8 x 2.8 µm, $\overline{Q}$= 2.5, n=50); 6.8-7.5 x 3.0-3.4 µm in CR ($\overline{x}$= 6.6 x 2.9 µm, $\overline{Q}$= 2.2, n= 10), aseptate to rarely one-septate, hyaline, lacking guttulae in all mountants, also when fresh in water, smooth, thin-walled, septum unstained in CB, MLZ. *Paraphyses* cylindrical, not protruding above the level of the asci (Fig. 3.5), simple, rarely branched, thin-walled, lacking guttulation or pigment in all mountants (Figs. 3.8d and 3.9a), also when fresh in water, not forming an epithecium; terminal cells 23-42 x 1.2-2.0 µm ($\overline{x}$= 31 x 1.6 µm, n=10) in CB and MLZ.

Anamorph: Growth on CMA, and PDA covering no more than 75% of the petri plate regardless of age. Colonial morphology: on CMA, aerial hyphae fertile, white, produced in dense tufts from the surface of the mycelium; on PDA, colony raised and floccose in the centre becoming flat and smooth textured, grading from mostly white to grey-brown close to the edge with a narrow, submerged lighter coloured margin; characteristic deep radial sulcae often split open with age; on MEA, colony mostly submerged, dark greenish-black, aerial hyphae paler and sparse. Vegetative hyphae smooth, hyaline, 1.5-2.5 µm wide,

becoming dematiaceous with age; abundant toruloid cells and dematiaceous strands developing in older preparations. Fertile hyphae undifferentiated from vegetative hyphae; segmenting into smooth, hyaline, cylindrical arthroconidia of variable lengths, 3-10 μm, which separate via schizolytic dehiscence but often remain connected in zig-zag chains. Arthroconidial production abundant on CMA, sparse on MEA and Melin's agar, and absent on PDA. The conidial state is identified as *Scytalidium vaccinii* Dalpé, Litten & Sigler, Mycotaxon 35:372. Holotype: U.S.A., Maine, Washington County, isolated from the cortical root cells of *Vaccinium angustifolium* Ait., April 1986 Dalpé (DAOM 196925). (DAOM = Canadian Collection of Fungus Cultures, Ottawa, Canada)

As Straker (1996) summarizes in his review paper, the identification of the dark, sterile fungi isolated from ericoid mycorrhizas is becoming increasingly problematic for researchers in diverse geographic locations. Though the fungi isolated are apparently culturally similar to *Hymenoscyphus ericae*, a variety of molecular and biochemical approaches indicates a heterogeneous assemblage of fungi (Hutton et al., 1994; Perotto et al., 1996).

The results of previous research (Hambleton and Currah, 1997) suggested that amongst a group of culturally similar strains of root endophytes isolated from the Ericaceae, there were both subspecific and supraspecific differences based on RFLP (restriction fragment length polymorphism) analysis. Isolates identified as *Hymenoscyphus ericae* comprised two genotypes whose distribution correlated with habitat. Type I, from upland sites, varied in arthroconidial production while Type II, from an acidic peatland, produced conidia readily in culture. In addition, the analysis delimited a separate and as-yet unidentified taxon (Variable White Taxon) which never formed conidia. These results highlight the hazard of relying on cultural morphology for the identification of sterile fungi, though they do not negate the validity of the research that has been done on the functioning of ericoid mycorrhizas in ecosystems (Read, 1991).

Obtaining the teleomorph in culture is a rare occurrence. Of 30 strains of *H. ericae* (including the ex-type strains of *H. ericae* and *S. vaccinii*) that were grown on Melin's agar as described, only one, UAMH 8680, formed apothecia. Consequently, it is usually necessary to identify this ericoid endophyte on the basis of other characters. In the absence of ascomata, appropriate cultural conditions to obtain the anamorph, combined with molecular characterization, should be used. The latter approach may be more advantageous, since conidiogenesis is often difficult to induce. RFLP digests of the same portion of nuclear ribosomal DNA using the same restriction enzymes can be compared to those

published by Hambleton and Currah (1997). For strains which do not readily form arthroconidia in slide culture preparations, or on CER or CMA, prolonged growth on CMA at room temperature, up to four months or more, will eventually result in the formation of conidia submerged in the medium, though production can often be sparse.

The characteristics of the hyphae and conidia in UAMH 8680 are comparable to those given the original description of *Scytalidium vaccinii* (Dalpé et al., 1989) even though there are minor genetic differences between these two collections. However, UAMH 8680 is representative of a distinct ecotype, comprising strains isolated from an acidic peatland (Type II). This ecotype differs in cultural morphology, as well as in the prolific production of arthroconidia on CMA and in slide culture, as compared to the ex-type strain of either *S. vaccinii* (UAMH 5828) or *Hymenoscyphus ericae* (UAMH 6735), both having Type I restriction digest profiles. On CMA, Type II strains produce abundant white and fertile aerial hyphae whereas Type I isolates produce conidia less reliably from the predominantly submerged growth of the mycelium. Pigmentation on PDA is variable but Type II colonies are generally lighter in colour and of a smoother texture. In addition, Type II isolates lack a restriction site found in Type I isolates when rDNA is digested with *Rsa*I (Hambleton and Currah, 1997). Egger and Sigler (1993) and Hambleton and Currah (1997) provide further discussions of the cultural and vegetative characteristics of strains of *H. ericae* and include light micrographs of the conidial state.

Details of the apothecia and ascospores given here differ slightly from the original prepared by Read (1974). Spore length in our material was shorter than that given by Read (1974). In addition, young apothecia have a hyaloscyphaceous appearance due to the long marginal hyphal ends and the short clavate cells on the flanks, while Read's drawing appears to portray a typically helotiaceous fungus. In our material, the number of clavate hyphal ends on flanks varies among individual apothecia, and in Read's the apothecia illustrated were more expanded. These differences might be attributed to the effect of agar versus pot culture. In spite of these apparent differences, Type I and Type II are presumed to be conspecific. It would seem ill-advised to describe a new species in this taxon based on the differences discussed, especially given the fact that all isolates have so far been obtained from roots of the Ericales.

The most appropriate genus for the disposition of *Hymenoscyphus ericae* has been and continues to be unclear. The original placement in *Pezizella* Fuckel was later changed by Korf and Kernan (in Kernan and Finocchio, 1983) because of the non-gelatinized excipular structures. Since then however, the genus *Pezizella* has been variously delimited

(Svrček, 1983; Arendholz, 1989; Baral, 1994). Recently Baral (1994) concluded, in contrast to Arendholz (1989), that *Pezizella* is a synonym of *Calycina* Nees ex Gray (type species *C. herbarum* (Pers.) Gray), a taxon which differs substantially from the genus *Hymenoscyphus* Gray (type species *H. fructigenus* (Bull.:Fr.) S. F. Gray). Considering the characters listed typical for *Calycina* by Baral and Krieglsteiner (1985), our material is similar to *Calycina* with respect to excipular structure and in ascal reaction (Baral and Krieglsteiner, 1985; Triebel and Baral, 1996). However, no obvious or refractive contents, often present in *Calycina* (Baral and Krieglsteiner, 1985), were noted in the paraphyses or marginal hyphae of our material of *H. ericae*. Considering that the systematics of these inoperculate genera are in a state of flux, it seems imprudent to burden an ecologically interesting species with new taxonomic placement.

Our description is based on living material for the most part. Ascospores and asci are larger when measured in living material compared to specimens preserved by drying. Read's (1974) measurements may have been from fresh specimens but the smaller ascal and ascospore measurements would indicate a study based on dried material or otherwise dead cells. The simple septate ascal bases with "downward protuberances" remaining separate from the stalk cell (= preceding cell) were typical in some taxa in *Hyaloscypha* Boud. (Huhtinen, 1990). This character is linked with simple-septate (aporhynchous) ascal bases and is relatively stable among populations. In *Hyaloscypha* it was a useful character at the species level. However, some simple-septate taxa never showed this character and in some taxa many populations showed these "downward protuberances" in every apothecium (but not in every ascus). Details of ascal bases have recently received taxonomic weight exemplified in the treatments of Weber (1992), Huhtinen (1994), Hengstmengel (1996) and Triebel and Baral (1996), but more data are needed for species of *Hymenoscyphus* in order to evaluate the usefulness of this character here.

Type material was not studied. In a letter to L. Sigler (Curator, UAMH; pers. comm.), Dr. B. M. Spooner at K states that although the isotype consists of dried specimens on *Calluna*, the material is scanty and those apothecia which have been examined do not contain asci or spores. It was not deemed appropriate at this time to further damage the isotype. The holotype in IMI, consisting of two dried cultures and a slide, is devoid of a teleomorph (Dr. John David, pers. comm.). Consequently, the type material is inadequate for comparative purposes and the illustrations by Read (1974) lack sufficient detail to serve as a lectotype. The best approach to retypification would be to select an ample collection from the wild, if one could be found. This would then serve as an epitype (Art. 9.7 of the Code, Greuter et al., 1994). Alternatively, attempts could be

made to regrow the teleomorph from the freeze dried culture of the holotype in IMI. However, our attempts to induce fruiting of the ex-type strain have so far been unsuccessful. Litten (1985) reported the fruiting of an authentic strain of *H. ericae* in resynthesis trials using the procedure of Moore-Parkhurst and Englander (1981) but there was no indication that specimens were preserved.

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Figures 3.1 - 3.7.

Microscopic and cultural morphology of *Hymenoscyphus ericae.*

Fig. 3.1. Fresh "hair" root of *Ledum groenlandicum* (S-277) showing hyphal complexes, stained with trypan-blue/glycerol/lactic acid, in epidermal cells. Note the presence of narrow arthroconidia typical of *H. ericae* within cells (arrowhead) as well as external to the root (arrow). Bar = 10 μm.

Figs. 3.2-3.7. UAMH 8680.

Fig. 3.2. Apothecia produced beyond the visible margin of the colony from submerged growth, on Melin's agar after 4 months. Colony sulcate, raised in the centre, smooth and pale-brown grading to floccose and dark-brown at the margin. Bar = 1 cm.

Fig. 3.3. Detail of two apothecia on Melin's agar; one is a young fertile cupulate apothecium while the margin of the other disc is reflexed back onto the agar surface with age. Bar = 250 μm.

Fig. 3.4. SEM micrograph of an apothecium before the disc is fully expanded. Bar = 50 μm.

Fig. 3.5. SEM micrograph showing intermingled ascus tips and paraphyses on the upper surface of the hymenium. Bar = 5 μm.

Fig. 3.6. SEM micrograph showing detail of ascospores, ascal tips and paraphyses. Bar = 2 μm.

Fig. 3.7. Light micrograph of an ascus with 8 ascospores, from an unstained squash mount of an apothecium. Bar = 10 μm.

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Figure 3.8.

Hymenoscyphus ericae (UAMH 8680).
Illustrated in vital condition in water, unless otherwise indicated. CB = Cotton Blue in lactic acid; CR = ammoniacal Congo Red; KOH = 10% potassiom hydroxide; LUG = Lugol's solution with 1% iodine; MLZ = Melzer's reagent. Bar = 50 μm except in e) bar = 200 μm and c) bar = 5 μm.

a) cylindrical hyphal apices from cup margin
b) ascospores
c) ascus apex showing the apical ring
d) paraphyses and asci; arrow indicates downward protuberance observed on some asci
e) fresh apothecia.

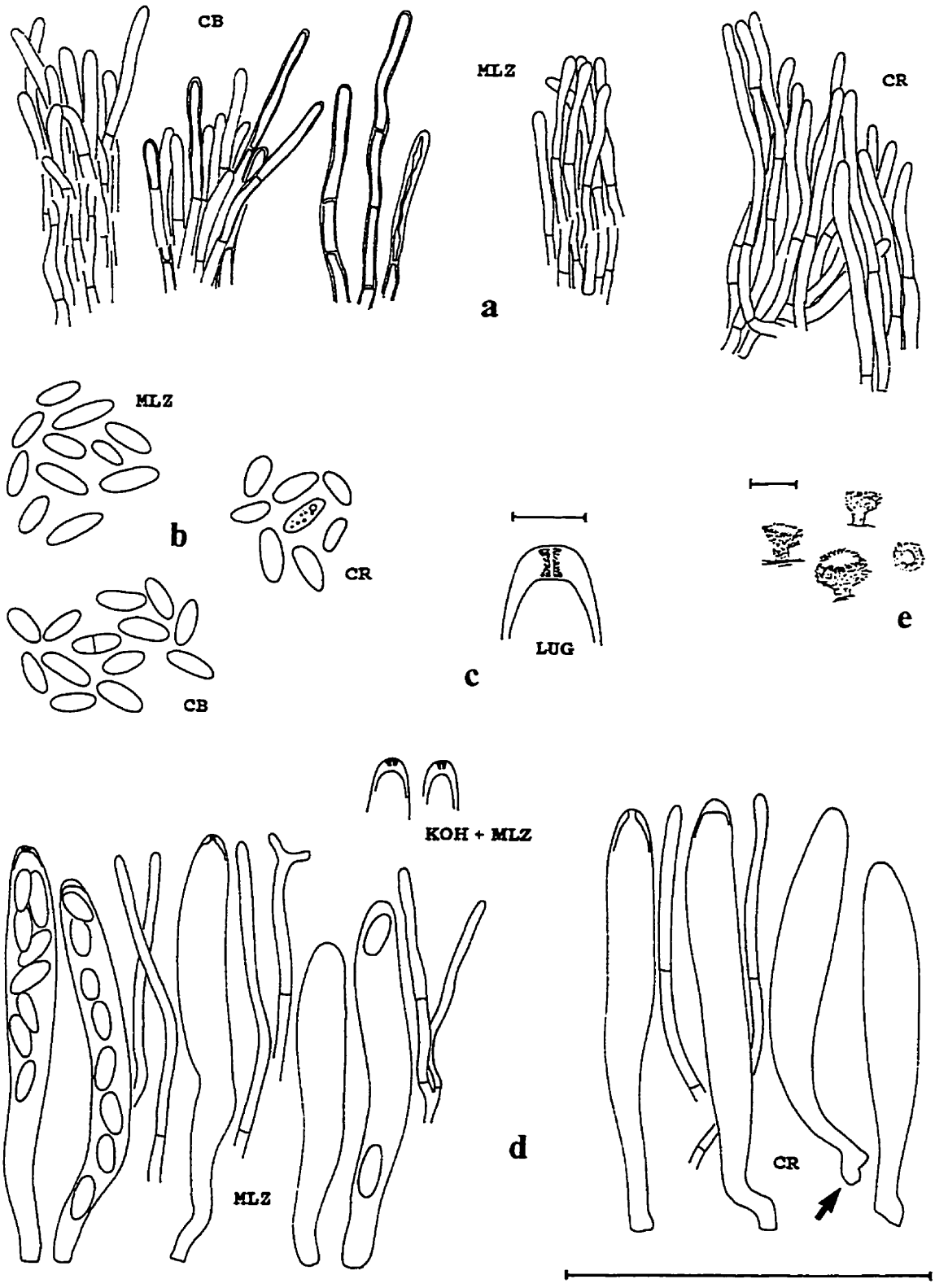
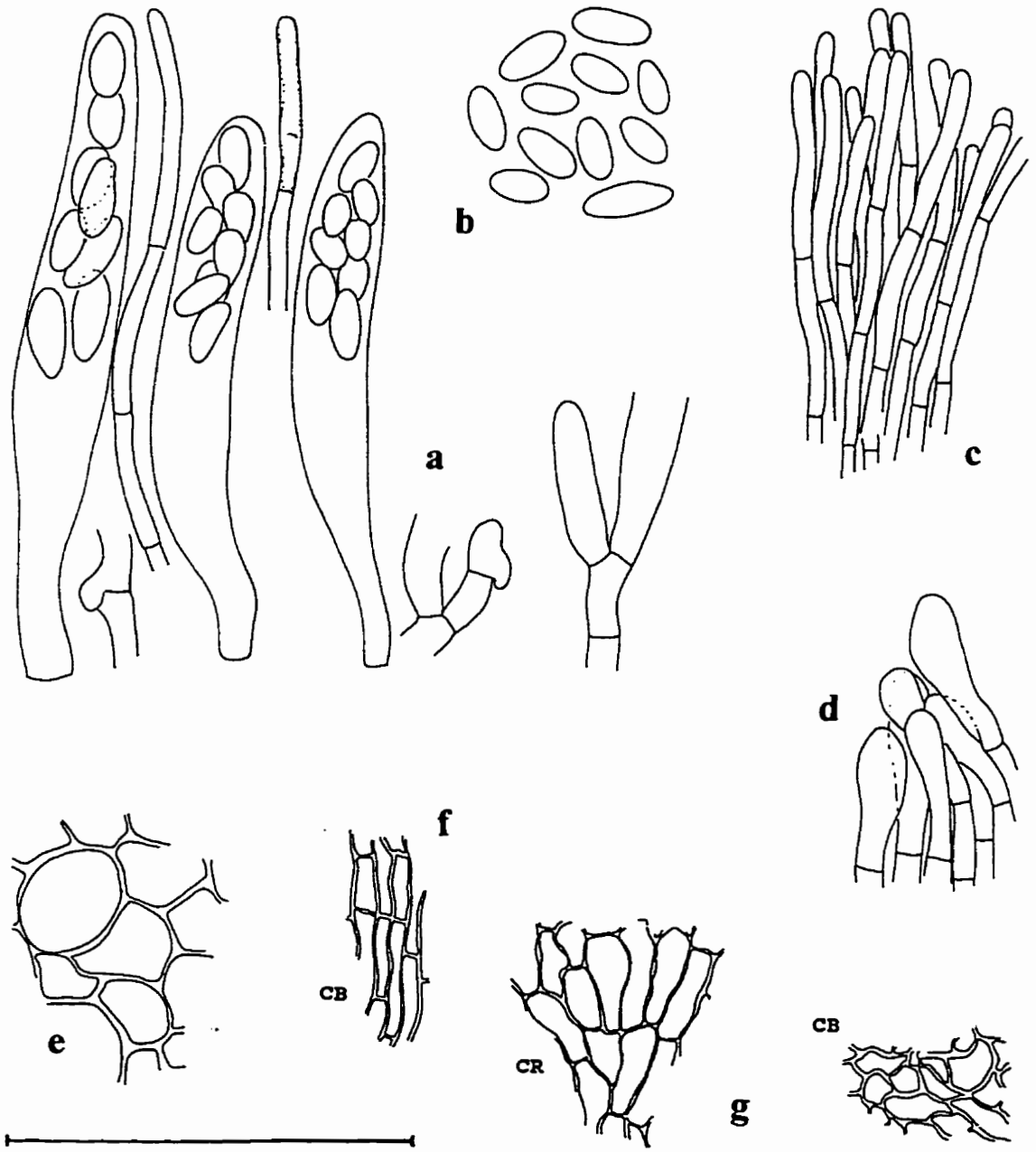

CB
MLZ
CR
a
MLZ
b
CR
CB
LUG
c
e
KOH + MLZ
MLZ
d
CR

Figure 3.9.

Hymenoscyphus ericae (UAMH 8680).
Illustrated in vital condition in water, unless otherwise indicated. CB = Cotton Blue in lactic acid; CR = ammoniacal Congo Red. Bar = 50 μm.

a) asci and paraphyses
b) spores
c) detail from cup margin
d) detail from lower flanks
e) lower flank excipulum, surface view
f) medial flank excipulum, surface view
g) lower flank excipulum, surface view.


a
b
c
d
e
f
g
CB
CR
CB

Chapter 4

The Genus *Oidiodendron*: Species Delimitation and Phylogenetic Relationships Based on Nuclear Ribosomal DNA Analysis [1]

Introduction

Species of *Oidiodendron* Robak (1932) (Hyphomycetes) are commonly recovered from humus or decaying wood and bark. The genus is readily identified by the distinctive arborescent conidiogenous apparatus. Erect, dematiaceous conidiophores terminate in a complex branching head of fertile hyphae that segment basipetally into arthroconidia. The monograph of Barron (1962) provides a thorough discussion of conidiogenesis with descriptions of nine species. The most comprehensive key to the genus includes 13 species (Domsch et al., 1980) and a further six have been listed in the Index of Fungi, International Mycological Institute, Vol. 1-6, 1940-1996. The genus is putatively placed within the Myxotrichaceae (Onygenales) since the only teleomorphic species known to have *Oidiodendron* states (*Myxotrichum arcticum*, *M. cancellatum*, *M. setosum*, and *Byssoascus striatosporus*) belong to this family (Hawksworth et al., 1995, p. 320).

Though some *Oidiodendron* species have additional distinctive features, the primary characters used for identification are conidium size, shape and ornamentation as well as conidiophore length and cultural morphology. All these vary even on one culture medium and the ranges can overlap among species (Barron, 1962). Such variability in morphology leads to confusion about species delimitation and uncertainty as to which characteristics should be emphasized for identification purposes.

Three culturally similar species with intergrading features are *Oidiodendron maius*, *O. griseum*, and *O. tenuissimum*, the first two of which have been documented as ericoid mycorrhizal fungi and isolated from field-collected roots. In our recent study of the root endophytes of the Ericaceae in Alberta, Canada, a large number of *Oidiodendron* isolates was recovered (Hambleton and Currah, 1997). Most were identified as *O. maius*, possessing the very long conidiophores and undulating branches of fertile hyphae that are

[1] A version of this chapter has been accepted for publication. Hambleton, S., Egger, K. N., and R. S. Currah. 1998. Mycologia 90(5).

distinctive for the species. Some strains were intermediate between *O. maius* and *O. griseum* based on conidiophore characteristics, though the conidia more closely matched those of *O. maius*.

Restriction fragment length polymorphism (RFLP) analysis of PCR-amplified ITS regions was used to identify strains of *O. maius* that were morphologically atypical (Hambleton and Currah, 1997). The ubiquitous presence of *O. maius* in the roots of Ericaceae of boreal habitats, and the difficulties with identifications based on morphology, led to speculation that historical records identifying *O. griseum* as an ericoid mycorrhizal fungus may have been confounded by ambiguous features.

Pigment production on agar media is another character that may not be as informative as once thought. The production of a dark brown diffusing pigment cited in the original description of *O. fuscum* (Robak, 1932, p. 251) (= *O. tenuissimum*; Hughes, 1958), which was used as a key character for an intraspecific cultural grouping in the key of Domsch et al. (1980), was also reported for *O. griseum* by Tokumasu (1973) and Hambleton and Currah (1997).

For one of the ericoid endophytic isolates of Hambleton and Currah (UAMH 8925), and for a strain examined by Tokumasu as well, the identification was more problematic because the upper range of the conidiophore lengths was greater than described in the original description of *O. griseum* (Melin and Nannfeldt, 1934, p. 440). Barron (1962) had already revised the conidiophore length range for *O. griseum* upwards with the result that it overlapped with the ranges of both *O. tenuissimum* and *O. maius*. Unfortunately, in this case, RFLP analysis was unable to differentiate *O. griseum* from *O. tenuissimum* (Hambleton, unpub. data). Further, RFLP analysis of the genus as a whole suggested that for some pairs of species, morphological variation might be significant only at the subspecies level.

The first objective of this study was to clarify species concepts within the genus *Oidiodendron*, with emphasis on the three target species (*O. griseum, O. tenuissimum* and *O. maius*), using sequence analysis of the internal transcribed spacer regions of the nuclear ribosomal RNA gene (ITS-1 and ITS-2) as a basis for species delimitation. The data were then used to assess the stability of some key morphological characters for identification purposes, notably conidiophore length and pigment production. Secondly, morphological assessment and RFLP analysis were used to examine whether five historically important

ericoid mycorrhizal *Oidiodendron* strains are conspecific. The third objective was to test the hypothesis that species of *Oidiodendron* represent taxa within the Myxotrichaceae.

Materials and Methods

Molecular

Fungal strains chosen for this study (listed in Table 4.1) included 15 species of *Oidiodendron* (14 described species and one new species revealed by the analysis), seven species of *Myxotrichum*, and a single strain for each of *Byssoascus striatosporus*, *Gymnostellatospora japonica* and *Pseudogymnoascus roseus*. All four genera in the Myxotrichaceae were represented. The only well-recognized species in the family not included were *M. ochraceum* Berkeley & Broome, *G. frigida* Uchiyama, Kamiya & Udagawa, and three species for which there are no cultures available, *M. aeruginosum* Montagne, *M. bicolor* (Ehrenberg) Fries, and *M. berkeleyi* Apinis. Ex-type and authentic strains were used where possible as species reference standards for both the molecular and morphological analyses.

Fungi were grown on thin plates of E-strain agar (Egger and Fortin, 1990) for 3 wk in the dark at room temperature. Eight plugs of mycelium, 0.5 cm diam with a minimum of agar attached, were used for DNA extraction following the chloroform/isopropanol protocol of Gardes and Bruns (1993) with minor modifications (Hambleton and Currah, 1997).

Primers ITS1 and ITS4 (White et al., 1990) were used to amplify a portion of the nuclear ribosomal RNA genes (rDNA) including both ITS spacer regions and the 5.8S subunit. Nucleotide sequences for the primers used are given in Appendix 1a with a diagram illustrating their relative priming locations. PCR reaction volumes of 100 µL contained PCR buffer, 25 mM $MgCl_2$, 50 µM each of dGTP, dATP, dTTP, and dCTP (Boehringer Mannheim Biochemica), 0.4 µM of each primer and 2 units of *Taq* DNA polymerase. One µL of diluted DNA was used as the template for each reaction except for a DNA-free, negative control. PCR amplifications were carried out on a DNA Thermal Cycler (Gene E, Techne Ltd., Princeton, New York) using the following cycle parameters: 94 C denaturation for 1 min, 55 C annealing for 1 min, 72 C extension for 2 min. The total number of cycles was 30 with an initial denaturation step of 2 min at 94 C and a final extension at 72 C for 7 min. Amplified PCR products were purified using Wizard PCR Preps columns (Promega, Madison, Wisconsin) following the manufacturer's instructions.

Automated DNA sequencing reactions were performed using standardized methods, then processed and analysed on a ABI 373A automatic DNA sequencer (Perkin-Elmer: Applied Biosystems, Foster, California) following the protocols suggested by the manufacturer. Sequences of complementary strands were determined for those strains serving as the reference standards of the target species as well as any strains for which the initial sequence was ambiguous. Consensus sequences were determined for these and used in the analyses. Preliminary alignments of DNA sequences were performed using the automatic DNA sequencer software package SeqEd ver. 1.0. Final alignments were optimized by hand.

Two data matrices were used for analysis. The first alignment (*Oidiodendron* analysis) was generated using the sequences of all 23 strains of *Oidiodendron* listed in Table 4.1, with *Pseudogymnoascus roseus* as the outgroup taxon. The second alignment (Myxotrichaceae analysis) was generated using the sequences of the 15 *Oidiodendron* spp. and the 10 teleomorphic species of the Myxotrichaceae listed in Table 4.1. For *O. griseum*, *O. tenuissimum*, and *O. maius* only the ex-type strains were included. The last five nucleotide positions at the 3' end of the 18S small subunit were used as the starting point of the aligned matrices. Sequences were coded to remove ambiguities in the alignment, in the manner described by Bruns et al. (1992), and gaps were counted as a fifth character. For both *P. roseus* and *G. japonica* the first 58 bases were coded as missing since the alignment was too ambiguous to perform with confidence. Though coding did not significantly affect the overall topology of the resulting trees, it was used as a check for the validity of the support indices. Sequences have been deposited in GenBank (Table 4.1) and the alignments used are deposited in TreeBASE (http://herbaria.harvard.edu/treebase/). The coded data matrices used in the *Oidiodendron* and Myxotrichaceae analyses are reproduced in Appendix 5 and 6 respectively.

Sequence dissimilarity measures were determined from the comparison of 500 uncoded aligned nucleotides at two taxonomic levels. At the intraspecific level, strains within the monophyletic clades containing the ex-type strains of *O. griseum*, *O. tenuissimum* and *O. maius* were compared. At the interspecific level, pairwise comparisons were made between meiotic and/or mitotic species.

Data matrices were analysed using the maximum parsimony program PAUP 3.1.1 (Swofford, 1993). Parsimony trees were constructed from a bootstrap analysis of each data matrix: 1000 replicate searches were carried out using the heuristic search algorithm, tree bisection-reconnection branch swapping, and random stepwise sequence addition. For the

Oidiodendron data matrix, a second analysis using the branch and bound search algorithm was performed. To permit timely execution of the data analysis, the dataset was slightly reduced by excluding two strains of *O. griseum* (UAMH 1693, 4080) and three of *O. maius* (UAMH 8529, 8921, 8922).

Restriction fragment length polymorphism analysis was carried out on five historically important *Oidiodendron* strains that have been isolated from ericoid mycorrhizas, deposited in culture collections and documented in the literature (Table 4.2). Methods followed those previously detailed in Hambleton and Currah (1997) using four restriction enzymes *Rsa*I, *Alu*I, *Hha*I, and *Hin*fI. Two separate extractions of different subcultures were tested for these five strains to provide confidence in the results. UAMH 8529 was also included in the *Oidiodendron* sequence analysis.

Morphological

All strains originally received as *Oidiodendron griseum* (UAMH 1403, 1693, 4080, 6514, 7022, 8507, 8529, 8925), *O. tenuissimum* (UAMH 8511, 8512, 8513, 8528), and *O. maius* (UAMH 1540, 8442, 8921, 8922) (Table 4.1 and 4.2) were examined for seven morphological characters: colonial morphology; the production of diffusing pigment on agar; the range in length of conidiophores; and size, shape, color and surface texture of the conidia. These data were compared to the original descriptions of these species (Table 4.3).

Pigment production and colonial morphology were characterized on potato dextrose agar (PDA: Difco-Bacto) and malt agar (MEA: 2% malt extract, Difco-Bacto). Cereal (CER) slide culture mounts (Sigler, 1993), 16 to 28 d old, were used to measure conidiophore length and characterize the conidia. Conidiophore length ranges were based on at least 25 random choices per slide. To standardize the results, only the portion of the conidiophore that was darkly pigmented was included in the measurement. Pigmentation was typically present for the entire length of the conidiophore and ceased at the septum below the lowermost conidiogenous branches (distance varied), which are hyaline. Size, shape, color and surface texture of conidia (under oil, at 1000X magnification) were documented for 10 mature conidia randomly chosen on the slide.

Other *Oidiodendron* species and teleomorphic taxa were grown in CER slide culture preparations in order to compare the observed anamorphic characteristics with herbarium documentation (University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta) and relevant discussions in the literature (Orr et al., 1963; Sigler and Carmichael, 1976; Currah, 1985, Udagawa et al., 1993, 1994).

Results

Oidiodendron analysis

The aligned DNA sequence data matrix of 15 *Oidiodendron* species (23 strains) and *Pseudogymnoascus roseus* comprised 540 bases of which 218 were variable and 79 were parsimony informative. The analysis was run with *P. roseus* and *Gymnostellatospora japonica* (both lack an *Oidiodendron* anamorph state) as the outgroup but each one placed the root at the same position (data not shown). Attempts were made to include a taxon from one of the other three families in the Onygenales (Gymnoascaceae, Arthrodermataceae, Onygenaceae) in the analysis. Although these families are hypothesized to represent the closest sister group to the Myxotrichaceae according to traditional classification schemes, the sequences of selected taxa were too divergent to be aligned successfully. Since the identity of the true sister group to the Myxotrichaceae is unclear, *P. roseus*, from within the family, was chosen as outgroup taxon.

The 50% majority rule consensus tree generated by the bootstrap analysis is presented in Fig. 4.1 (308 steps). One of three most parsimonious trees (279 steps; consistency index 0.65) generated by the branch and bound search algorithm is shown in Fig. 4.2. Branch lengths are proportional to the number of nucleotide changes along that branch. The overall topology of the two trees is the same and, while species relationships are clarified in Fig. 4.2, the lack of resolution in the bootstrap analysis indicates that some of these branches are not well-supported.

Although the inferred phylogenies did not resolve all the interspecific relationships in the genus, the ex-type strains of the target species of *O. griseum*, *O. maius* and *O. tenuissimum* were delimited as distinct in separate monophyletic clades (Fig. 4.1). Similarly, several pairings of species with high bootstrap support and low interspecific sequence divergence were revealed: *O. griseum*/*O. flavum*, *O. maius*/*O. citrinum* and *O. chlamydosporicum*/*O. scytaloides* (Fig. 4.2). Sequence divergence between species outside these pairings ranged from 6 to 10%, except for *O. echinulatum*/*O. cerealis* at 4%.

The "griseum" clade (bootstrap 98; Fig. 4.1) comprised UAMH 1403, an authentic strain; UAMH 4080; two strains that produced a dark amber diffusing pigment, UAMH 1693 and UAMH 8925; and UAMH 8528, which was received as *O. tenuissimum*. Percent sequence dissimilarity between these strains was at most 1%. *O. flavum* clusters with the *O. griseum* isolates and differs from the ex-type strain at 7 nucleotide positions (1.4%; Fig. 4.2).

The "maius" clade (bootstrap 100; Fig. 4.1) comprised UAMH 1540, the ex-type strain, as well as three strains isolated from ericoid mycorrhizas, UAMH 8921, UAMH 8922, and UAMH 8529 (which was received as *O. griseum*). Percent sequence dissimilarity between these strains was at most 0.8%. *O. citrinum* also clusters with *O. maius* and differs from the ex-type strain at 5 nucleotide positions (1%; Fig. 4.2), of which one is an insertion/deletion event which is probably due to replication slippage.

The "tenuissimum" clade (bootstrap 60; Fig. 4.1) comprised UAMH 8511, the ex-type strain; a second strain, UAMH 8512; and the ex-type strain of *O. setiferum*. Percent sequence dissimilarity of the latter two with UAMH 8511 was less than or equal to 2.2% (Fig. 4.2) with 10 and 7 nucleotide differences respectively.

Aside from these groupings, *O. chlamydosporicum* and *O. scytaloides* formed a closely related species pair (Figs. 4.1, 4.2; bootstrap 100) with only 4 nucleotide differences between them (0.8%), of which three are insertion/deletion events (two were coded out in the data matrix).

Myxotrichaceae analysis

The aligned DNA sequence data matrix generated using the 25 species listed in Table 4.1 comprised 547 bases of which 227 were variable and 129 were parsimony informative. The 50% majority rule consensus tree generated by the bootstrap analysis with *Pseudogymnoascus roseus* as outgroup taxon is presented in Fig. 4.3 (446 steps). *Oidiodendron*, *Byssoascus*, and *Myxotrichum* formed a monophyletic group within the Myxotrichaceae, while *Gymnostellatospora japonica* clustered with the outgroup taxon *P. roseus*, with high support (bootstrap 100). *M. deflexum* was basal to the rest of the species in the "Myxotrichum" ingroup. Sequence divergence between *P. roseus* and *G. japonica* was 8.2% but much higher at 19% when either species was compared to *M. deflexum*.

Several terminal clusters of species received high support. The species pairs *O. chlamydosporicum*/*O. scytaloides*, *O. tenuissimum*/*O. setiferum*, *O. griseum*/*O. flavum*, and *O. maius*/*O. citrinum*, observed in the *Oidiodendron* analysis, were repeated here. Three species of *Myxotrichum* clustered with anamorphic taxa: *M. arcticum* with *O. griseum*/*O. flavum* (bootstrap 96), *M. cancellatum* with *O. echinulatum* (bootstrap 97), and *M. setosum* with *O. truncatum* (bootstrap 89). For the first two, the percent sequence divergence from their associated *Oidiodendron* species is very low (Fig. 4.3). Three other *Myxotrichum* species formed a well supported divergent clade (bootstrap 100) and

sequence divergence amongst these species was much higher at 7.0 to 8.5%. Sequence divergence overall between species of *Myxotrichum* ranged from 6 to 12%.

Restriction Fragment Length Polymorphism Analysis

The RFLP patterns of all five strains in Table 4.2, and for both extractions, were identical and matched those published by Hambleton and Currah (1997) for *O. maius*.

Morphology

A summary of the information from the original species descriptions for the characters measured in the morphological examination of strains of *Oidiodendron griseum*, *O. tenuissimum* and *O. maius* is given in Table 4.3. Results of the morphological assessment of multiple strains of these species is given in Table 4.4; strains are grouped according to their re-identification based on the molecular analyses. Slight variations between the media used for the original diagnoses and the commercial preparations used in this study may have affected colony characteristics somewhat. The upper end of the range in conidiophore length was considered more informative since a small proportion of the conidiophores in all strains were extremely short (< 50 μm).

For the ex-type strains of all three species, the results for each character were within the range of variability initially described (compare Table 4.3, 4.4). The results for most other strains were also congruent within each of the three species after grouping based on the molecular assessment as summarized in Table 4.4. UAMH 8507, *O. maius*, did not produce conidia on agar or in slide culture. UAMH 8513, *Oidiodendron* sp., was different from all other strains for conidium color, conidium ornamentation and cultural morphology.

A few strains were atypical for conidiphore length: UAMH 8528 and 8925, long for *O. griseum* at 100/150 to 225/250 μm; UAMH 8511, short for *O. tenuissimum* at less than 100 μm; UAMH 8529 and 8921 short for *O. maius* at 100 to 200/250 μm. Conidia of *O. griseum* and *O. tenuissimum* were distinctly pale brown when grouped in masses, but less distinct individually. Conidia of *O. maius* were more frequently hyaline but sometimes pale brown in masses. Conidium surface texture was smooth for *O. maius* while finely roughened for the other two species, though this distinction was not apparent except at high magnification.

Conidium size exhibited a high degree of overlap but was useful for *O. maius* when paired with conidium shape. As well as the subglobose and ovoid conidia seen in the other two species, in *O. maius* the variable shapes included cylindrical conidia, and odd-shaped conidia formed by septation and disarticulation of the fertile hyphae across branching points.

Colony size for all strains on MEA and PDA was restricted to approximately half of the petri plate, regardless of age. The production of an amber diffusing pigment into the rest of the agar was observed on both media for two strains of *O. griseum* (UAMH 1693 and 8925) but was absent for the others. For both strains of *O. tenuissimum*, pigment production was strong on MEA but slight on PDA. Strains of *O. maius* produced an orange-brown diffusing pigment on MEA that was diagnostic but only after prolonged incubation (four weeks or more); no diffusing pigment was produced on PDA.

Anamorph states for species in the Myxotrichaceae have been reported and were observed as follows: *Myxotrichum carminoparum*, *M. chartarum*, *M. deflexum*, *M. stipitatum*: absent to infrequent arthroconidia to chains of arthroconidia assignable to the genus *Malbranchea*; *M. arcticum*, *M. cancellatum*, *M. setosum*, *Byssoascus striatosporus*: *Oidiodendron*; *Gymnostellatospora japonica*: lacking in the type specimen, *Ovadendron*-like in this strain; *P. roseus*: *Geomyces*.

Discussion

Intraspecific analysis of Oidiodendron

The combined molecular and morphological examination of multiple strains of three species in this study highlights some species delimitation problems. *Oidiodendron griseum*, *O. tenuissimum* and *O. maius*, as circumscribed, share similar cultural morphology and conidium color, and exhibit overlapping characteristics for conidium size, shape and surface texture (Table 4.3). In spite of these morphological similarities, the inferred phylogeny generated from the analysis of ITS sequences indicates that each species is distinct phylogenetically; the ex-type strains serving as species standards are resolved in three different monophyletic clades (Fig. 4.1). Bootstrap support for the cluster of strains within the clades containing these reference strains is robust for *O. griseum* and *O. maius* (98 and 100 respectively) and in addition, the measures of sequence dissimilarity for the two species are very low (1% and 0.8% respectively).

Conidiophore length has been considered a useful key character, especially for *O. maius* which is typified by very tall conidiophores (up to 500 μm) and long, undulate fertile hyphae at the apex. *O. griseum* is described as having very short conidiophores, most less than 100 μm, with shorter, straight fertile hyphae, while *O. tenuissimum* is intermediate in conidiophore length. In this study, conidiophore measurements were made under standardized growth conditions, and those of the ex-type strains matched published measurements. While the morphological and molecular assessments of most of the isolates sampled were congruent, the "maius" clade and the "griseum" clade, as delimited by sequence analysis, both contain strains exhibiting atypical conidiophore ranges.

The "maius" clade contains an isolate (UAMH 8529) derived from the first study to document *O. griseum* as an ericoid mycorrhizal fungus (Couture et al., 1983). The identification of this strain as *O. griseum* is not surprising since conidiophore lengths for UAMH 8529 (one of three strains from that study, all reported to look similar) are less than 200 μm, rather than up to 500 μm as is typical for *O. maius*. Another ericoid isolate, UAMH 8921, also produces conidiophores which are relatively short, yet in the molecular analysis these two strains cluster with *O. maius* with less than 1% sequence divergence.

There are three other studies documenting the isolation of *O. griseum* from roots of the Ericaceae, while only one study recovered *O. maius* (Table 4.2). RFLP analysis confirms the morphological assessment that these strains are all conspecific with *O. maius*. Conidium shape and ornamentation, and cultural morphology are consistent with the ex-type strain of *O. maius*. UAMH 8507 has degenerated and no longer forms conidia but culturally it exhibits similarities to *O. maius* (orange-brown diffusing pigment on MEA). Hambleton and Currah (1997) showed that the RFLP patterns of *O. maius* and *O. griseum* are distinct from each other for the four restriction enzymes used. Within the genus *Oidiodendron*, these RFLP profiles of *O. maius* are diagnostic for the species though the same cannot be said for *O. griseum* (Hambleton, unpub. data).

All of the major records of ericoid mycorrhizal *Oidiodendron* isolates are now confirmed as strains of *O. maius* (Burgeff, 1961; Couture et al., 1983; Douglas et al., 1989; Stoyke and Currah, 1991; Xiao and Berch, 1992; Hambleton and Currah, 1997) though for two of those studies not all of the deposited strains were examined (Couture et al., 1983; Xiao and Berch, 1992). The importance of this clarification is two-fold. The strains that have been used in experiments aimed at elucidating the role of the fungus as a mycobiont (Dalpé, 1986; Abuzinadah and Read, 1989; Leake and Read, 1990; Haselwandter et al., 1990, Xiao and Berch, 1995) have been those isolated by Couture et

al. (1983) (though specific strain identity was not always cited) or by Xiao and Berch (1992), giving the species *O. griseum* an undeserved reputation as a mycobiont. Results may also have been misinterpreted when bonafide strains of *O. griseum* were not observed to form ericoid mycorrhizas in pot cultures (for instance, UAMH 1403 in Douglas et al., 1989).

Secondly, the results indicate that *O. maius* is the only *Oidiodendron* species to have been confirmed from field-collected mycorrhizas. Hambleton and Currah (1997) reported very few strains of *O. griseum* (for example, UAMH 8925) in their study but speculated that this extremely low recovery indicated that these were rhizosphere fungi that occasionally survived the root sterilization process. Preliminary results of experiments in progress to test whether this strain forms mycorrhizas in axenic resynthesis with the host species indicate that it does not (Hambleton, unpub. data).

The "griseum" clade contains strains isolated from a wider range of substrates, of which two produce a striking amber diffusing pigment on PDA and MEA. For one of these, UAMH 8925, and for the isolate received as *O. tenuissimum*, UAMH 8528, conidiophore lengths ranged up to 225-250 μm. The molecular analysis, then, shows that conidiophore length is not a reliable character for these species: *O. maius* may produce very short conidiophores in the range of *O. griseum* and *O. tenuissimum* and vice-versa. Since conidiophore length range for *O. tenuissimum* is reported as intermediate, all three species overlap for this character.

In the original description of *O. tenuissimum* (= *O. fuscum*; Hughes, 1958, p. 790) one important feature noted as absent from *O. griseum* was the production of a dark, diffusing pigment on agar. Unfortunately, surface ornamentation of the conidia was not mentioned, and indeed this is a difficult character to resolve for such small conidia using the light microscope. Barron (1962), after identifying numerous strains isolated from soil as *O. tenuissimum*, noted that the species was highly variable for pigment production but could be distinguished from *O. griseum* by dark and echinulate conidia. Although UAMH 8513, received as *O. tenuissimum*, demonstrates these conidium characteristics and is also positive for pigment production, it diverges significantly from the "tenuissimum" clade in the phylogenetic analyses (Figs. 4.1, 4.2).

In the ex-type strain UAMH 8511, and UAMH 8512, the conidia were pale brown and only slightly roughened and in these respects similar to *O. griseum*. Based on the divergent sequences and on other distinctive morphological features, UAMH 8513 is

considered to represent an undescribed species. The production of a dark diffusing pigment on agar media is not a species specific attribute since it has been observed for several species in addition to *O. tenuissimum* and *O. griseum*, but because production varies depending on agar type, it still may be useful for sorting species groups.

Overall, the results indicate that there are few morphological characters that can be used reliably to identify *Oidiodendron griseum*, *O. tenuissimum* and *O. maius* and yet the ITS analysis provides unambiguous support for their validity as distinct phylogenetic species. Scanning electron microscopy of the conidia may provide new useful characters. In our preliminary examination of this approach, differences in surface texture have been consistent with the molecular analysis for strains of *O. griseum* and *O. maius*. Careful observations of colonial features, in particular color, also appear predictive but these may be difficult to standardize for general use.

Interspecific relationships within Oidiodendron

Molecular analysis reveals that within the genus *Oidiodendron* there is a high degree of sequence substitution in the ITS region of the rDNA. Sequence divergence varies from 6 to 10% among species at the internal nodes of the phylogenetic trees shown in Figs. 4.1 and 2 but a lack of resolution of these interspecific relationships is evident from the number of branches which received bootstrap support of less than 50. The relatively low consistency index of the tree in Fig. 4.2 indicates that data from a more conserved region, such as the 28S subunit, are needed to resolve the phylogeny. In contrast, several species pairs at the terminal nodes are supported by high bootstrap values and also exhibit low sequence dissimilarity measures, similar to those observed at the intraspecific level.

For *O. griseum*/*O. flavum*, *O. maius*/*O. citrinum*, and *O. chlamydosporicum*/*O. scytaloides*, sequence divergence between the paired taxa is very low, less than 1.5%, and bootstrap support is high (98 or 100) for their clustering. *O. flavum* and *O. citrinum* are distinguished from their paired species primarily by a yellow conidium color (Barron, 1962), whereas the difference between *O. chlamydosporicum* and *O. scytaloides* is based on the size of chlamydospores and conidia (Gams and Söderström, 1983). The molecular data suggest that these morphological criteria are too variable to be used as key characters or are only significant at the subspecies level.

In the "tenuissimum" clade, *O. setiferum* is more closely related phylogenetically to the ex-type strain of *O. tenuissimum* than is a second strain, and although the sequence divergence is still under 2.5% for that species, bootstrap support for the clade is low. In

many respects the description of *O. setiferum* is similar to that of *O. tenuissimum* but the species is unique in its production of dematiaceous seti at the apex of the conidiophore (Udagawa and Toyazaki, 1987). More sequence data are needed for both species in order to evaluate the cohesiveness of their inferred relationship.

In this study molecular characters have been used to clarify taxonomic decisions that were made based on morphology and to impose a phylogenetic perspective on species concepts in the genus *Oidiodendron*. Such an approach involves making decisions about the level of sequence variation that characterizes each taxonomic level. Seifert et al. (1995) have surveyed sequence divergence measures reported for ascomycetous fungi in the ITS regions of rDNA. The wide variation and often overlapping measures for intra- versus interspecific values is thought to be due to inconsistency in the rate of evolution of these regions (Seifert et al., 1995). Therefore taxonomic rankings based on molecular analysis should be attempted with caution and linked to an assessment of other characters common to the taxa within monophyletic groups.

A comparison of the divergence measures of other ascomycetous taxa based on both ITS1 and ITS2 with the 5.8S gene, such as 6.3 % for *Metarhizium anisopliae* (Curran et al., 1994), 10.5% for *Beauveria brongniartii* (Neuvéglise et al., 1994) and 0% for three species of *Penicillium* (LoBuglio et al., 1994), indicates that the intraspecific values for *Oidiodendron griseum* and *O. maius* (1%) are relatively conservative. By contrast, the much higher interspecific sequence divergence measures for *Oidiodendron* (6 to 10%) and *Myxotrichum* (6 to 12%) suggest that sequence divergence is useful for distinguishing phylogenetic groups at the two taxonomic levels in this group of fungi.

Without the resorting of strains based on the molecular analysis, the intra- and interspecific measures for the genus *Oidiodendron* would have overlapped. In our view, this resorting is validated by the correlation of several morphological characters and mycorrhizal lifestyle with the phylogenetic species groupings of *O. griseum* and *O. maius* (Table 4.4). Similarly, Kuhls et al. (1997) reported intraspecific divergence between 15 strains of *Hypocrea schweinitzii* as 6.1%. When strains were sorted into groups based on sequence type, the divergence within each group was at most 0.3%, which supported morphological and isozyme data suggesting that *H. schweinitzii* comprises several phylogenetic species.

Based on the low sequence divergence and an evaluation of the morphological criteria used to delimit these species, *O. chlamydosporicum* and *O. scytaloides* are assessed

as conspecific. Chlamydospore production is a unique attribute within *Oidiodendron* and the recorded variation in chlamydospore and conidium dimensions are similar to the levels of size variation seen in the genus as a whole. Similarly, *O. maius* and *O. citrinum* are assessed to be conspecific. The yellow conidium color may be a phenotypic response to characteristics of the original substrate, since for some strains it has been observed to fade in storage but this morphological variant could be given variety status. While similar justification could be used for merging *O. flavum* with *O. griseum*, judgment is reserved pending analysis of more strains.

The taxonomic placement of *Oidiodendron cerealis* has been the subject of some debate, such that strains are currently found deposited also as *Stephanosporium cerealis* (Thüm.) Swart. Either placement can be found in the hyphomycete literature (compare Domsch et al., 1980, p. 518 with Ellis, 1971, p. 35). Barron (1962) placed the species in *Oidiodendron* because conidiogenesis follows the same ontogeny as other species in the genus. Later, Swart (1965) emphasized the unusual lens-shaped conidia with a dark equatorial band in erecting a new combination, *S. cerealis*, to deal with several conspecific strains that had been given names in three genera. The analyses presented here suggest that a separate monotypic genus is not appropriate for this taxon as it clusters with high support with the other *Oidiodendron* species in both the *Oidiodendron* and the Myxotrichaceae analyses.

Anamorph-teleomorph relationships

Within the Myxotrichaceae, species of *Oidiodendron*, *Myxotrichum* and *Byssoascus* form a well-supported monophyletic group that has diverged significantly from the two other genera in the family. We judged *Pseudogymnoascus roseus* and *Gymnostellatospora japonica* to be equally effective as outgroup taxa. They were less divergent from each other than from *Myxotrichum deflexum*, the most basal species of the ingroup, indicating a distant relationship and exclusion from the ingroup. In addition, neither produces an *Oidiodendron* anamorph state.

Relationships of the teleomorphic taxa within the monophyletic clade appear correlated with the anamorph state produced. *Myxotrichum deflexum*, which produces infrequent alternate arthroconidia, is basal, while three *Myxotrichum* species with either no anamorph state or a *Malbranchea*-like anamorph state (*M. carminoparum*, *M. chartarum* and *M. stipitatum*) cluster together in a well-supported clade excluding all species of *Oidiodendron*. *Byssoascus striatosporus*, *M. arcticum*, *M. cancellatum*, and *M. setosum*,

all with distinct or reported *Oidiodendron* anamorph states, are nested with the *Oidiodendron* species.

Both *M. arcticum* and *M. cancellatum* exhibit less than 1% sequence divergence from an anamorphic species, *O. griseum* and *O. echinulatum* respectively (Fig. 4.3). Based on earlier arguments, this result could indicate conspecific identity for these taxa. *M. arcticum* produces an *Oidiodendron* state that is morphologically typical of *O. griseum* though some conidiophores form an unusual geniculate head with single conidia produced over the surface (Udagawa et al., 1994). The conidial state of *M. cancellatum* was observed to be very similar to the strain of *O. echinulatum* used in this study but neither taxon produced the distinctly roughened conidia reported for the latter species. In both cases, more strains need to be examined before any judgments concerning conspecificity are made.

The position of *Byssoascus striatosporus* within the "Myxotrichum" clade is interesting because it suggests that the generic concepts of *Myxotrichum* and *Byssoascus* need to be re-examined. *Byssoascus striatosporus* forms ascomata atypical for the family (Currah, 1985) in which the fruiting body is a cobweb-like envelope (telaperidium) of thin-walled hyphae scarcely differentiated from the vegetative hyphae. Species of *Myxotrichum* form a distinctive reticuloperidium made up of a mesh of thick-walled, dematiaceous peridial hyphae, often ornamented with pigmented appendages.

Byssoascus striatosporus (a monotypic genus) was erected by von Arx (1971) but Sigler and Carmichael (1976) recommended that the taxon be transferred to *Myxotrichum*. In addition to the marked similarities in ascospore morphology and cellulolytic ability of the two genera, this recommendation was based on observed ascospore production amongst blunt, dematiaceous, thick-walled hyphae on cereal agar, similar to the peridial hyphae of *Myxotrichum* ascomata. Due to the lack of a distinct peridium, though, the transfer was not adopted in subsequent monographs in which fruiting body characteristics were emphasized at the genus level (Benny and Kimbrough, 1980; Currah, 1985).

Historically, *M. deflexum* was renamed as *Eidamella deflexa* (also a monotypic genus) by Benjamin (1956) with the statement that it did not fit the then-current concept of the genus *Myxotrichum*, but more recent treatments do not concur (Orr et al., 1963; Benny and Kimbrough, 1980; Currah, 1985). Though Benjamin's criteria were not clearly defined, they were apparently based on whether appendages are differentiated from the peridial hyphae. However, appendage characteristics have since been used for interspecific

distinctions. Data from more strains and from more conserved regions of DNA need to be evaluated to assess the phylogenetic stability of ascomatal characters for inferring relationships in this group.

Sigler and Carmichael (1976) noted similarities between the *Oidiodendron* state of *Byssoascus striatosporus* and that of *M. setosum* in which the conidia are described as barrel-shaped. Though conidiophore production was poor for the strain of *M. setosum* examined, interestingly it is paired in the phylogenetic analysis with *O. truncatum* (4% sequence divergence), with relatively high support. In *O. truncatum* conidia are barrel-shaped rather than the more commonly seen ovoid or cylindrical shapes.

The molecular analysis confirms the initial hypothesis that the hyphomycete genus *Oidiodendron* is, as a group, phylogenetically a member of the Myxotrichaceae, and is closely related to the genera *Byssoascus* and *Myxotrichum*, especially those species with *Oidiodendron* states. It is unknown whether the anamorphic taxa have lost their sexual stage, but it does suggest that conidiophore production elevating the arthroconidial apparatus above the surface of the mycelium may have conferred an advantage for dispersal and permitted a proliferation of anamorphic forms.

The inferred phylogeny also indicates a marked divergence within the Myxotrichaceae between the genus *Myxotrichum* (and *Byssoascus*), and the other two genera in the family. Sequence analysis of a more conserved portion of the rDNA gene is underway to determine whether *Myxotrichum* shares closer evolutionary ties with taxa outside the current circumscription of the Myxotrichaceae.

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Table 4.1. Name, strain number, origin, analysis performed and GenBank accession number of fungi used for sequence analysis.

Species	Strain[a]	Origin[b]	Data Matrix[c]	GenBank No.
Oidiodendron Robak				
O. cerealis (Thum.) Barron	UAMH 1522	peat soil; ON Canada	Oid, Myx	AF062788
O. chlamydosporicum Morrall	UAMH 6520	T, soil ex boreal forest; SK Canada	Oid, Myx	AF062789
O. citrinum Barron	UAMH 1525	T, soil ex cedar bog; ON Canada	Oid, Myx	AF062790
O. echinulatum Barron	IMI 110132 (UAMH 8467)	A, soil ex cedar bog; ON Canada	Oid, Myx	AF062791
O. flavum Szilvinyi	UAMH 1524	soil ex cedar bog; ON Canada	Oid, Myx	AF062792
O. griseum Robak	CBS 249.33 (UAMH 1403)	A, wood pulp; Sweden (Melin and Nannfeldt, 1934)	Oid, Myx	AF062793
	UAMH 1693	douglas fir timber; BC Canada	Oid[d]	AF062794
	UAMH 4080	wood chips and bark ex logging truck; AB Canada	Oid[d]	AF062795
(received as *O. tenuissimum*)	DAOM 51071 (UAMH 8528)	*Pinus contorta*; AB Canada	Oid	AF062796
	UAMH 8925	ex roots *Vaccinium myrtilloides*; AB Canada (Hambleton and Currah, 1997)	Oid	AF062797
O. maius Barron	UAMH 1540	T, soil ex cedar bog; ON Canada (Barron, 1962)	Oid, Myx	AF062798
(received as *O. griseum*)	DAOM 184107 (UAMH 8529)	ex roots *Vaccinium corymbosum*; PQ Canada (Couture et al., 1983)	Oid[d]	AF062799
	UAMH 8921	ex roots *Vaccinium myrtilloides*; AB Canada (Hambleton and Currah, 1997)	Oid[d]	AF062800
	UAMH 8922	ex roots *Vaccinium vitis-idaea*; AB Canada (Hambleton and Currah, 1997)	Oid[d]	AF062801
O. periconioides Morrall	DAOM 197506 (UAMH 8527)	T, soil; SK, Canada	Oid, Myx	AF062802
O. pilicola Kobayasi	UAMH 7526	forest soil; Sweden	Oid, Myx	AF062787
O. rhodogenum Robak	UAMH 1405	A, pulp in sludge strainers; Norway	Oid, Myx	AF062803
O. scytaloides Gams & Söderström	UAMH 6521	T, forest soil; Sweden	Oid, Myx	AF062804
O. setiferum Udagawa & Toyazaki	UAMH 5715	T, house dust; Japan	Oid, Myx	AF062805

Table 4.1. Continued

Species	Strain[a]	Origin[b]	Data Matrix[c]	GenBank No.
O. tenuissimum (Peck) Hughes	CBS 238.31 (UAMH 8511)	T of *O. fuscum*, wood pulp; Norway (Robak, 1932) (= *O. tenuissimum*, Barron, 1962)	Oid, Myx	AF062807
	CBS 920.73 (UAMH 8512)	forest soil; Sweden	Oid	AF062808
O. truncatum Barron	UAMH 1399	T, soil ex mixed woods; ON Canada	Oid, Myx	AF062809
O. sp. nov. (received as *O. tenuissimum*)	CBS 315.95 (UAMH 8513)	leaf litter; Canary Islands Spain	Oid, Myx	AF062806
Myxotrichum Kunze				
M. arcticum Udagawa, Uchiyama & Kamiya[e]	UAMH 7565	T, forest soil; Alaska USA	Myx	AF062810
M. cancellatum Phillips[e]	UAMH 1911	frozen blueberry pastry; NJ USA	Myx	AF062811
M. carminoparum Robak	UAMH 1597	T, wood pulp; Norway	Myx	AF062812
M. chartarum (Nees) Kunze	UAMH 1997	soil; Japan	Myx	AF062813
M. deflexum Berkeley	UAMH 6365	soil; ON Canada	Myx	AF062814
M. setosum (Eidam) Orr & Plunkett[e]	UAMH 3835	soil; AB Canada	Myx	AF062815
M. stipitatum (Lindfors) Orr & Kuehn	UAMH 1510	N, sand dune; England	Myx	AF062816
Byssoascus striatosporus (Barron & Booth) von Arx[e]	UAMH 3572	T, soil; ON Canada	Myx	AF062817
Gymnostellatospora japonica Udagawa Uchiyama, & Kamiya	UAMH 8899	white spruce log, decay stage 5, fire site; AB Canada	Myx	AF062818
Pseudogymnoascus roseus Raillo	UAMH 9163	ex roots *Abies lasiocarpa*; AB Canada	Oid, Myx	AF062819

[a] Fungi were obtained from culture collections and original isolation work of S.H. Culture collection origin is noted for strains not previously at UAMH. CBS = Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. DAOM = Canadian Collection of Fungal Cultures, Ottawa, Canada. IMI = International Mycological Institute, Egham, United Kingdom. UAMH = University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada.
[b] Ex-type (T), authentic (A) or neotype (N) strains were used as standards for the species wherever possible.
[c] Strains were included in one or both data matrices: Oid = *Oidiodendron*, Myx = Myxotrichaceae.
[d] Strains of *Oidiodendron* excluded from the branch-and-bound analysis of the *Oidiodendron* data matrix.
[e] Teleomorphic species with an *Oidiodendron* state.

Table 4.2. Strain number and origin of previously documented ericoid mycorrhizal fungi that have been identified as *Oidiodendron maius* using RFLP analysis.

Strain[a]	Origin	Received as
UAMH 6514	ex roots *Loiseleuria procumbens*; AB, Canada (Stoyke and Currah, 1991)	*O. griseum*
UAMH 7022	ex roots *Gaultheria shallon*; BC, Canada (Xiao and Berch, 1992; one of five *O. griseum* strains deposited from the study)	*O. griseum*
ATCC 66504 (UAMH 8442)	ex roots *Rhododendron* sp cv. Pink Pearl; Ireland (Douglas et al., 1989)	*O. maius*
CBS 334.52 (UAMH 8507)	ex roots Ericaceae; Sweden (Burgeff, 1961)	*O. griseum*
DAOM 184107[b] (UAMH 8529)	ex roots *Vaccinium corymbosum*; PQ Canada (Couture et al., 1983; one of three *O. griseum* strains deposited from the study)	*O. griseum*

[a] ATCC= American Type Culture Collection, Rockville, Maryland, USA. CBS = Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. DAOM = Canadian Collection of Fungal Cultures, Ottawa, Canada. UAMH = University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada. Culture collection origin is noted for strains not previously at UAMH.
[b] This strain was also included in the sequence analysis.

Table 4.3. Comparison of morphological characteristics of *Oidiodendron griseum*, *O. tenuissimum* and *O. maius* taken from the original descriptions of the type specimens.

	O.griseum Melin & Nannfeldt 1934	*O. tenuissimum* (=*O. fuscum*) Robak 1932	*O. maius* Barron 1962
conidiophore length	40-150 µm (90-100)	to 300 µm (110-150)	to 500 µm (250-350)
conidium size	1.6-2 X 2-3.6 µm	1.2-2.2 X 1.6-3.6 µm	2-2.5 X 2.5-4 µm
conidium shape	globose, sub-globose, ovoid to short-cylindric	globose, sub-globose, ovoid	globose, sub-globose, short-cylindric
conidium colour	hyaline to dilute greenish-grey	hyaline to brownish-green	hyaline
conidium surface texture	smooth to roughened	not indicated	smooth to roughened
MEA: colonial morphology	olive-grey to greenish grey	brown to grey brown	brown grey
PDA: colonial morphology	not indicated	not indicated	pale-grey to dirty-white

Table 4.4. Summary of morphological data for strains of *Oidiodendron griseum*, *O. tenuissimum* and *O. maius* used in sequence or RFLP analysis, grouped according to their re-identification based on the molecular analyses.

	conidiophore length (µm)[a]	conidium size (µm)[a]	conidium shape[b]	conidium color[c]	conidium surface texture[d]	diffusing pigment[e] MEA/PDA[f]	colonial morphology MEA/PDA[f]
O. griseum							
UAMH 1403[g]	+	+	G, sG, shC	pbr	fine rough	- / -	MEA: submerged growth olive-
UAMH 1693	+	+	G, sG	pbr	fine rough	A / A	black at center, hyaline at margin;
UAMH 4080	+	+	G, sG, shC	pbr	fine rough	- / -	conidiogenous layer grey-brown
UAMH 8528	100-225	+	shC	pbr	fine rough	- / -	PDA: varied, similar to *O. maius* but
UAMH 8925	150-250	+	shC	pbr	fine rough	A / A	with smoother surface texture
O. maius							
UAMH 1540[g]	+	+	shC, C, B	hyl-pbr	smooth	O / -	MEA: orange-brown cast to agar
UAMH 6514	+	+	shC, C, B	hyl-pbr	smooth	O / -	and colonies after 3+ wk;
UAMH 7022	+	+	shC, C, B	hyl-pbr	smooth	O / -	conidiogenous layer brownish
UAMH 8442	+	+	shC, C, B	hyl	smooth	O / -	(except 8529, lighter grey-
UAMH 8507	-	-	-	-	-	O / -	brown, smoother)
UAMH 8529	100-200	+	shC, C, B	hyl-pbr	smooth	O / -	PDA: submerged mycelium dark;
UAMH 8921	100-250	+	shC, C, B	hyl	smooth	O / -	brownish-grey, granular
UAMH 8922	+	+	shC, C, B	hyl-pbr	smooth	O / -	surface texture
O. tenuissimum							
UAMH 8511[g]	few, < 100	+	G, sG	pbr	fine rough	A / sl	MEA: dark grey-brown
UAMH 8512	+	+	G, sG	pbr	fine rough	A / -	PDA: mostly submerged, grey-purplish-black
Oidiodendron sp.							
UAMH 8513	80-200	2.0 X 2-3.2	G	dkbr	echinulate	A / sl	MEA: dark brown, granular PDA: dark brown, raised center

[a] + = within ranges given in original species description, - = missing data
[b] G = globose, sG = subglobose, shC = short-cylindric, C = cylindric, B = odd shapes due to branching of fertile hyphae
[c] pbr = pale brown, hyl = hyaline, dkbr = dark brown
[d] examined under oil at 1000X magnification
[e] A = dark amber pigment, O = orange-brown pigment after 4+ wk, sl = slight amount of amber pigment, - = pigment absent
[f] MEA = malt agar, PDA = potato dextrose agar
[g] ex-type or authentic strain for the species

Figure 4.1. Majority rule consensus tree (308 steps) resulting from 1000 bootstrap replications of maximum parsimony analysis of the *Oidiodendron* data set using the heuristic search algorithm of PAUP 3.1.1. Bootstrap values above 50% are given adjacent to the corresponding node. UAMH numbers are given to identify multiple strains for a species where needed and ex-type (T) and authentic (A) strains are indicated.

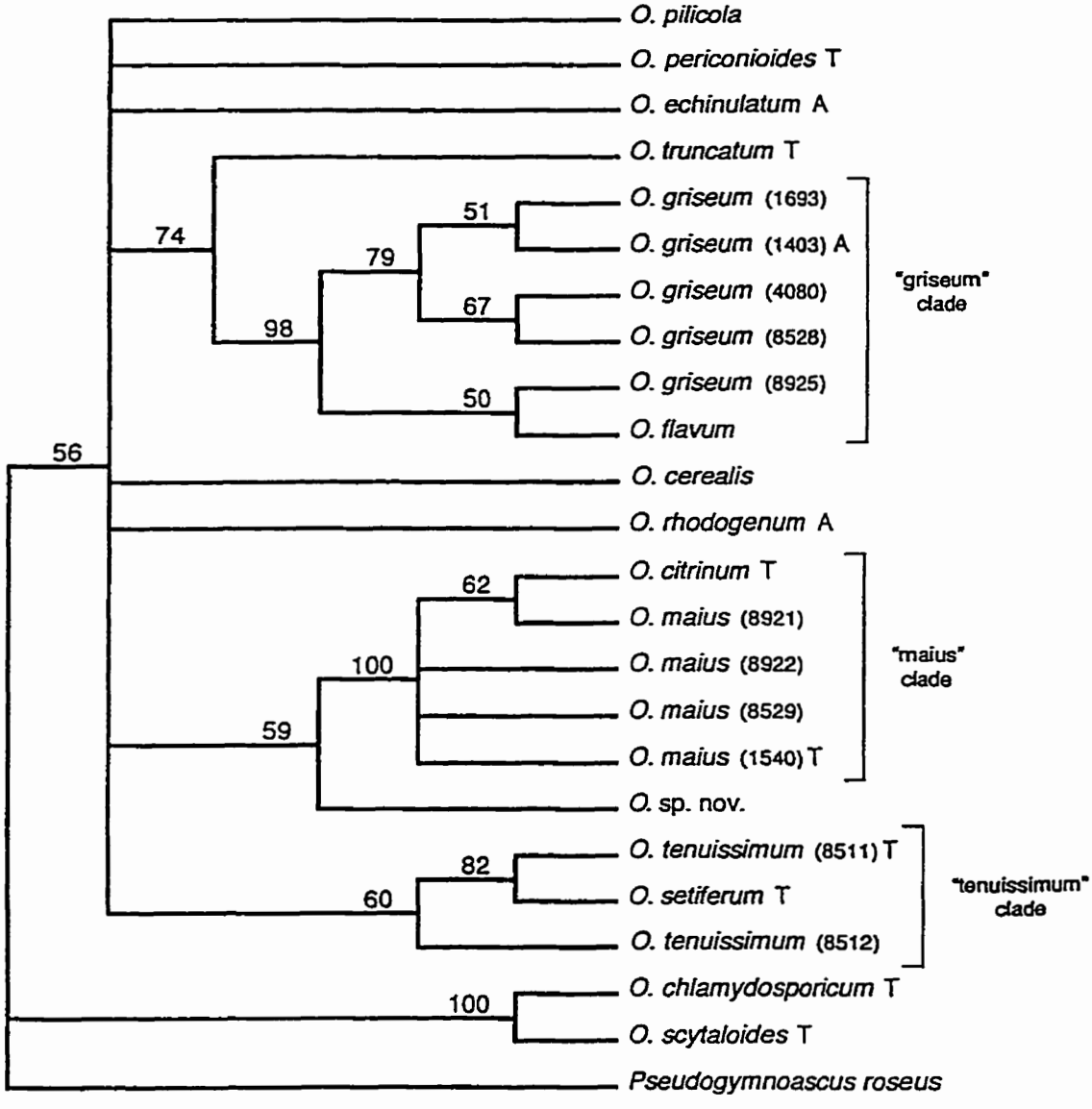


Figure 4.2. One of three most parsimonious trees (279 steps, CI = 0.65) resulting from a maximum parsimony analysis of the *Oidiodendron* data set (excluding two strains of *O. griseum* and three of *O. maius*) using the branch and bound algorithm of PAUP 3.1.1. Numbers above the branches indicate the number of nucleotide changes along each branch. UAMH numbers are given to identify multiple strains for a species where needed and ex-type (T) and authentic (A) strains are indicated. Sequence divergence measures for four monophyletic clades are indicated.

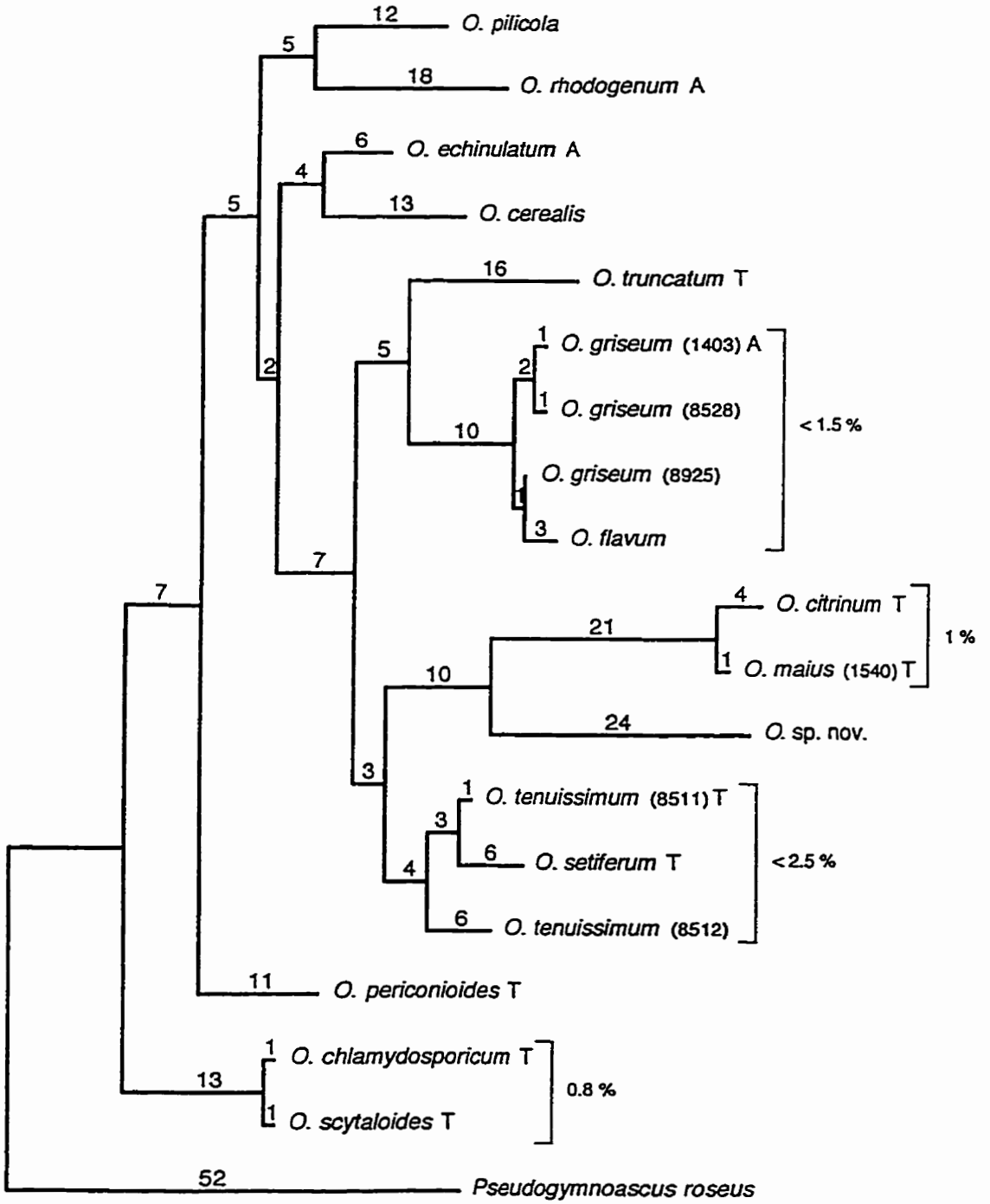
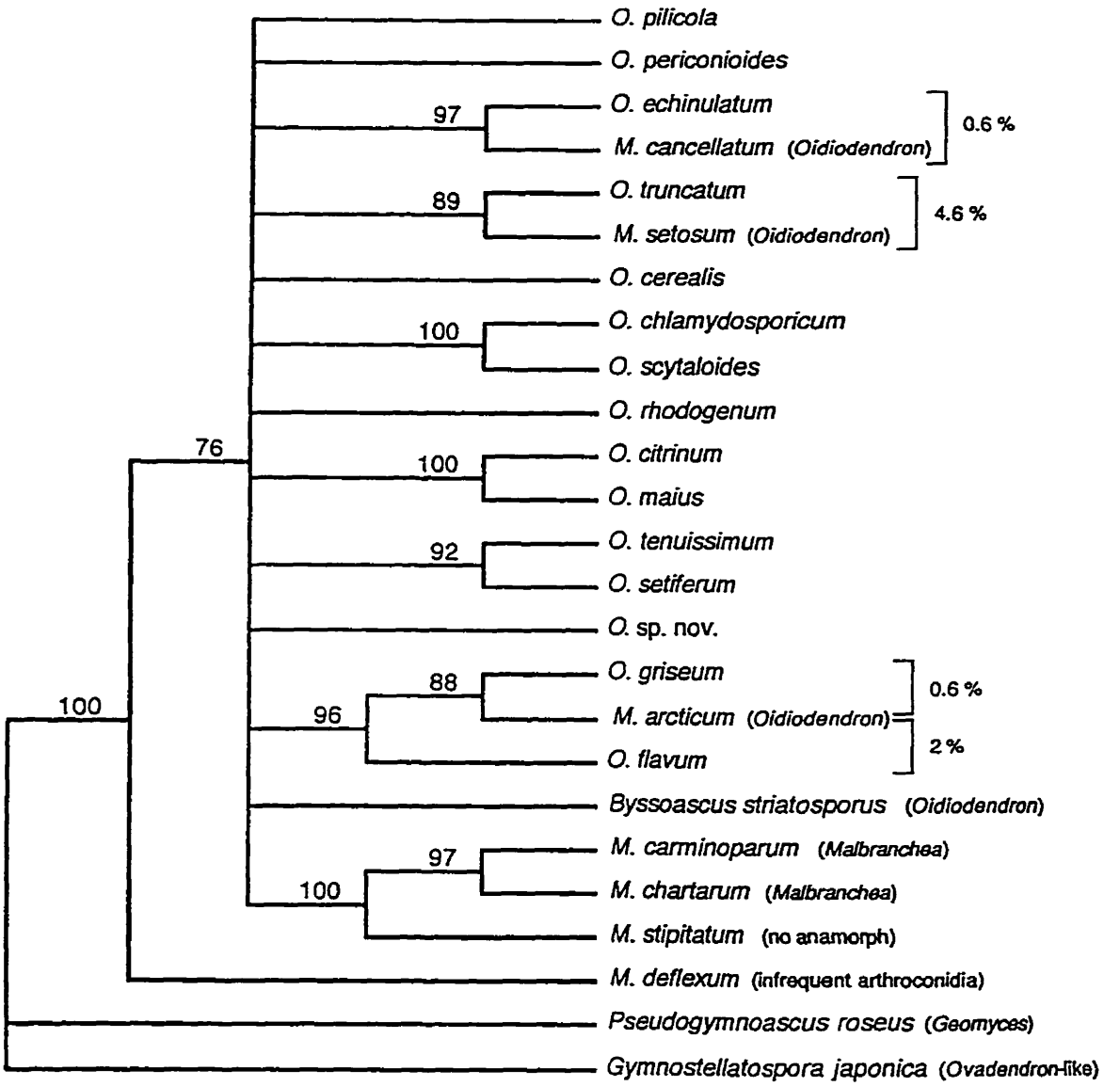


Figure 4.3. Majority rule consensus tree (446 steps) resulting from 1000 bootstrap replications of maximum parsimony analysis of the Myxotrichaceae data set using the heuristic search algorithm of PAUP 3.1.1. Bootstrap values above 50% are given adjacent to the corresponding node. Anamorph states for the teleomorphic taxa are mapped on the tree and sequence divergence measures for four meiotic/mitotic species pairs are given.



Chapter 5

Phylogenetic Affinity of Ericoid Mycorrhizal Fungi to the Leotiales

Introduction

The preceeding three chapters have provided isolation data for four fungal root endophytes of the Ericaeae isolated from plants collected in natural habitats of Alberta; *Scytalidium vaccinii*, *Oidiodendron maius*, *Phialocephala fortinii* and a fungus as-yet unidentified, Variable White Taxon (VWT). Issues concerning their characterization, identification and taxonomic ranking have been addressed using various culturing techniques and molecular markers, and for *S. vaccinii* and *O. maius*, affinities to teleomorphic taxa have been clarified.

As discussed in Chapter 2, the role these fungi play in marginal habitats is not well-understood, except for *S. vaccinii* (which will be referred to as *Hymenoscyphus ericae* based on the confirmed anamorph-teleomorph connection; Chapter 3) for which there is ample experimental evidence of mycorrhizal status. *Oidiodendron maius* is also considered to be a mycorrhizal fungus based on its frequent isolation from ericaceous roots and its ability to form typical ericoid mycorrhizas when grown in axenic culture with appropriate plants.

Though molecular analysis (Hambleton and Currah, 1997; Chapter 2) indicates that VWT is distinct from *H. ericae*, the level of significance of the data taxonomically is difficult to evaluate without further study. In preliminary resynthesis trials pairing VWT with *Vaccinium myrtilloides*, morphologically similar hyphal complexes were observed in root cells, though in fewer cells per root sample compared to observations of mycorrhizas from trials with either *H. ericae* or *O. maius* (unpub. data). This is an indication that VWT may function as an ericoid mycorrhizal fungus as well. On the other hand, restriction endonuclease digest patterns for strains of culturally similar, sterile fungi isolated from serially washed mycorrhizas of *Picea mariana* by Summerbell (1987) match those of VWT (unpub. data), suggesting that these sterile fungi are conspecific and that their ecological role is more cosmopolitan. More isolation and experimental data as well as an understanding of its phylogenetic affinity to a teleomorphic group are needed to evaluate further the role of VWT.

Investigating the ecological role of *Phialocephala fortinii* has been the challenge of several studies since the fungus was first described (Wang and Wilcox, 1985; Wilcox and

Wang, 1987; Stoyke and Currah, 1993; Fernando and Currah, 1996; O'Dell et al., 1993). The subject continues to confound researchers but is not a focus of this thesis. Of interest here is the phylogenetic placement of *P. fortinii*, given the stated hypothesis, the level of interest in the taxon ecologically, and a previous suggestion that it belongs with the inoperculate discomycetes based on the production of sterile discocarps by a single strain grown in pot culture with *Rhododendron brachycarpum* (Currah et al., 1993).

The objective of the research presented in this chapter was to investigate the hypothesis that the fungal taxa that are apparently involved in a highly specialized and specific mycorrhizal association, with a monophyletic group of plant species in the Ericales, share a recent common ancestor. Parsimony-based cladistic analysis of small subunit nuclear ribosomal DNA sequences was used to test this hypothesis. Of primary interest is the relationship of *H. ericae*, a member of the inoperculate discomycete order, the Leotiales, to *O. maius*, inferred to be phylogenetically a member of the cleistothecial order, the Onygenales (Hambleton et al., 1998). Secondarily, the phylogenetic affinities of the other two endophytic fungi of boreal and alpine Ericaceae, VWT and *P. fortinii*, can best be inferred using molecular analysis, and representatives of these taxa were also included in the analysis.

Materials and Methods

New sequence data were obtained for a representative strain of each of the root endophytes *Hymenoscyphus ericae*, UAMH 8873 (S-42b); *Oidiodendron maius*, UAMH 8920 (S-10Aa); VWT, UAMH 8861 (S-70Ac); and *Phialocephala fortinii*, S-7Aa (see Appendix 1 for collection and isolation data). Representatives of three of the four families in the Onygenales sensu Currah (1985) were also sequenced: *Myxotrichum arcticum* (UAMH 7565), Myxotrichaceae; *Gymnoascus reessii* (UAMH 4809), Gymnoascaceae; and *Uncinocarpus uncinatus* (UAMH 3913), Onygenaceae.

DNA was extracted from living mycelium grown in culture, using the methods outlined in Hambleton and Currah (1997; Chapter 2). Four primer pairs were used to amplify overlapping portions of the small subunit (18S) nuclear ribosomal gene from the 5' to 3' direction: NS1-NS2, NS11mun-NS12mun, NS13mun-NS6 and NS151mun-NS8. Nucleotide sequences for the primers used are given in Appendix 1b with a diagram illustrating their relative priming locations. Primers NS1, NS2, NS6 and NS8 were published in White et al. (1990). Primers NS11mun, NS12mun, NS13mun and

NS151mun were developed by K.N. Egger at the University of Northern British Columbia, Prince George, BC Canada.

Methods for DNA amplification, purification and automated sequencing follow those detailed in Hambleton et al. (1998; Chapter 4). Nucleotide sequences were determined for only one DNA complement, except in those cases where the presence of relatively large insertions required data for the reverse complement to obtain the complete sequence. The insertions are hypothesized to be Group I introns based on reports of the frequency with which these introns occur in rDNA (Gargas et al., 1995). Several other strains of *H. ericae*, *O. maius*, and *Myxotrichum* spp. were amplified in an attempt to minimize the problem. Strains sampled, and the approximate sizes of the fragments amplified by each primer pair are given in Table 5.1. Intron sequences for the strains of *H. ericae*, *O. maius* and *P. fortinii* chosen for sequencing were located during alignment and deleted from the data matrix. Consensus sequences for each fungal strain were determined from overlapping alignments of the sequences generated from the four separate amplifications.

Sequences newly generated in this study were aligned with sequences obtained from GenBank for fungi chosen as representative of the major ascomycete lineages and as availability permitted. The fungi used in the analysis are listed in Table 5.2 according to their current classification (Hawksworth et al., 1995). Sequence alignment was performed using the automatic DNA sequencer software package SeqEd. ver. 1.0 (Applied Biosystems, Foster, California). Final alignments were optimized by hand.

The data matrix comprised 30 taxa, and was analysed using the maximum parsimony program PAUP 3.1.1 (Swofford, 1993). Thirty replicate searches using the heuristic algorithm with random stepwise sequence addition and tree bisection-reconnection (TBR) branch swapping specified were performed to determine the most parsimonious phylogenetic reconstruction. To determine the level of support for the topology of the resulting inferred phylogeny, a bootstrap analysis was performed on the same data matrix using the heuristic search algorithm with 500 replicate searches of random stepwise sequence addition and TBR branch swapping. The data matrix used in the analysis is reproduced in Appendix 7.

Results

The aligned DNA sequence data matrix comprised 1683 bases. This represents over 90% of the length of the 18S subunit as compared to the size of most eukaryotic small

subunit rDNA sequences reported to be approximately 1800 bases long (Gargas and Taylor, 1995). There were a total of 614 variable sites and 328 parsimony informative sites. The analysis was run with *Saccharomyces cerevisiae* (Saccharomycetales, Ascomycotina) as outgroup based on the results of previous molecular phylogenetic studies showing *S. cerevisiae* to be basal to the filamentous ascomycete lineages or euascomycetes (Bruns et al., 1992; Landvik et al., 1993; Spatafora, 1994). The sizes of the introns spliced out of the sequences were 330 nucleotides from NS151-8 for *H. ericae*, 360 from NS13-6 for *O. maius*, 299 from NS13-6 and 217 from NS151-8 for *P. fortinii*.

Thirty replicates of a heuristic search resulted in a single most parsimonious tree (MPT) (Fig. 5.1) of 917 steps with a consistency index of 0.63. Branch lengths correspond to the number of changes along each branch and bootstrap support values over 50% are indicated. Four main clades, of which two can be further subdivided, corresponded to traditional ascomycete taxonomic groups. Three were supported by bootstrap values of 100%: the Onygenales and Eurotiales clade (plectomycetes or cleistothecial fungi), the Sordariales clade (pyrenomycetes or unitunicate perithecial fungi), and the Dothideales clade (loculoascomycetes or bitunicate ascostromatic fungi). Within the Onygenales/Eurotiales clade, four monophyletic groups corresponded to the Eurotiales (bootstrap 100) and three of the four families in the Onygenales, the Onygenaceae (bootstrap 96), the Arthrodermataceae (bootstrap 87), and the Gymnoascaceae (bootstrap 98), though this clade also included *U. uncinatus* of the Onygenaceae.

The remaining large clade comprised all the apothecia-forming taxa analyzed (discomycetes), and was resolved into two monophyletic sister groups that corresponded to the Pezizales (operculate discomycetes), weakly supported by bootstrap (60), and the Leotiales (inoperculate discomycetes). *M. arcticum* and *O. maius* (members of the fourth family in the Onygenales, the Myxotrichaceae), *P. fortinii*, VWT as well as *H. ericae* cluster with the inoperculate discomycete genera *Leotia* (type genus of the order Leotiales), *Spathularia*, *Cudonia* and *Sclerotinia*, with the first three leotialian genera resolved as basal to the other taxa.

Discussion

The inferred phylogeny generated from the analysis of small subunit rDNA sequences suggests that a monophyletic Leotiales includes not only the ericoid mycorrhizal fungus *H. ericae* and other inoperculate discomycetes but also the mycobiont *O. maius* and the cleistothecial *M. arcticum* (Onygenales), plus the unclassified root endophytes *P.*

fortinii and VWT. The results confirm the original hypothesis that the ericoid mycorrhizal and endophytic fungi tested are closely related phylogenetically, and further indicate that these taxa have evolved from within the inoperculate discomycete lineage. In addition, the Onygenales is revealed as polyphyletic, as is the genus *Uncinocarpus*.

The low level of bootstrap support for the inoperculate discomycete clade, seen here represented by Leotialean taxa from four different families, concurs with other molecular analyses based on small subunit rDNA sequences (Spatafora, 1994; Gargas and Taylor, 1995; Landvik et al., 1996). Unfortunately there is as yet a limited number of inoperculate discomycete sequences available in GenBank for this gene and until a more comprehensive analysis of the group is done, support for subordinal relationships will be lacking. The Leotiales is a large and poorly characterized order comprising 13 families and 400 genera (Hawksworth et al., 1995), united taxonomically by small apothecia, simple paraphyses, thin-walled asci that open by means of a simple pore (inoperculate), and ascospores that are usually small, hyaline and smooth. Current systematic efforts to define characters that would be diagnostic for natural groupings of taxa are focused on characters associated with the structure of the ascus apical apparatus, aspects of ascus development, and histochemical reactions. Recent comprehensive treatments of these fungi such as those by Huhtinen (1989), and Verkley (1995) will help to inform the decisions to be made about taxon sampling for future molecular phylogenetic studies.

The well-supported exclusion of the Myxotrichaceae from the Onygenales and its inferred placement within the Leotiales indicates that the cleistothecial fruiting body morphology has arisen more than once and that it represents a derived state. In this case, the results further suggest that ecological role and substrate preferences may be more predictive of phylogenetic relationships than ascocarp morphology. The Myxotrichaceae was placed in the Onygenales because of the mesh-like structure of the peridium (ascomatal hyphal wall) and arthroconidial anamorphs (Currah, 1985). In contrast to the other Onygenalean families, Myxotrichaceous species are cellulolytic, have stipitate asci and small, hyaline, fusiform ascospores, characteristics found among fungi in the Leotiales. Ascospore shape may be an important unifying characteristic. For Leotialian taxa, spores are "usually not quite longitudinally symmetrical" (p. 239, Hawksworth et al., 1995), a description that can be applied to those of *Myxotrichum* spp. which are boat-shaped rather than strictly fusiform.

While the Onygenaceae and the Arthrodermataceae are distinguished by ascospore morphology and anamorph type, taxa in both families degrade keratin, a unique enzymatic

capability among fungi, considered to be indicative of a monophyletic lineage (Currah, 1985). The Gymnoascaceae is less well-delimited but not keratinolytic (Currah, 1985). *Uncinocarpus reesii* (Onygenaceae), which is isolated from keratinous materials, is resolved with high support in the Onygenaceae clade. On the other hand, a second species, *U. uncinatus*, clusters with the Gymnoascaceae as does another strain of this species, UAMH 8530 (data not shown), again with high support. *Uncinocarpus uncinatus* was previously disposed in the genus Gymnoascus (Gymnoascaceae), but was moved to *Uncinocarpus* by Currah (1985) because of ascocarp morphology and ascospore characteristics. Recent experiments using hair degradation assays have shown that neither strain of *U. uncinatus* is able to break down keratin (T. Lumley, pers. comm.) which provides support for the molecular analysis in suggesting that the taxon is misplaced in the Onygenaceae. By implication, the results further indicate convergence in some of the morphological characters that define *Uncinocarpus*, such as punctate ascospores and hooked appendages, and provide support for the notion that substrate affinity or ecological role has predictive value for developing phylogenetic hypotheses. Relationships among the four monophyletic groups within the plectomycete clade are largely unsupported by bootstrap values (Fig. 5.1). An analysis including more taxa is needed to evaluate whether the Arthrodermataceae and the Onygenaceae are sister groups within a single monophyletic lineage.

The overall topology of the most parsimonious tree (MPT) presented here indicates general support for traditional ascomycete classification at the order level and this finding has been discussed elsewhere (Berbee and Taylor, 1992; Spatafora, 1994; Gargas and Taylor, 1995) but the universality of this congruence depends to a great extent on taxon sampling (Gargas and Taylor, 1995). Sampling for previous studies focusing on the Onygenales has largely centered on the human pathogenic taxa and their hypothesized closest relatives classified in the Onygenaceae and the Arthrodermataceae (LeClerc et al., 1994; Bowman et al., 1996). The molecular analysis presented here provides unequivocal support for the reclassification of the Myxotrichaceae as suggested by Currah (1994) but it is premature to advocate ordinal status. Analysis of species relationships using the more rapidly evolving ITS regions of rDNA revealed substantial genetic divergence within the family (Hambleton et al., 1998; Chapter 4), suggesting that the Myxotrichaceae, at least including the genera *Pseudogymnoascus* and *Gymnostellatospora*, may not be a monophyletic group.

The idea that fruiting body morphology is subject to convergence is not new (Cain, 1972; Malloch, 1981). As early as 1932, Nannfeldt proposed that relatively

undifferentiated apothecia gave rise to derived cleistothecia and perithecia (from Gargas and Taylor, 1995), and this view has so far been supported by molecular analyses (Spatafora, 1994; Gargas and Taylor, 1995; Landvik et al., 1996; Wedin and Tibell, 1997). There is molecular evidence that two other cleistothecial fungi, *Blumeria graminis* f.sp. *hordei* (Erysiphales; Saentz et al., 1994) and *Amylocarpus encephaloides* (unclassified ascomycete; Landvik et al., 1996) are phylogenetically related to the Leotiales. Interestingly, both species produce stalked asci which bear a morphological ressemblance to the cylindrical asci of discomycetous fungi, as do species of *Myxotrichum* (Currah, pers. comm.; Lumley and Currah, 1995).

By definition, the term cleistothecium refers to a closed fruiting body with no predefined opening (Hawksworth et al., 1995, p. 96). When used to describe the fruiting bodies of the Onygenales, it includes a variety of forms in which the peridial wall is not closed but variously modified and often reduced to a network of loosely woven or mesh-like hyphae surrounding the asci. For many cleistothecial fungi (including the Onygenales), the asci have a scattered distribution within the ascoma and the characteristics of passive spore release from globose evanescent asci is also implied, though not all fungi with evanescent asci form cleistothecia. Evanescent asci have previously been shown to have arisen in six independent lineages, outside of the plectomycetes, within perithecial fungi (Blackwell and Spatafora, 1994) and two more within lichenized fungal groups (Wedin and Tibell, 1997). A case has been made that associations with arthropods and other animals are the driving force behind this convergence (Blackwell and Spatafora, 1994). For Myxotrichaceous taxa (and generally within the Onygenales), morphological adaptations in the form of elaborate hooked and barbed ascomatal appendages promote animal-mediated dispersal and compensate for the loss of forcible spore discharge (Currah, 1985).

Preliminary evidence is presented here that the two hyphomycetous root endophytes, Variable White Taxon and *Phialocephala fortinii* find their phylogenetic placement among the discomycetes. As discussed in Hambleton and Currah (1997; Chapter 2), VWT apparently represents a taxon distinct from *H. ericae* based on RFLP analysis of the ITS regions of rDNA, though the level of taxonomic significance is unknown. In this analysis of 18S sequences, VWT does not form a monophyletic group with *H. ericae*, in spite of the relatively short branches separating them; *H. ericae* is basal to VWT which is basal to a clade uniting *P. fortinii* and *Sclerotinia sclerotiorum*. This lack of monophyly is in contrast to two other well-supported clades within the inoperculate discomycetes, one uniting *Myxotrichum arcticum* and *Oidiodendron maius* of the Myxotrichaceae, and the other uniting *Cudonia confusa* and *Spathularia flavida*, both members of the

Geoglossaceae. The results support the findings of the ITS analysis of VWT, and suggest that this fungus may belong to a different genus, and not to *Hymenoscyphus*.

While strains of *Phialocephala fortinii* may be identified solely by vegetative characteristics, the strain used here was positively identified after conidiogenesis was induced in cold storage. The inferred phylogenetic placement of *P. fortinii* in the MPT as sister taxon to *Sclerotinia sclerotiorum* (Sclerotiniaceae) is interesting though it lacks bootstrap support. *P. fortinii* exhibits some of the characteristics of *S. sclerotiorum* (Sclerotiniaceae), giving intuitive support for this hypothesized relationship. Taxa in the Sclerotiniaceae are stromatic, forming sclerotia or mummified host tissue, are pathogenic or saprobic on various plant parts including roots, with anamorphs that are often spermatial (Hawksworth et al, 1995). *P. fortinii* forms dematiaceous sclerotial plaques on the surface of roots and sclerotia within plant root cells as well as in culture (Currah and Tsuneda, 1993), and is reported to be pathogenic under some experimental conditions. Though it is unknown whether the conidia of *P. fortinii* also function as spermatia, the small size of the phialoconidia makes them potential candidates (Currah, pers. comm.). Though *P. fortinii* is known primarily as a sterile dematiaceous root-associate, the partial success of Currah et al. (1993) in obtaining the teleomorph indicates that viable fruiting bodies may yet be obtained; parsimony analysis supports the suggestion that the teleomorph is an inoperculate discomycete.

In summary, the molecular analysis confirms the original speculation that fungal taxa which are apparently involved in a highly specialized mycorrhizal relationship with a monophyletic group of plant species, would themselves share a recent common ancestor. Both ericoid mycorrhizal fungi, *Hymenoscyphus ericae* and *Oidiodendron maius*, are nested within the inoperculate discomycete clade. Further molecular studies are needed to clarify this relationship, and to examine the monophyly of the Myxotrichaceae.

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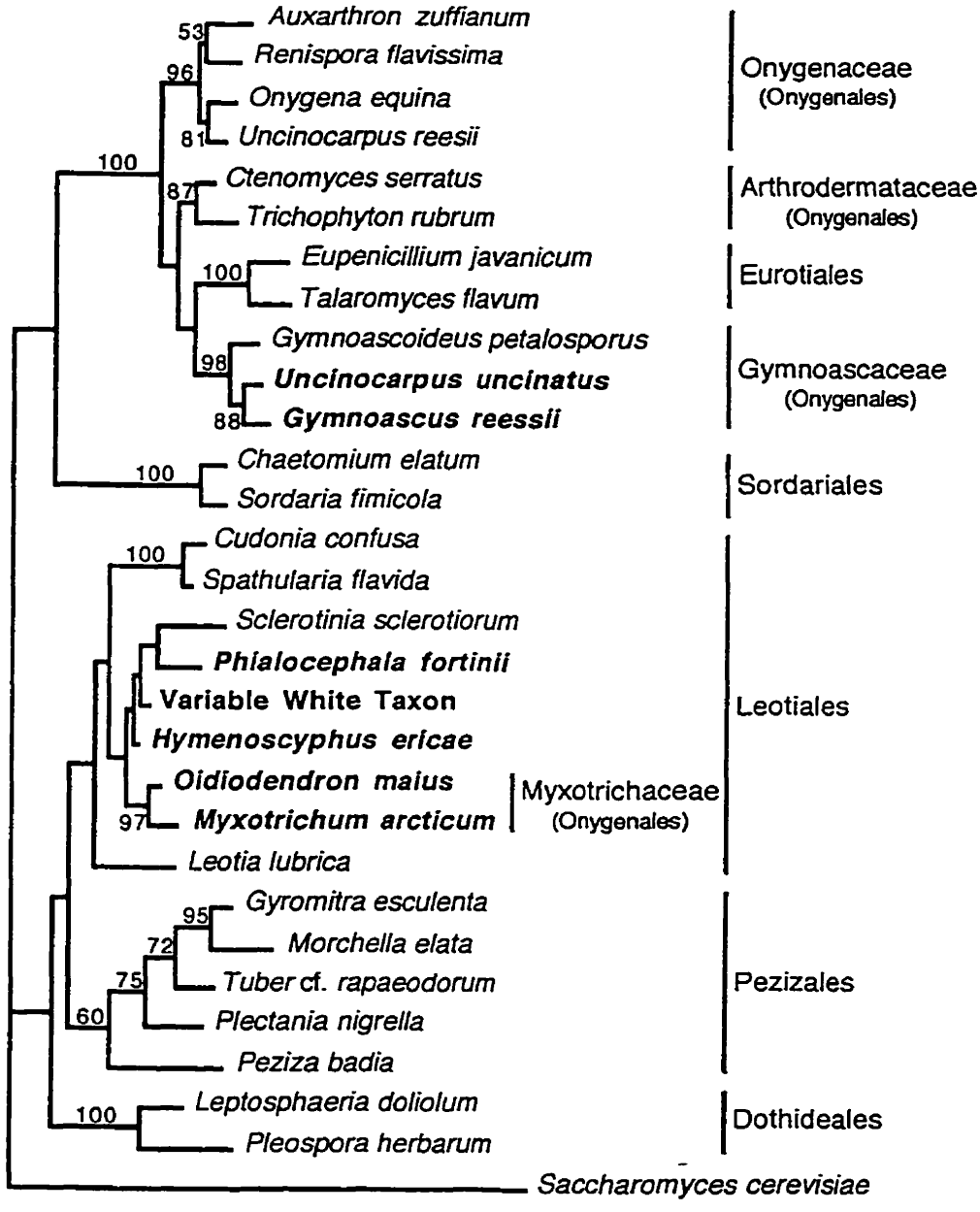
Table 5.1. Name and strain number of the fungal DNA amplified with the approximate size of the fragment of small subunit rDNA resulting from each primer pair given in basepairs (bp). Larger than normal fragment sizes are estimated for the applicable strains, indicating the presence of insertions in that portion of rDNA. Sizes were estimated from a visual comparison of amplified fragments with a size standard on ethidium bromide-stained agarose gels. Shaded lines indicate the strains chosen for sequencing.

	rDNA Small Subunit Primer Pairs			
Amplified Strains	NS1-2 ~ 500bp	NS11-12 ~ 500bp	NS13-6 ~ 600bp	NS151-8 ~ 600bp
G. reessii UAMH 4809	√	√	√	√
H. ericae UAMH 8873	√	√	√	~ 900
H. ericae UAMH 6735	√	~ 800	~ 1300	√
H. ericae UAMH 8680	√	√	-	~ 900
O. maius UAMH 8920	√	√	~ 1000	√
O. maius UAMH 8922	√	~ 800	~1500	-
O. maius UAMH 8923	√	~ 800	~1500	-
M. arcticum UAMH 7565	√	√	√	√
M. cancellatum UAMH 1911	√	√	√	√
M. chartarum UAMH 6505	√	~ 800	~ 1200	~ 1200
M. deflexum UAMH 6365	√	√	√	√
P. fortinii S-7Aa	√	√	~ 900	~ 900
U. uncinatus UAMH 3913	√	√	√	√
VWT UAMH 8861	√	√	√	√

Table 5.2. Fungi used for sequence analysis, listed according to their current classification. Shaded lines indicate the taxa newly sequenced with their UAMH deposition numbers (University of Alberta Microfungus Collection and Herbarium, Edmonton, AB) or the original isolation number in the case of *P. fortinii*. Genbank deposition numbers are given for the other taxa.

Dothideales		
Leptosphaeriaceae	*Leptosphaeria doliolum*	U04205
Pleosporaceae	*Pleospora herbarum*	U05201
Eurotiales		
Tricocomaceae	*Eupenicillium javanicum*	U21298
Tricocomaceae	*Talaromyces flavus*	M83262
Leotiales		
Geoglossaceae	*Cudonia confusa*	Z30240
Geoglossaceae	*Spathularia flavida*	Z30239
Hymenoscyphaceae	*Hymenoscyphus ericae*	UAMH 8873
Leotiaceae	*Leotia lubrica*	L37536
Sclerotiniaceae	*Sclerotinia sclerotiorum*	L37541
Onygenales		
Arthrodermataceae	*Ctenomyces serratus*	U29391
Arthrodermataceae	*Trichophyton rubrum*	X58570
Gymnoascaceae	*Gymnoascoideus petalosporus*	U29392
Gymnoascaceae	*Gymnoascus reessii*	UAMH 4809
Myxotrichaceae	*Myxotrichum arcticum*	UAMH 7565
Onygenaceae	*Auxarthrom zuffianum*	L28062
Onygenaceae	*Onygena equina*	U45442
Onygenaceae	*Renispora flavissima*	U29393
Onygenaceae	*Uncinocarpus reesii*	U29394
Onygenaceae	*Uncinocarpus uncinatus*	UAMH 3913
Pezizales		
Discinaceae	*Gyromitra esculenta*	Z30238
Morchellaceae	*Morchella elata*	L37537
Pezizaceae	*Peziza badia*	L37539
Sarcosomataceae	*Plectania nigrella*	Z27408
Tuberaceae	*Tuber cf rapaeodorum*	Z49755
Saccharomycetales	*Saccharomyces cerevisiae*	M27607
Sordariales		
Sordariaceae	*Chaetomium elatum*	M83257
Sordariaceae	*Sordaria fimicola*	X69851
Asexual taxa	*Oidiodendron maius*	UAMH 8920
	Phialocephala fortinii	S-7Aa
	Variable White Taxon	UAMH 8861

Figure 5.1. Single most parsimonious tree (917 steps; CI 0.63) resulting from 30 replicate searches using the heuristic search algorithm of PAUP 3.1.1. Branch lengths correspond to the number of changes along each branch. Bootstrap support values over 50% are indicated adjacent to the relevant node. Species names in bold type indicate fungi newly sequenced for this study.



Chapter 6

Summary

Historically, research on ericoid mycorrhizas has been driven by a plant-centered view of the association. A large number of the questions asked were focused on what benefit the plant partner received from the symbiosis, with the goal of extrapolating from laboratory experiments an understanding of the dynamics at play in the ecosystems in which the Ericales are dominant. When the story is reviewed from a mycological standpoint, it is less satisfying because of the lack of careful documentation of the precise fungal strains used in many of these experiments. In 1993, as I began the research presented in this dissertation, it was clear that more than one fungal species was involved; the first questions that needed answers were how many are there, how can they be identified and characterized, and how are they related to one another.

A comprehensive field-based survey of fungal root endophytes of the Ericales, isolated from 19 species native to Alberta, was conducted, providing a bank of isolates for further analysis. Habitats in which the Ericales thrive and can become the dominant flora are commonly referred to as heathlands, as typified by the *Calluna* heathlands of Great Britain and Northern Europe in which the acidic mor humus soil has low mineral nutrient availability for plant growth. In Alberta the equivalent habitats include alpine heathlands, acidic peatlands and sand dunes. Plants were collected from sites representing these three habitats at differing times over three growing seasons.

Four fungal taxa were recovered with enough frequency to warrant their consideration as common and ubiquitous root endophytes in these sites: *Scytalidium vaccinii*, considered here to be conspecific with *Hymenoscyphus ericae* based on RFLP analyses and the production of both anamorph and teleomorph in culture by one isolate from *Ledum groenlandicum*; *Oidiodendron maius*, whose phylogenetic placement within the Myxotrichaceae was inferred from parsimony analysis of ITS sequence data; a taxon provisionally named Variable White Taxon (VWT), delimited as distinct from *Hymenoscyphus ericae* using RFLP analysis, a distinction supported with small subunit rDNA sequence analysis; and *Phialocephala fortinii*, a root endophyte of diverse plant families growing in cold, infertile soils.

The two approaches used for taxonomy and systematics, molecular analysis and the more traditional morphological characterizations, served to inform and clarify each other. In some cases, a hypothesis developed from comparing morphological data was given support

by the molecular evidence. At other times, the results of the molecular analysis initiated a resolution of the confusing array of morphological characters. Authentic cultures from type specimens or well-vouchered strains were obtained for the species being examined. It was deemed important that the type specimen be used as the species reference and standard wherever possible, especially for the intraspecific molecular analyses.

A major contribution of the research has been to provide molecular markers for the two ericoid mycorrhizal taxa, *Hymenoscyphus ericae* and *Oidiodendron maius* as well as the third potential mycobiont, VWT. The published molecular markers can be used for comparative purposes by other researchers. Given the degree of uncertainty reported in the literature about the identity of the sterile isolates obtained from roots of the Ericales in disparate geographic locations, molecular analysis provides the best option for resolving the identification problems in order to be able to answer questions about the global distribution and diversity of ericoid endophytic fungi.

Molecular analysis was especially useful for distinguishing two taxa, *H. ericae* and VWT, within a group of morphologically intergrading isolates. As well, RFLP analysis was used to clarify species delimitation within the genus *Oidiodendron*, resulting in the re-identification of several historically important ericoid *Oidiodendron* strains as *O. maius*. This information highlights the discrepancy between reports that numerous *Oidiodendron* species are able to form mycorrhizas in axenic culture with the plants in the Ericaceae, while only *O. maius* has been isolated from field-collected roots. The reasons for this discrepancy are not clear but it would be useful to repeat the axenic resynthesis trials with strains characterized using molecular markers.

Hymenoscyphus ericae, *Oidiodendron maius* and VWT were recovered from all three sites and from most plant species sampled, at times from the same small portion of root processed. Previously the name *Hymenoscyphus ericae* had become synonymous with the concept of the ericoid mycobiont though there was evidence that the genus *Oidiodendron* was also implicated. The results presented here indicate that the fungal contribution to the association is very complex. The bank of identified and preserved isolates provides a springboard for experimentation that looks closely at the potentially different roles of the mycobionts, and for comparisons with additional taxa that may be recovered in other parts of the world or using other isolation techniques.

The new record of *Hymenoscyphus ericae* from western Canada, the preservation of teleomorphic material and the expanded description of the holomorph contributes in a

significant way to our understanding of this renowned and ecologically interesting fungus. This material will be available for further study should fruiting bodies ever be found in the field and for any monographic treatment of the genus *Hymenoscyphus* and its relatives.

Another major contribution of the research was the use of molecular analysis for the delimitation of species within the genus *Oidiodendron* and the subsequent correlation of morphological characters with three monophyletic species groups. The comparison of sequence divergence at the inter- and intra-specific levels was found to be informative for inferring species groups whereas the morphological data did not clearly resolve these groups. This approach required a comprehensive study of multiple strains, using the type specimen that defines the species as the reference genotype. There are relatively few characters that can be used for species distinctions in hyphomycetous fungi because they are known only in their asexual stage, and in some cases a single distinctive character, such as the colour of one attribute, is used. The analysis showed that several such characters useful for defining morphological species within *Oidiodendron*, are not indicative of natural relationships within the group. This work is a prerequisite to a monographic revision of the genus with the development of a new key for identification.

Molecular analysis was also used to confirm the anamorph-teleomorph connection of *Oidiodendron* spp. to taxa in the Myxotrichaceae. Hyphomycetous fungi are classifed in form-genera, outside of the taxonomic hierarchy which is based primarily on the characters associated with sexual reproduction. Although this connection had been hypothesized previously and many hypotheses about the phylogenetic relationships of hyphomyceteous taxa to teleomorphic groups are made, support from molecular studies is needed in order to achieve an integrated classification system for all fungi.

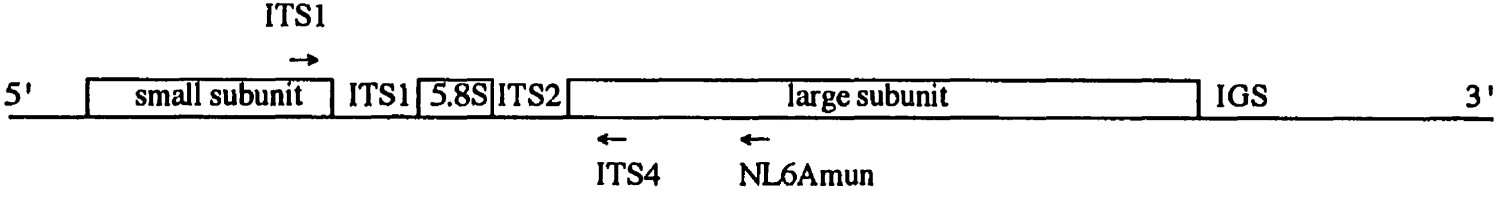
The final molecular analysis, that tested a hypothesis about the phylogenetic relatedness of the fungi endophytic in ericaceous roots of boreal habitats, contributes significantly to a major research thrust in systematics. This thrust, which has accelerated with the advent of methodologies for molecular systematics, is the gradual revision of taxonomic hierarchies to reflect natural relationships, rather than morphological similarities which were emphasized for fungal groups in the past. Several important findings resulted from the parsimony analysis of small subunit ribosomal DNA sequences. The unequivocal exclusion of the cleistothecial genus *Myxotrichum* from the Onygenales, which is now revealed as polyphyletic, and its inclusion in the discomycete clade is a new striking example of convergence in fruiting body morphology. The results of DNA sequence analyses suggest that the reduction in form of an open fruiting body with forcible discharge

of spores (as exemplified by the inoperculate discomycetes) to a spherical enclosure with passive spore release (as exemplified by *Myxotrichum*) has occurred repeatedly within fungi. One of the challenges for molecular systematists is to uncover these evolutionary novelties. In this case, the next challenge is to find the closest sister taxa to *Myxotrichum* within that lineage.

Perhaps the most interesting finding relates to the molecular evidence suggesting that the four endophytic taxa initially isolated from roots, at times from the same root system, have apparently evolved from within the same fungal lineage though much work remains to be done to clarify those phylogenetic relationships. There are many questions to ask about the precise ecological roles of the different taxa, in particular for *Phialocephala fortinii* which is a root endophyte of other plant families. Nevertheless, one is intrigued by the possibility that these fungal root associates have evolved from a common apothecium-forming ancestor, in concert with a monophyletic lineage of plants, resulting in an adaptation to survival in marginal habitats for all the partners.

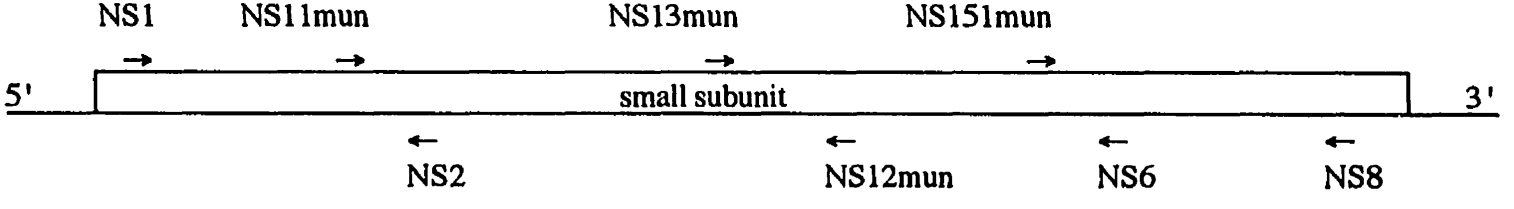
Appendix 1. Diagrammatic representation (not drawn to scale) of the eukaryotic nuclear ribosomal RNA gene. One repeat unit or cluster consists of the small subunit (18S), the 5.8S subunit and the large subunit (28S) separated by two internal transcribed spacers ITS1 and ITS2, and the intergenic spacer (IGS). a) The upper diagram shows the annealing locations of the primers ITS1 - NL6Amun used to amplify rDNA for the RFLP analyses presented in Chapter 2 and 4, and the primer pair ITS1 - ITS4 used to amplify the rDNA for the sequence analyses presented in Chapter 4. b) The lower diagram is an enlargement of the small subunit and shows the relative annealing locations of the primers used to amplify small subunit rDNA for the molecular analysis presented in Chapter 5. Arrows indicate the direction of extension during amplification. All primer sequences are given below, written in the 5' to 3' direction.

a)



ITS1	TCC GTA GGT GAA CCT GCG G	NL6Amun	CAA GTG CTT CCC TTT CAA CA
ITS4	TCC TCC GCT TAT TGA TAT GC		

b)



NS1	GTA GTC ATA TGC TTG TCT C	NS13mun	TGG TTT CTA GGA CCG CCG T
NS2	GGC TGC TGG CAC CAG ACT TGC	NS6	GCA TCA CAG ACC TGT TAT TGC CTC
NS11mun	GCA AAT TAC CCA ATC CCG AC	NS151mun	GAA ACT CAC CAG GTC CAG ACA
NS12mun	AAT CCA AGA ATT TCA CCT CT	NS8	TCC GCA GGT TCA CCT ACG GA

Appendix 2. Fungal strains from original isolation work used for RFLP analyses (Chapter 2) with their corresponding deposition numbers at the University of Alberta Microfungus Collection and Herbarium (UAMH), University of Alberta, Edmonton, AB.

Hymenoscyphus ericae (*Scytalidium vaccinii*); Type I:

S-76Bc	UAMH 8868
S-77Ba	UAMH 8867
S-85Ac	UAMH 8866
S-87Bb	UAMH 8865
S-109Aa	UAMH 8869
S-120a	UAMH 8870

Hymenoscyphus ericae (*Scytalidium vaccinii*); Type II:

S-14Bb	UAMH 8680 and UAMH 8685
S-24Ab	UAMH 8872
S-42b	UAMH 8873
S-355b	UAMH 8871

Oidiodendron griseum:

S-31b	UAMH 8925
S-266b	UAMH 8926

Oidiodendron maius:

S-9Da	UAMH 8919
S-10Aa	UAMH 8920
S-38a	UAMH 8932
S-27b	UAMH 8921
S-357Ca	UAMH 8922
S-74Aa	UAMH 8923
S-77Aa	UAMH 8933
S-81Bc	UAMH 8924

Variable White Taxon (VWT)

S-70Ac	UAMH 8861
S-71Aa	UAMH 8862
S-77Ac	UAMH 8863
S-86Ae	UAMH 8864

Appendix 3. Summary of plant collections sorted by species, site (A = alpine, B = bog, S = sand dune) , and time of collection (Spr = spring, Sum = mid-summer, Fall = late summer or fall) in the growing season. Isolation results are given for each of the four major endophytes discussed in Chapter 2, *Oidiodendron maius*, *Scytalidium vaccinii*, Variable White Taxon (VWT), *Phialocephala fortinii*.

"+" indicates that the taxon was isolated at least once from the root segment(s) processed for that collection.

O. griseum was isolated from only four collections (Coll #'s 31, 73, 266, 357); *O. g.* is entered in the *O. maius* column for each of these collections.

	Coll. #	Species of Ericaceae	Site	Time	*O. maius*	*S. vaccinii*	VWT	*P. fortinii*
1.	132	Andromeda polifolia	B	Spr	+	+	+	
2.	133	Andromeda polifolia	B	Spr	+	+	+	
3.	134	Andromeda polifolia	B	Spr		+		
4.	135	Andromeda polifolia	B	Spr	+	+		
5.	136	Andromeda polifolia	B	Spr		+	+	
6.	152	Andromeda polifolia	B	Spr		+		
7.	153	Andromeda polifolia	B	Spr	+	+		
8.	168	Andromeda polifolia	B	Spr	+	+	+	
9.	169	Andromeda polifolia	B	Spr	+	+		
10.	170	Andromeda polifolia	B	Spr		+	+	
11.	36	Andromeda polifolia	B	Sum				
12.	40	Andromeda polifolia	B	Sum	+	+	+	
13.	296	Andromeda polifolia	B	Sum	+	+		
14.	297	Andromeda polifolia	B	Sum	+	+		
15.	298	Andromeda polifolia	B	Sum	+	+		
16.	299	Andromeda polifolia	B	Sum	+	+		
17.	300	Andromeda polifolia	B	Sum	+		+	
18.	11	Andromeda polifolia	B	Fall	+			+
19.	187	Cassiope mertensiana	A	Spr			+	+
20.	188	Cassiope mertensiana	A	Spr		+		
21.	189	Cassiope mertensiana	A	Spr			+	+
22.	196	Cassiope mertensiana	A	Spr	+	+		
23.	220	Cassiope mertensiana	A	Spr			+	+
24.	233	Cassiope mertensiana	A	Spr		+		
25.	304	Cassiope mertensiana	A	Sum				
26.	78	Cassiope mertensiana	A	Fall		+		+
27.	79	Cassiope mertensiana	A	Fall		+		
28.	80	Cassiope mertensiana	A	Fall		+		+
29.	88	Cassiope mertensiana	A	Fall		+		+
30.	89	Cassiope mertensiana	A	Fall		+		
31.	342	Cassiope mertensiana	A	Fall				
32.	190	Cassiope tetragona	A	Spr		+		
33.	191	Cassiope tetragona	A	Spr		+		
34.	210	Cassiope tetragona	A	Spr			+	+
35.	216	Cassiope tetragona	A	Spr		+		
36.	226	Cassiope tetragona	A	Spr	+	+		
37.	229	Cassiope tetragona	A	Spr		+		+
38.	343	Cassiope tetragona	A	Fall				+
39.	147	Chamaedaphne calyculata	B	Spr		+	+	
40.	148	Chamaedaphne calyculata	B	Spr	+	+	+	

Appendix 3. Continued

	Coll. #	Species of Ericaceae	Site	Time	*O. maius*	*S. vaccinii*	VWT	*P. fortinii*
41.	149	Chamaedaphne calyculata	B	Spr	+	+	+	
42.	150	Chamaedaphne calyculata	B	Spr		+		
43.	151	Chamaedaphne calyculata	B	Spr	+	+		
44.	154	Chamaedaphne calyculata	B	Spr	+	+	+	
45.	155	Chamaedaphne calyculata	B	Spr		+		
46.	174	Chamaedaphne calyculata	B	Spr	+	+	+	
47.	175	Chamaedaphne calyculata	B	Spr		+		
48.	176	Chamaedaphne calyculata	B	Spr	+	+		
49.	37	Chamaedaphne calyculata	B	Sum				
50.	39	Chamaedaphne calyculata	B	Sum	+	+	+	
51.	42	Chamaedaphne calyculata	B	Sum	+	+		
52.	281	Chamaedaphne calyculata	B	Sum	+	+		
53.	282	Chamaedaphne calyculata	B	Sum		+		+
54.	284	Chamaedaphne calyculata	B	Sum		+		+
55.	285	Chamaedaphne calyculata	B	Sum	+	+	+	
56.	9	Chamaedaphne calyculata	B	Fall	+	+		
57.	177	Empetrum nigrum	A	Spr	+			
58.	178	Empetrum nigrum	A	Spr		+		+
59.	179	Empetrum nigrum	A	Spr		+		+
60.	180	Empetrum nigrum	A	Spr		+		+
61.	181	Empetrum nigrum	A	Spr		+		+
62.	303	Empetrum nigrum	A	Sum		+		
63.	310	Empetrum nigrum	A	Sum		+		
64.	83	Empetrum nigrum	A	Fall		+		
65.	84	Empetrum nigrum	A	Fall	+	+		+
66.	85	Empetrum nigrum	A	Fall		+	+	
67.	86	Empetrum nigrum	A	Fall	+	+		
68.	87	Empetrum nigrum	A	Fall	+	+		
69.	341	Empetrum nigrum	A	Fall		+		
70.	235	Gaultheria humifusa	A	Spr		+		+
71.	236	Gaultheria humifusa	A	Spr		+		
72.	312	Gaultheria humifusa	A	Sum	+	+		+
73.	335	Gaultheria humifusa	A	Fall		+		
74.	336	Gaultheria humifusa	A	Fall				
75.	207	Kalmia polifolia	A	Spr				+
76.	208	Kalmia polifolia	A	Spr		+		+
77.	209	Kalmia polifolia	A	Spr		+		+
78.	212	Kalmia polifolia	A	Spr		+		+
79.	219	Kalmia polifolia	A	Spr		+		+
80.	309	Kalmia polifolia	A	Sum		+	+	
81.	329	Kalmia polifolia	A	Fall				+
82.	142	Ledum groenlandicum	B	Spr	+	+		
83.	143	Ledum groenlandicum	B	Spr	+	+	+	
84.	144	Ledum groenlandicum	B	Spr	+	+	+	
85.	145	Ledum groenlandicum	B	Spr	+	+	+	
86.	146	Ledum groenlandicum	B	Spr	+	+	+	
87.	158	Ledum groenlandicum	B	Spr	+	+	+	
88.	159	Ledum groenlandicum	B	Spr	+	+		
89.	171	Ledum groenlandicum	B	Spr	+	+		
90.	172	Ledum groenlandicum	B	Spr	+	+	+	

Appendix 3. Continued

	Coll. #	Species of Ericaceae	Site	Time	*O. maius*	*S. vaccinii*	VWT	*P. fortinii*
91.	173	Ledum groenlandicum	B	Spr	+	+	+	
92.	38	Ledum groenlandicum	B	Sum	+			
93.	276	Ledum groenlandicum	B	Sum	+	+		
94.	277	Ledum groenlandicum	B	Sum	+	+		
95.	278	Ledum groenlandicum	B	Sum	+	+		
96.	279	Ledum groenlandicum	B	Sum	+		+	
97.	280	Ledum groenlandicum	B	Sum		+		
98.	14	Ledum groenlandicum	B	Fall	+	+		
99.	211	Loiseleuria procumbens	A	Spr	+	+		+
100.	213	Loiseleuria procumbens	A	Spr		+		+
101.	215	Loiseleuria procumbens	A	Spr				+
102.	218	Loiseleuria procumbens	A	Spr	+	+		+
103.	222	Loiseleuria procumbens	A	Spr		+		
104.	224	Loiseleuria procumbens	A	Spr		+	+	+
105.	225	Loiseleuria procumbens	A	Spr		+		+
106.	227	Loiseleuria procumbens	A	Spr		+		+
107.	314	Loiseleuria procumbens	A	Sum				+
108.	324	Loiseleuria procumbens	A	Fall		+		+
109.	328	Loiseleuria procumbens	A	Fall		+		+
110.	331	Loiseleuria procumbens	A	Fall				+
111.	333	Loiseleuria procumbens	A	Fall				+
112.	334	Loiseleuria procumbens	A	Fall				+
113.	205	Menziesia ferruginea	A	Spr		+		
114.	206	Menziesia ferruginea	A	Spr		+		+
115.	317	Menziesia ferruginea	A	Sum				+
116.	96	Menziesia ferruginea	A	Fall		+		
117.	338	Menziesia ferruginea	A	Fall				+
118.	127	Oxycoccus quadripetalus	B	Spr		+	+	
119.	128	Oxycoccus quadripetalus	B	Spr		+	+	
120.	129	Oxycoccus quadripetalus	B	Spr		+	+	
121.	130	Oxycoccus quadripetalus	B	Spr		+	+	
122.	131	Oxycoccus quadripetalus	B	Spr		+		
123.	156	Oxycoccus quadripetalus	B	Spr		+		
124.	157	Oxycoccus quadripetalus	B	Spr	+	+	+	
125.	165	Oxycoccus quadripetalus	B	Spr		+		
126.	166	Oxycoccus quadripetalus	B	Spr	+	+	+	
127.	167	Oxycoccus quadripetalus	B	Spr	+	+		
128.	41	Oxycoccus quadripetalus	B	Sum	+	+		
129.	286	Oxycoccus quadripetalus	B	Sum		+		
130.	287	Oxycoccus quadripetalus	B	Sum	+	+		
131.	288	Oxycoccus quadripetalus	B	Sum		+		
132.	289	Oxycoccus quadripetalus	B	Sum		+		
133.	290	Oxycoccus quadripetalus	B	Sum		+		
134.	10	Oxycoccus quadripetalus	B	Fall	+	+		
135.	15	Oxycoccus quadripetalus	B	Fall	+			
136.	20	Oxycoccus quadripetalus	B	Fall	+	+		
137.	22	Oxycoccus quadripetalus	B	Fall	+	+		
138.	24	Oxycoccus quadripetalus	B	Fall	+	+		
139.	183	Phyllodoce empetriformis	A	Spr		+		+
140.	185	Phyllodoce empetriformis	A	Spr		+		+

Appendix 3. Continued

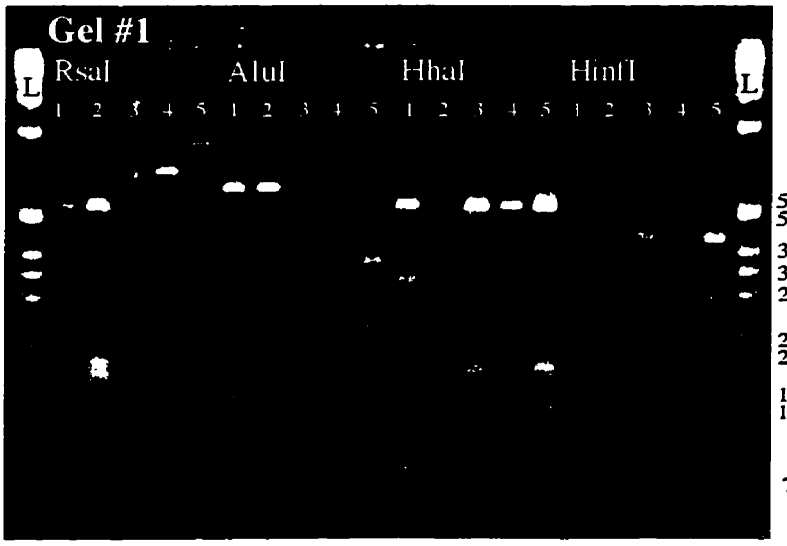
	Coll. #	Species of Ericaceae	Site	Time	*O. maius*	*S. vaccinii*	VWT	*P. fortinii*
141.	186	Phyllodoce empetriformis	A	Spr		+		+
142.	232	Phyllodoce empetriformis	A	Spr		+		+
143.	308	Phyllodoce empetriformis	A	Sum			+	+
144.	75	Phyllodoce empetriformis	A	Fall		+		
145.	76	Phyllodoce empetriformis	A	Fall	+	+		+
146.	77	Phyllodoce empetriformis	A	Fall	+	+		+
147.	93	Phyllodoce empetriformis	A	Fall		+	+	
148.	94	Phyllodoce empetriformis	A	Fall		+		
149.	339	Phyllodoce empetriformis	A	Fall		+		+
150.	217	Phyllodoce glanduliflora	A	Spr				+
151.	223	Phyllodoce glanduliflora	A	Spr		+		+
152.	228	Phyllodoce glanduliflora	A	Spr		+		
153.	302	Phyllodoce glanduliflora	A	Sum				
154.	73	Phyllodoce glanduliflora	A	Fall	+;*O. g.*	+		+
155.	74	Phyllodoce glanduliflora	A	Fall	+	+		
156.	90	Phyllodoce glanduliflora	A	Fall	+	+		+
157.	346	Phyllodoce glanduliflora	A	Fall		+		
158.	202	Rhododendron albiflorum	A	Spr				+
159.	203	Rhododendron albiflorum	A	Spr			+	+
160.	204	Rhododendron albiflorum	A	Spr	+	+	+	
161.	316	Rhododendron albiflorum	A	Sum				
162.	70	Rhododendron albiflorum	A	Fall		+	+	+
163.	95	Rhododendron albiflorum	A	Fall		+		
164.	323	Rhododendron albiflorum	A	Fall				+
165.	197	Vaccinium membranaceum	A	Spr		+		+
166.	198	Vaccinium membranaceum	A	Spr		+		+
167.	199	Vaccinium membranaceum	A	Spr			+	
168.	200	Vaccinium membranaceum	A	Spr		+		+
169.	201	Vaccinium membranaceum	A	Spr		+	+	+
170.	307	Vaccinium membranaceum	A	Sum				
171.	313	Vaccinium membranaceum	A	Sum				+
172.	71	Vaccinium membranaceum	A	Fall		+	+	
173.	72	Vaccinium membranaceum	A	Fall		+		+
174.	91	Vaccinium membranaceum	A	Fall				+
175.	92	Vaccinium membranaceum	A	Fall				+
176.	344	Vaccinium membranaceum	A	Fall				
177.	101	Vaccinium myrtilloides	S	Spr		+		+
178.	102	Vaccinium myrtilloides	S	Spr			+	+
179.	103	Vaccinium myrtilloides	S	Spr	+	+	+	+
180.	104	Vaccinium myrtilloides	S	Spr		+		+
181.	105	Vaccinium myrtilloides	S	Spr				+
182.	109	Vaccinium myrtilloides	S	Spr		+		
183.	111	Vaccinium myrtilloides	S	Spr				+
184.	113	Vaccinium myrtilloides	S	Spr				+
185.	118	Vaccinium myrtilloides	S	Spr				+
186.	121	Vaccinium myrtilloides	S	Spr	+	+		+
187.	25	Vaccinium myrtilloides	S	Sum				+
188.	26	Vaccinium myrtilloides	S	Sum				+
189.	29	Vaccinium myrtilloides	S	Sum				+
190.	31	Vaccinium myrtilloides	S	Sum	+;*O. g.*			+

Appendix 3. Continued

	Coll. #	Species of Ericaceae	Site	Time	*O. maius*	*S. vaccinii*	VWT	*P. fortinii*
191.	238	Vaccinium myrtilloides	S	Sum				+
192.	239	Vaccinium myrtilloides	S	Sum		+		+
193.	240	Vaccinium myrtilloides	S	Sum				+
194.	242	Vaccinium myrtilloides	S	Sum		+		+
195.	243	Vaccinium myrtilloides	S	Sum	+	+		+
196.	244	Vaccinium myrtilloides	S	Sum				+
197.	245	Vaccinium myrtilloides	S	Sum				+
198.	246	Vaccinium myrtilloides	S	Sum				+
199.	271	Vaccinium myrtilloides	S	Sum			+	
200.	272	Vaccinium myrtilloides	S	Sum	+			+
201.	347	Vaccinium myrtilloides	S	Fall				+
202.	348	Vaccinium myrtilloides	S	Fall	+			+
203.	351	Vaccinium myrtilloides	S	Fall	+	+	+	+
204.	352	Vaccinium myrtilloides	S	Fall		+		+
205.	353	Vaccinium myrtilloides	S	Fall	+			+
206.	359	Vaccinium myrtilloides	S	Fall	+			+
207.	214	Vaccinium scoparium	A	Spr				+
208.	230	Vaccinium scoparium	A	Spr		+		
209.	231	Vaccinium scoparium	A	Spr				+
210.	306	Vaccinium scoparium	A	Sum				+
211.	315	Vaccinium scoparium	A	Sum				+
212.	81	Vaccinium scoparium	A	Fall	+	+		
213.	82	Vaccinium scoparium	A	Fall		+		
214.	325	Vaccinium scoparium	A	Fall		+	+	+
215.	345	Vaccinium scoparium	A	Fall	+			+
216.	311	Vaccinium uliginosum	A	Sum		+		+
217.	326	Vaccinium uliginosum	A	Fall				+
218.	330	Vaccinium uliginosum	A	Fall		+		+
219.	332	Vaccinium uliginosum	A	Fall	+	+		+
220.	337	Vaccinium uliginosum	A	Fall				
221.	192	Vaccinium vitis-idaea	A	Spr		+		
222.	193	Vaccinium vitis-idaea	A	Spr		+		
223.	194	Vaccinium vitis-idaea	A	Spr		+		+
224.	195	Vaccinium vitis-idaea	A	Spr		+		+
225.	305	Vaccinium vitis-idaea	A	Sum				
226.	137	Vaccinium vitis-idaea	B	Spr	+	+		
227.	138	Vaccinium vitis-idaea	B	Spr	+	+	+	
228.	139	Vaccinium vitis-idaea	B	Spr	+	+		
229.	140	Vaccinium vitis-idaea	B	Spr	+	+		
230.	141	Vaccinium vitis-idaea	B	Spr	+	+		
231.	160	Vaccinium vitis-idaea	B	Spr		+		
232.	161	Vaccinium vitis-idaea	B	Spr	+	+	+	
233.	162	Vaccinium vitis-idaea	B	Spr	+	+	+	
234.	163	Vaccinium vitis-idaea	B	Spr	+	+	+	
235.	164	Vaccinium vitis-idaea	B	Spr		+	+	
236.	291	Vaccinium vitis-idaea	B	Sum		+	+	
237.	292	Vaccinium vitis-idaea	B	Sum	+	+		
238.	293	Vaccinium vitis-idaea	B	Sum		+	+	
239.	294	Vaccinium vitis-idaea	B	Sum	+	+	+	
240.	295	Vaccinium vitis-idaea	B	Sum		+		

Appendix 3. Continued

	Coll. #	Species of Ericaceae	Site	Time	*O. maius*	*S. vaccinii*	VWT	*P. fortinii*
241.	19	Vaccinium vitis-idaea	B	Fall	+	+		
242.	23	Vaccinium vitis-idaea	B	Fall	+	+		
243.	106	Vaccinium vitis-idaea	S	Spr				+
244.	107	Vaccinium vitis-idaea	S	Spr				+
245.	110	Vaccinium vitis-idaea	S	Spr				+
246.	112	Vaccinium vitis-idaea	S	Spr				+
247.	114	Vaccinium vitis-idaea	S	Spr		+		+
248.	115	Vaccinium vitis-idaea	S	Spr				+
249.	117	Vaccinium vitis-idaea	S	Spr				+
250.	120	Vaccinium vitis-idaea	S	Spr		+		+
251.	122	Vaccinium vitis-idaea	S	Spr	+			+
252.	124	Vaccinium vitis-idaea	S	Spr		+		+
253.	27	Vaccinium vitis-idaea	S	Sum	+	+		+
254.	258	Vaccinium vitis-idaea	S	Sum		+		+
255.	259	Vaccinium vitis-idaea	S	Sum				+
256.	260	Vaccinium vitis-idaea	S	Sum	+	+		+
257.	261	Vaccinium vitis-idaea	S	Sum		+		+
258.	262	Vaccinium vitis-idaea	S	Sum	+	+		+
259.	263	Vaccinium vitis-idaea	S	Sum				+
260.	264	Vaccinium vitis-idaea	S	Sum		+		+
261.	265	Vaccinium vitis-idaea	S	Sum	+			+
262.	266	Vaccinium vitis-idaea	S	Sum	*O. g.*			+
263.	267	Vaccinium vitis-idaea	S	Sum		+		
264.	349	Vaccinium vitis-idaea	S	Fall				+
265.	350	Vaccinium vitis-idaea	S	Fall				
266.	354	Vaccinium vitis-idaea	S	Fall				+
267.	355	Vaccinium vitis-idaea	S	Fall	+	+		+
268.	356	Vaccinium vitis-idaea	S	Fall	+			+
269.	357	Vaccinium vitis-idaea	S	Fall	+;*O. g.*		+	+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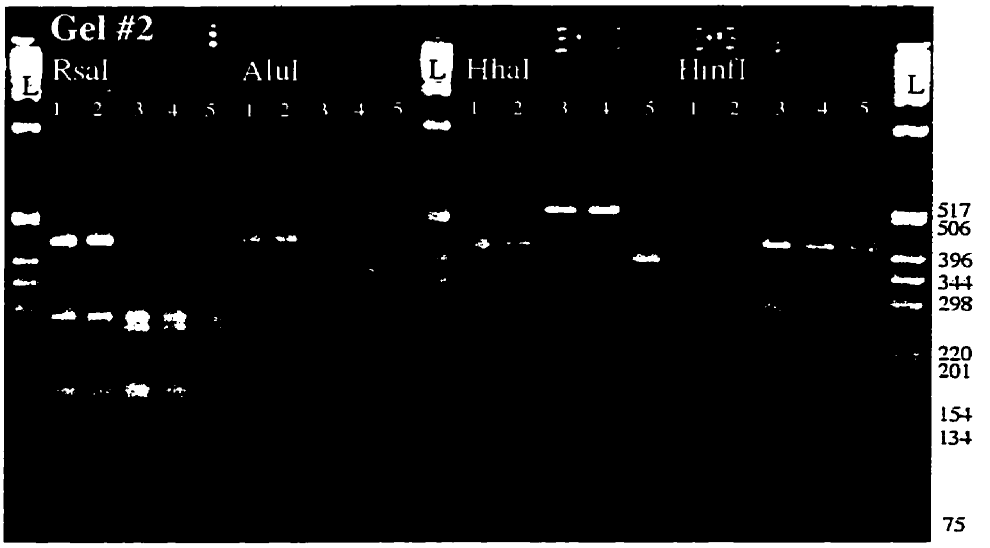
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AluI
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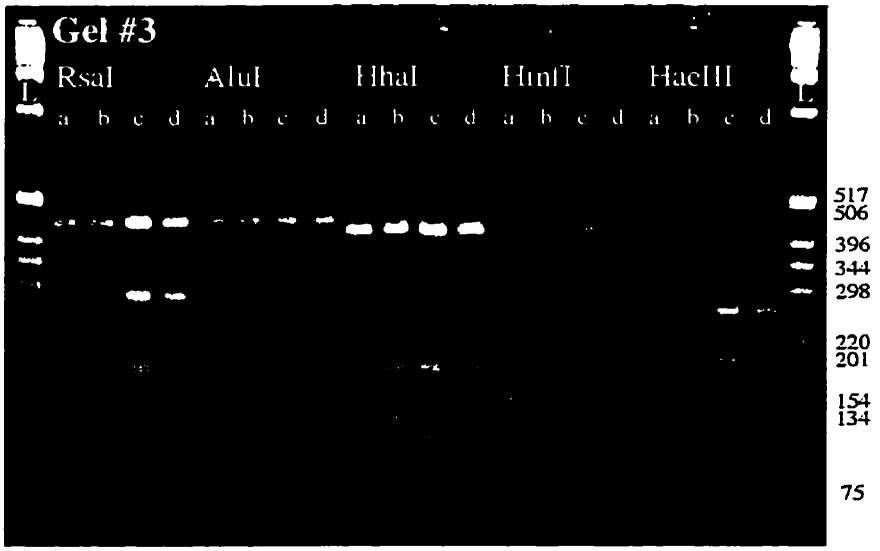
Gel #3
RsaI
AluI
HhaI
HinfI
HaeIII
L
a b c d a b c d a b c d a b c d a b c d
517
506
396
344
298
220
201
154
134
75

Appendix 5. *Oidiodendron* coded data matrix used to generate the inferred phylogenetic reconstructions shown in Figure 4.1 and 4.2 23 strains of 15 *Oidiodendron* species, and *Pseudogymnoascus roseus*. Nucleotide positions are numbered from 1 to 540 as indicated above the data matrix and the location of ITS1, the 5.8S subunit, ITS2 and the 28S subunit are indicated. Dots indicate that the nucleotid in that position for that taxon matches the nucleotide noted for *O. pilicola*; "-" indicates a gap introduced for alignment purposes and counted as a fifth character; "?" denotes missing data either from missing bases in the sequences or introduced during the process o: coding the data matrix to reduce ambiguities in the alignment.

```
                                ITS1⇒
                           1    ↓                                                                          80
O. pilicola                CATTACAGAGTTCTCGCCCTCGCGGGTAGATCTCCCACCCACTGTTATCGTTACTATCGTTGCTTTGGCGGGCCGCCGGG
O. periconioides           ........????......................................C...CG.....T..............T...
O. echinulatum             ....................AC..........................T.C....G........................
O. truncatum               .....................A........-..............G..T.....CG....................T...
O. cerealis                .............AT.....A...........................T......G........................
O. chlamydosporicum        ................................................T.C...CG.-...............T..T...
O. scytaloides             ????????????..............Y.....................T.C...CG.-...............T..T...
O. rhodogenum              .........S..........TA.................................G.....T..................
O. citrinum                ........???..AT......-...................T..C...AAC....C....................T...
O. maius (8922)            .............AT...Y..-...........................AC....C...................?T...
O. maius (8529)            .............AT......-............................C....C....................T...
O. maius (8921)            .............AT......-......................C....AC....C........................
O. maius (1540)            .............AT......-...........................AC....C....................T...
O. tenuissimum (8511)      ....................T...........................T.A....G.-......................
O. tenuissimum (8512)      .....................-.......................G..T.A....G.-......................
O. setiferum               .....................A..........................T.A....G.-......................
O. sp. nov. (8513)         ...................G.C.......................G..T.C...CG.-...T..................
O. griseum (1693)          .............................................G..T.C...CG.-..................T...
O. griseum (1403)          .............................................G..T.C...CG.-..................T...
O. griseum (4080)          .........???.................................G..T.C...CG.-......................
O. griseum (8925)          .............................................G..T.C...CG.-......................
O. griseum (8528)          .............................................G..T.C...CG.-......................
O. flavum                  .............................................A..T.C...CG.-......................
P. roseus                  ??????????????????????????????????????????????????????????...........AA...?-T.-?
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Appendix 5. Continued

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                          81                                                                                                160
O. pilicola               TCCTGCCCGGCCCCC-GGC-?CCC-?GGCTGGGGTGCGCCCGCCAGAGGCCCTACAAACTCTGAATGTCAGTGTCGTCTG
O. periconioides          C..........G..-...-?T..-?......C.C.............A...CC.............T..........
O. echinulatum            C..........G..-...-?...-?......C.C..........A......C-T.........................
O. truncatum              C..A..T.A...A..-...-?...-?......T.C...............T.CG........TG......AC........
O. cerealis               C..A..T.....G..-...-?T..-?......C.C...............T.-.............T..........
O. chlamydosporicum       C.TA........G..-...-?T..-?......C.C.?-.............G.....T.....T..............
O. scytaloides            C.TA........G..-...-?T..-?...C..C.C...............G.....T.....T..............
O. rhodogenum             ...-?.......G..-...GT.TAATA.GC..C.C...............T.-.............T..........
O. citrinum               C..........G..-...-?...-?......C.C.T..........A..TC...G..........T.........C.
O. maius (8922)           C..........G..-...-?...-?..?...C.C.T..........A..TC...G..........T.........C.
O. maius (8529)           C..........G..-...-?...-?......C.C.T..........A..TC...G..........T.........C.
O. maius (8921)           C..........G..-...-?...-?......C.C.T..........A..TC...G..........T.........C.
O. maius (1540)           C..........G..-...-?...-?......C.C.T..........A..TC...G..........T.........C.
O. tenuissimum (8511)     C.....T.....G..-...-?T..-?......C.C..............T.CG..................C........
O. tenuissimum (8512)     C........T..G..-...-?T..-?......C.C..............T.CG.........................
O. setiferum              C..C..T.....G..-...-?T..-?......C.C..............T.CG..................C........
O. sp. nov. (8513)        C..........G..A...GA...-?C..C..C.C..............T.CC.....C..................C.
O. griseum (1693)         C..C....C...G..-...-?...-?...C..C.C.T..........A....CG..................C........
O. griseum (1403)         C..A....C...G..-...-?...-?...C..C.C.T..........A....CG..................C........
O. griseum (4080)         C..A....C...G..-...-?...-?...C..C.C.T..........A....CG.......C.........C........
O. griseum (8925)         C..A....A...G..-...-?...-?...C..C.C.T..........A....CG..................C........
O. griseum (8528)         C..A....C...G..-...-?...-?...C..C.C.T..........A....CG.......C.........C........
O. flavum                 C..A....-...G..-...-?...-?...C..C.C.T..........A....CG..................C........
P. roseus                 C..?????...TG.T-...-?T..-?...C..C.A....TT........A...-?........TT....TA.ACT.....
```

Appendix 5. Continued

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                                          5.8S⇒
                       161                  ↓                                                            240
O. pilicola            AGTA-CTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
O. periconioides       ....-.....C.....................................................................
O. echinulatum         ....-.....C.....................................................................
O. truncatum           ....-...........................................................................
O. cerealis            ....-.....C.....................................................................
O. chlamydosporicum    ....-...........................................................................
O. scytaloides         ....-...........................................................................
O. rhodogenum          ....-...........................................................................
O. citrinum            ....A.....C...C.................................................................
O. maius (8922)        ....A.....C...C.................................................................
O. maius (8529)        ....A.........C.................................................................
O. maius (8921)        ....A.....C...C.................................................................
O. maius (1540)        ....A.....C...C.................................................................
O. tenuissimum (8511)  ....-...........................................................................
O. tenuissimum (8512)  ....-...........................................................................
O. setiferum           ..C.-...........................................................................
O. sp. nov. (8513)     ....A.-.C.C.....................................................................
O. griseum (1693)      ....-...........................................................................
O. griseum (1403)      ....-...........................................................................
O. griseum (4080)      ....-...........................................................................
O. griseum (8925)      ....-...........................................................................
O. griseum (8528)      ....-...........................................................................
O. flavum              ....-...........................................................................
P. roseus              ....-...........................................................................
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Appendix 5. Continued

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                          241                                                                                  320
O. pilicola               AGTAATGCGAATTGCAGAATTCAGTGAGTCATCGAATCTTTGAACGCACATTGCGCCCTGTGGTATTCCGCAGGGCATGC
O. periconioides          ................................................................................
O. echinulatum            ................................................................................
O. truncatum              ................................................................................
O. cerealis               ................................................................................
O. chlamydosporicum       ................................................................................
O. scytaloides            ................................................................................
O. rhodogenum             ................................................................................
O. citrinum               ................................................................................
O. maius (8922)           ................................................................................
O. maius (8529)           ................................................................................
O. maius (8921)           ................................................................................
O. maius (1540)           ................................................................................
O. tenuissimum (8511)     ................................................................................
O. tenuissimum (8512)     ................................................................................
O. setiferum              ................................................................................
O. sp. nov. (8513)        ................................................................................
O. griseum (1693)         ................................................................................
O. griseum (1403)         ................................................................................
O. griseum (4080)         ................................................................................
O. griseum (8925)         ................................................................................
O. griseum (8528)         ................................................................................
O. flavum                 ................................................................................
P. roseus                 .......T..................A.............................CC..........GG........
```

Appendix 5. Continued

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                              ITS2⇒
                         321  ↓                                                                           400
O. pilicola               CTGTTCGAGCGTCATTTCAACCCTCAAGCACTGCTTGGTGTTGGGCCCTGCCCG-CCGCGGCCGGCCCTAAAGACAGTGG
O. periconioides          .............................T..................A.....-T............C...........
O. echinulatum            .............................T.................AC....?-T............C....C......
O. truncatum              .............................T.C.....................?-.............C...........
O. cerealis               .............................T.................AC....?-.............C....CT.....
O. chlamydosporicum       .............................T..................C.....CT........................
O. scytaloides            .............................T..................C.....CT........................
O. rhodogenum             .............................T................T.C....?-...T.........C....T......
O. citrinum               .............................CTC......................-...................T.....
O. maius (8922)           .............................CTC......................-...................T.....
O. maius (8529)           .............................CTC......................-...................T.....
O. maius (8921)           .............................CTC......................-...................T.....
O. maius (1540)           .............................CTC......................-.........................
O. tenuissimum (8511)     .............................T.......................?-.........................
O. tenuissimum (8512)     .............................T........................CT........................
O. setiferum              .............................T.C.....................?-.........................
O. sp. nov. (8513)        .............................C.................GG.....-T........................
O. griseum (1693)         .............................T........................-.T...........C...........
O. griseum (1403)         .............................T........................-.T...........C...........
O. griseum (4080)         .............................T........................-.T...........C...........
O. griseum (8925)         .............................T........................-.T.......................
O. griseum (8528)         .............................T........................-.T...........C...........
O. flavum                 .............................T........................-.T.......................
P. roseus                 ....C...........A............T.A.......A........C...GA-???....G..........T......
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Appendix 5. Continued

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                          401                                                                                                  480
O. pilicola               CGGCGCCGTCTGGCTCTAAGCGTAGTACAACT-CTCGCTCTGGAGTCC-?GCGGT?-GCTTGCCAGAACCCC-?AA-TCT
O. periconioides          ..........G...C.............T..-........A.G.C..-?.T...?-.........C.A...-?..C..C
O. echinulatum            ..........C.................T..-........A.G.C..-G....C?-..C............-A..C...
O. truncatum              ........CTC...C.............T..-........A.GAC..-G.....?-..C............-A..C..A
O. cerealis               ...T........................T..-........A.G.C..-G.....?-.............?-AC.C...
O. chlamydosporicum       ....AG..C.......C...........G..-.........G.CT?-G.....?-..C............-???C.TC
O. scytaloides            ....AG..C.......C...........G..-.........G.CT?-G.....?-..C.....?......-???C.TC
O. rhodogenum             ............................T..-........A.G....-?.....?-..............-?..C.TC
O. citrinum               ........C.....C..C..........G..-.......CA.G....-G....C?-..C............CC..C.??
O. maius (8922)           ........C.....C..C..........G..-.......CA.G....-G....C?-..C............-?..C.??
O. maius (8529)           ........C.....C..C..........G..-.......CA.G....-G....C?-..C............CC..C.??
O. maius (8921)           ........C.....C..C..........G..-.......CA.G....-G....C?-..C............CC..C.??
O. maius (1540)           ........C.....C..C..........G..-.......CA.G....-G....C?-..C............-?..C.??
O. tenuissimum (8511)     ............................T..-........A.C.C..-G.....?-..C............-?..C.??
O. tenuissimum (8512)     ............................T..-........A.C....-G.....?-..CC...........-?..C.??
O. setiferum              ............................T..-........A.C.C.T-G.....?-..C............CC..C.??
O. sp. nov. (8513)        ........CTC......C..........C.C-......GC..T-C..GG....C?-..CC...........-?G.C.??
O. griseum (1693)         ...T....C.....C..G..........T..-.......CA.T....-G.....?-..C...........?-A..C...
O. griseum (1403)         ...T....C.....C..G..........T..-.......CA.T....-G.....?-..C...........?-A..C...
O. griseum (4080)         ...T....C.....C..G..........T..-.......CA.T....-G.....?-..C...........?-A..C...
O. griseum (8925)         ...T....C.....C..G..........T..-.......CA.T.C..-G.....?-..C............-?..C...
O. griseum (8528)         ...T....C.....C..G..........T..-.......CA.T....-G.....?-..C...........?-A..C...
O. flavum                 ...T....C.....C..G..........T..-.......CA.T.C..-G.T...?-..C............-?..C...
P. roseus                 ...T......C.....CG......R..ATT..T...........GT.CG.TC..GT...C.....C.A...CC..T.T.
```

Appendix 5. Continued

```
                              28S⇒
                         481   ↓                                                         540
O. pilicola                TAT-?GGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCG
O. periconioides           ...-?.......................................................
O. echinulatum             ...-?.......................................................
O. truncatum               .G.-?.......................................................
O. cerealis                ...-?.......................................................
O. chlamydosporicum        ...-?.......................................................
O. scytaloides             ...-?.......................................................
O. rhodogenum              ...-?.......................................................
O. citrinum                -G.-?.......................................................
O. maius (8922)            -G.-?......................................?????????????????
O. maius (8529)            -G.-?..................................................?????
O. maius (8921)            -G.-?.......................................................
O. maius (1540)            -G.-?......................................................?
O. tenuissimum (8511)      -G.-?.......................................................
O. tenuissimum (8512)      -G.-?................K...K................................??
O. setiferum               -G.-?.......................................................
O. sp. nov. (8513)         -G.-?.......................................................
O. griseum (1693)          CG.-?.......................................................
O. griseum (1403)          CG.-?.......................................................
O. griseum (4080)          CG.-?.......................................................
O. griseum (8925)          CG.-?.......................................................
O. griseum (8528)          CG.-?.......................................................
O. flavum                  CG.-?.......................................................
P. roseus                  .????......................................................?
```

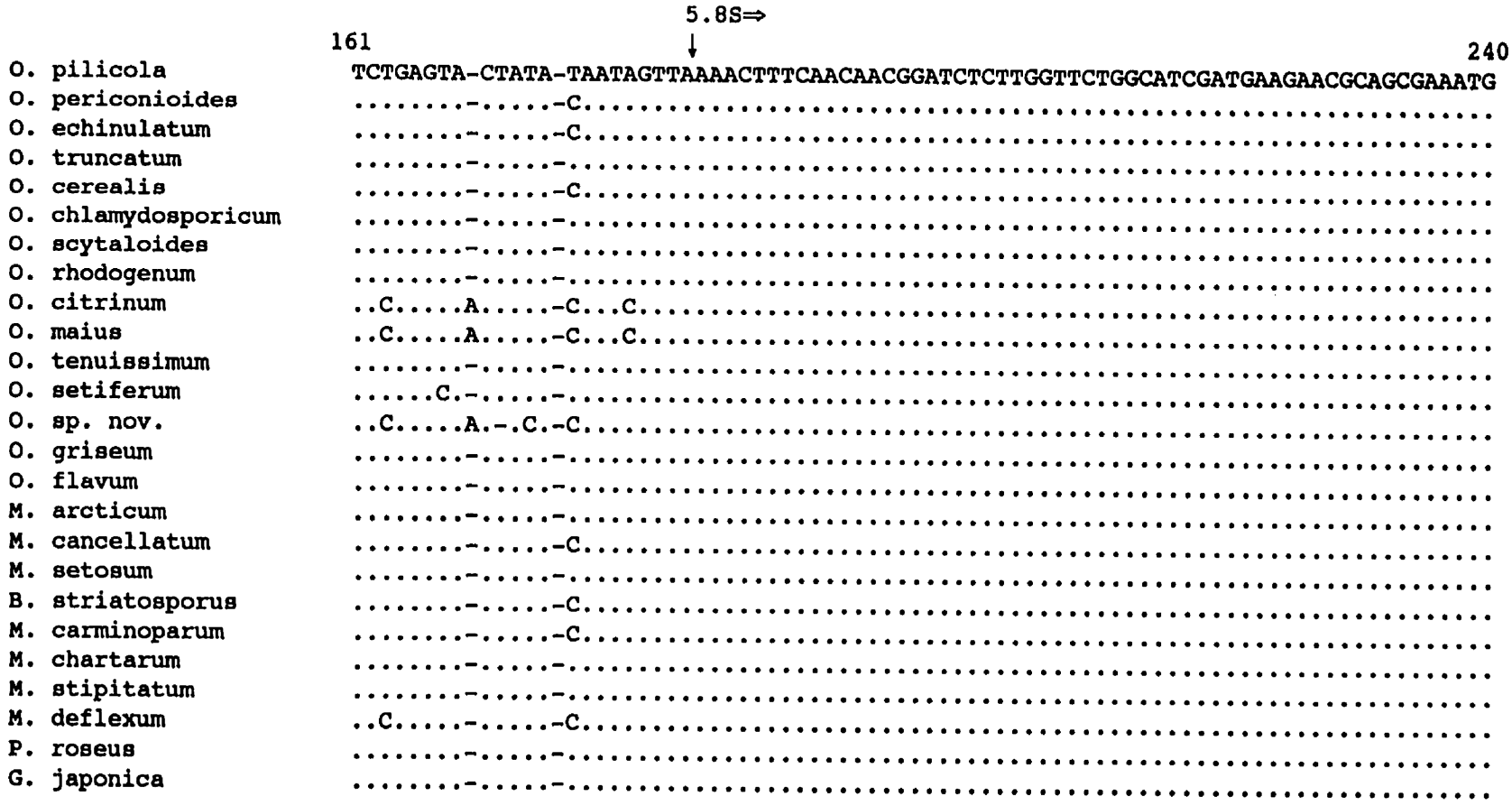
Appendix 6. Myxotrichaceae coded data matrix used to generate the inferred phylogenetic reconstruction shown in Figure 4.3: 15 *Oidiodendron* species, 10 *Myxotrichum* species, *Byssoascus striatosporus*, *Gymnostellatospora japonica* and *Pseudogymnoascus roseus*. Nucleotide positions are numbered from 1 to 547 as indicated above the data matrix and the location of ITS1, the 5.8S subunit, ITS2 and the 28S subunit are indicated. Dots indicate that the nucleotide in that position for that taxon matches the nucleotide noted for *O. pilicola*; "-" indicates a gap introduced for alignment purposes and counted as a fifth character; "?" denotes missing data either from missing bases in the sequences or introduced during the process of coding the data matrix to reduce ambiguities in the alignment.

```
                          ITS1⇒
                     1     ↓                                                                         80
O. pilicola          CATTACAGAGTTCTCGCCCTCGCGGGTAGATCTCCCACCCACTGTTATCGTTACTATCGTTGCTTTGGCGGGCCGCCGGG
O. periconioides     ........????......................................C...CG.....T..............T...
O. echinulatum       ....................AC..........................T.C....G........................
O. truncatum         .....................A........-..............G..T.....CG....................T...
O. cerealis          .............AT.....A...........................T......G........................
O. chlamydosporicum  ................................................T.C...CG.-...............T..T...
O. scytaloides       ????????????..............Y.....................T.C...CG.-...............T..T...
O. rhodogenum        .........S..........TA.................................G.....T..................
O. citrinum          ........???..AT......?-..................T..C...AAC....C....................T...
O. maius             .............AT......?-..........................AC....C....................T...
O. tenuissimum       ....................T...........................T.A....G.-......................
O. setiferum         .....................A..........................T.A....G.-......................
O. sp. nov.          ...................G.C.......................G..T.C...CG.-...T..................
O. griseum           .............................................G..T.C...CG.-..................T...
O. flavum            .............................................A..T.C...CG.-......................
M. arcticum          .........C...................................G..T.C...CG.-..................A...
M. cancellatum       ....................AC..........................T.C....G........................
M. setosum           ....................TA........-..............G..T.....CG....................T...
B. striatosporus     ......C............A.A..........................T.....CG...................TTA..
M. carminoparum      .............G....TCTA..................TG........C...CG-...................TA..
M. chartarum         .............G....T.TA..................TG........C...CG-...................TA..
M. stipitatum        ...........C.G..........................TG........C...CG-..................T....
M. deflexum          .............G......TAT.................TG..C......CC..G.-..................T...
P. roseus            ??????????????????????????????????????????????????????????...........AA...??-.CC
G. japonica          ??????????????????????????????????????????????????????????...........A....??-.CC
```

Appendix 6. Continued

	81 – 160
	TCC-??GCCCGGCCCCC-GGC-?CCC-??GGCTGGGGTGCGCCCGCCAGAGGCCCTAC-AAACTCTGAATGTCAGTGTCG
O. pilicola	TCC-??GCCCGGCCCCC-GGC-?CCC-??GGCTGGGGTGCGCCCGCCAGAGGCCCTAC-AAACTCTGAATGTCAGTGTCG
O. periconioides	C..-??........G..-...-?T..-??......C.C.............A...CC.-.............T.......
O. echinulatum	C..-??........G..-...-?...-??......C.C..........A......C??-.....................
O. truncatum	C..-??..T.A...A..-...-?...-??......T.C...............T.CG.-.......TG......AC....
O. cerealis	C..-??..T.....G..-...-?T..-??......C.C................T.??-.............T.......
O. chlamydosporicum	C.T-??........G..-...-?T..-??......C.C.?-..............G..-...T.....T...........
O. scytaloides	C.T-??........G..-...-?T..-??...C..C.C.................G..-...T.....T...........
O. rhodogenum	...-???.......G..-...??T.TAATA.GC..C.C................T.??-.............T.......
O. citrinum	C..-??........G..-...-?...-??......C.C.T............A..TC..-.G...........T.......
O. maius	C..-??........G..-...-?...-??......C.C.T............A..TC..-.G...........T.......
O. tenuissimum	C..-??..T.....G..-...-?T..-??......C.C...............T.CG.-................C....
O. setiferum	C..-??..T.....G..-...-?T..-??......C.C...............T.CG.-................C....
O. sp. nov.	C..-??........G..A...GA...-??C..C..C.C...............T.CC.-....C................
O. griseum	C..-??....C...G..-...-?...-??...C..C.C.T...........A....CG.-................C....
O. flavum	C..-??....-...G..-...-?...-??...C..C.C.T...........A....CG.-................C....
M. arcticum	C..-??....C...G..-...-?...-??...C..C.C.T...........A....CG.-................C....
M. cancellatum	C..-??........G..-...-?...-??......C.C..........A......-G.-.....................
M. setosum	CTT-??........A..-...-?...-??......T.C...............T.CG.-........G......AC....
B. striatosporus	C..TGT.....A..G..-...-?...-??...C..T.C....................G........C....T.......
M. carminoparum	CT.-??.G..A...A..-...-?T..-??...C..T.C.............A...CT?-?..TC....C...AC......
M. chartarum	CT.-??AG..T...A..-...-?T..-??...C..T.C.T............A...CC?-?T.TC....C.A.A.......
M. stipitatum	CT.-??A...C...G.T-...-?T..-??...C..C.C.............A...CC?-??.TC......A.AC..A...
M. deflexum	CT.-??A.......G..-...-?T..-??......C.C...............T...?-??.....TG....TT...AT.
P. roseus	CT.??????G...TG.T-...-?T..-??...C..C.A...TT.........A....?-??......TT....TA.ACT.
G. japonica	CT.??????G...TG.T-...-?T..-??...C..C.A.....T........A....?-??......TT....TA.AAT.

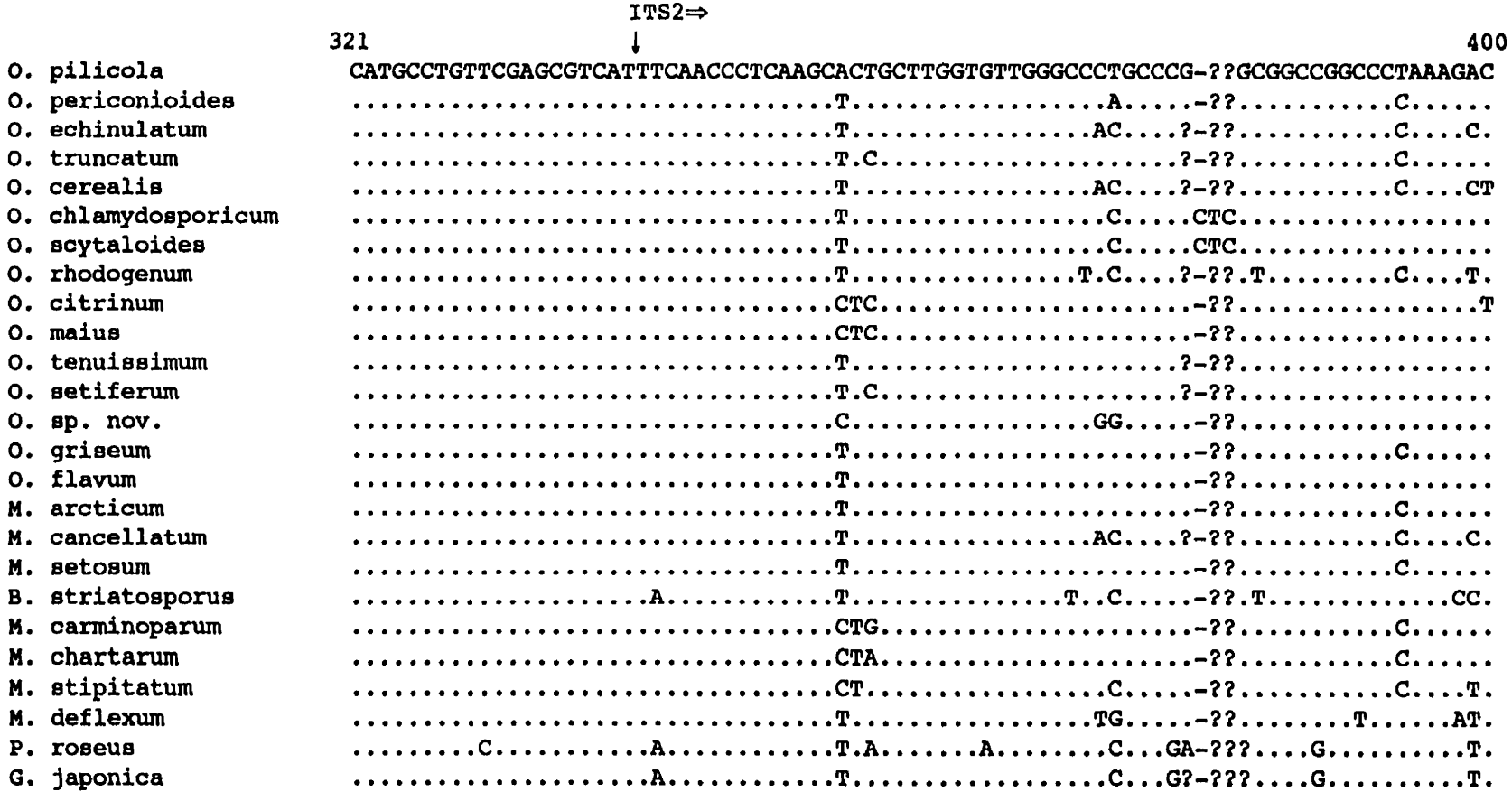
Appendix 6. Continued



Appendix 6. Continued

```
                    241                                                                                                320
O. pilicola         CGATAAGTAATGCGAATTGCAGAATTCAGTGAGTCATCGAATCTTTGAACGCACATTGCGCCCTGTGGTATTCCGCAGGG
O. periconioides    ................................................................................
O. echinulatum      ................................................................................
O. truncatum        ................................................................................
O. cerealis         ................................................................................
O. chlamydosporicum ................................................................................
O. scytaloides      ................................................................................
O. rhodogenum       ................................................................................
O. citrinum         ................................................................................
O. maius            ................................................................................
O. tenuissimum      ................................................................................
O. setiferum        ................................................................................
O. sp. nov.         ................................................................................
O. griseum          ................................................................................
O. flavum           ................................................................................
M. arcticum         ................................................................................
M. cancellatum      ................................................................................
M. setosum          ................................................................................
B. striatosporus    ..........................................................C.............G...
M. carminoparum     ................................................................................
M. chartarum        ................................................................................
M. stipitatum       ................................................................................
M. deflexum         ..............................................................C.................
P. roseus           ...........T.................A.............................CC.........GG...
G. japonica         ...........T.................A...........................T.CC.........GG.A.
```

Appendix 6. Continued



Appendix 6. Continued

```
                        401                                                                            480
O. pilicola             AGTGGCGGCGCCGTCTGGCTCTAAGCGTAGTACA-ACTCTCGCTCTGGAG-TC???GCGGT-?GCTTGCCAG-AACCC??
O. periconioides        ...............G...C..............-T..........A.G.-C.???.T...-?.........C.....??
O. echinulatum          ...............C..................-T..........A.G.-C.??G....C-?..C......-.....??
O. truncatum            .............CTC...C..............-T..........A.GA-C.??G.....-?..C......-.....??
O. cerealis             ........T.........................-T..........A.G.-C.??G.....-?.........-.....??
O. chlamydosporicum     .........AG..C.......C............-G............G.-CT??G.....-?..C......-.....??
O. scytaloides          .........AG..C.......C............-G............G.-CT??G.....-?..C.....?-.....??
O. rhodogenum           ..................................-T..........A.G.-..???.....-?.........-.....??
O. citrinum             .............C.....C..C...........-G.........CA.G.-..??G....C-?..C......-.....??
O. maius                .............C.....C..C...........-G.........CA.G.-..??G....C-?..C......-.....??
O. tenuissimum          ..................................-T..........A.C.-C.??G.....-?..C......-.....??
O. setiferum            ..................................-T..........A.C.-C.??G.....-?..C......-.....??
O. sp. nov.             .............CTC......C...........-C.C......GC..T?-C.CGG....C-?..CC.....-.....??
O. griseum              ........T....C.....C..G...........-T.........CA.T.-..??G.....-?..C......-.....??
O. flavum               ........T....C.....C..G...........-T.........CA.T.-C.??G.T...-?..C......-.....??
M. arcticum             ........T....C.....C..G...........-T.........CA.T.-..??G.....-?A.C......-.....??
M. cancellatum          .............C.C..................-T..........A.G.-C.??G....C-?..C......-.....??
M. setosum              ..................................-...............-C.??G.....-?C.C......-.....??
B. striatosporus        ........T..........C.............T-T.........CA.G.-..CCG.....-?..C......-.....??
M. carminoparum         .........A........TC............AT-G..........A.G.-..??G....?-?..AC.....C.....??
M. chartarum            .........A........TC............AT-G..........A.G.-..??G....?-?..A......C.....??
M. stipitatum           .........A....AC..TC............AT-..........CA.G.-C.???A...C-?..GC.....-.....??
M. deflexum             ........T....C..................AT-G..........A.G.-..??G.T...-?...G.....-.....??
P. roseus               ........T......C.....CG......R..ATTCT.............G..??G.TC..GT...C.....C.....??
G. japonica             ........T......C.....CG.........ATTTT.......T.....GC.CGG.-C.CGT...C.....TC....??
```

Appendix 6. Continued

```
                                 28S⇒
                      481          ↓                                                      547
O. pilicola           ??A-TCTT?-ATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCG
O. periconioides      ??.C..C.?-.........................................................
O. echinulatum        ??.C....?-.........................................................
O. truncatum          ??.C..A.?-G........................................................
O. cerealis           ??.C....?-.........................................................
O. chlamydosporicum   ???C.TC.?-.........................................................
O. scytaloides        ???C.TC.?-.........................................................
O. rhodogenum         ??.C.TC.?-.........................................................
O. citrinum           ??.C...??-G........................................................
O. maius              ??.C...??-G.......................................................?
O. tenuissimum        ??.C...??-G........................................................
O. setiferum          ??.C...??-G........................................................
O. sp. nov.           ??.C...??-G........................................................
O. griseum            ??.C...C?-G........................................................
O. flavum             ??.C...C?-G........................................................
M. arcticum           ??.C...C?-G........................................................
M. cancellatum        ??.C....?-.........................................................
M. setosum            ??.C.TC.?-.........................................................
B. striatosporus      ??.C....?-.....................................................????
M. carminoparum       ??.C..C.C-?........................................................
M. chartarum          ??.C.TC.?-.........................................................
M. stipitatum         ??.C..C.?-.........................................................
M. deflexum           ??.C.TC.?-.........................................................
P. roseus             ??.T.T..TTCA......................................................?
G. japonica           ??.T.T..T?CA.......M...................................???????????
```

Appendix 7. Small subunit rDNA data matrix used to generate the inferred phylogenetic reconstruction shown in Figure 5.1. Nucleotide positions are numbered from 1 to 1683 as indicated above the data matrix. Dots indicate that the nucleotide in that position for that taxon matches the nucleotide noted for *Auxarthron*. "?" denotes missing data either from missing bases in the sequences or introduced for alignment purposes. Fungi that were newly sequenced for this study are indicated by an "*" adjacent to the species name. Genbank accession numbers for the sequences of the other fungi analysed are given in Figure 5.2.

```
                          1                                                  100
Auxarthron zuffianum      GAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATTTGATAGTACCTT??GCTACATGG?ATACCTGTGGTAATTCTAGAGCTAATACATGCTAAAA
Chaetomium elatum         ..........................................................??A........?...A.C.....................................
Ctenomyces serratus       ..........................................................??A........?.....................................
Cudonia confusa           ..................................................T..??A........?...A.C.....................................
Eupenicillium javanicum   ..........................................................??A........?.....................................
Gymnoascoideus petalosporus ......................................................??A........?.....C...............................
Gyromitra esculenta       ..................................................C??AT...T...?...A.C.....................................
Leotia lubrica            ??????????????????????????...?.?.....................??A....T...?...A.C.....................................
Leptosphaeria doliolum    ..........................................................??A....T...?...A.C.....................G...
Morchella elata           ..........................................................??A....T...?...A.C.....................................
Onygena equina            ..................................................TTT.C......?.....C.....................................
Peziza badia              ????????????????.....................................??A....T...?...A.C.....................................
Plectania nigrella        ..........................................................??A....T...?...A.C.....................................
Pleospora herbarum        ..............................................A.......??A....T...?...A.C.....................G...
Renispora flavissima      ..........................................................??A........?.....C...............................
Sclerotinia sclerotiorum  ????????????T.....................................??A........?...A.C.....................................
Sordaria fimicola         ..........................................................??A........?...A.C.....................................
Spathularia flavida       ..................................................T..??A........?...A.C.....................................
Talaromyces flavum        ..................................................C.??A........?.....................................GC...
Trichophyton rubrum       ..........................................................??A........?.....................................
Tuber cf rapaeodorum      ..........................................................T?.....T...?...A.C.....................................
Uncinocarpus reesii       ..........................................................??A........?.....C...............................
* Hymenoscyphus ericae    ..........................................................??A....T...?...A.C.....................................
* Oidiodendron maius      ..........................................................??A....T...?...A.C.....................................
* Myxotrichum arcticum    ..........................................................??A....T...?...A.C.....................................
* Phialocephala fortinii  ..........................................................??A....T...?...A.C.....................................
* Variable White Taxon    ..........................................................??A....T...?...A.C.....................................
* Uncinocarpus uncinocarpus ........................................................??A........?.....C...............................
* Gymnoascus reessii      ..........................................................??A........?.....C...............................
Saccharomyces cerevisiae  ..................................................T....T?A........T...A.C.....................T...
```

Appendix 7. Continued

	101 200
Auxarthron zuffianum	ACCTCGACTTC??GGAAGGGGTGTATTTATTAGATAAAAAACCAATGCCCTTCGGGTCTCTTTGGTGATTCATAATAACTTGTCGAATCGCATGGCCTTG
Chaetomium elatum	.T.C.......??.......A..............T....................G....C...................C..........C.......
Ctenomyces serratus	...........??...G....C...................C..................
Cudonia confusa	...C.......???.G.A.....................................??...CC...................AA.................
Eupenicillium javanicum	...C.......A?................................C..........G...C.......A............AA.................
Gymnoascoideus petalosporus	...........??...G....C......A............C..................
Gyromitra esculenta	.T.C....CC.?T.......A...........................?.......CTCAC............G.......AA.................
Leotia lubrica	...C.......??...G...C...................AAA.................
Leptosphaeria doliolum	...C.A.....??..G.............................C..........G..TC............G.......TA..G..............
Morchella elata	...C.......AC?.G................................?....?..G.CTCC...........G.......AA.................
Onygena equina	...........??...G...C....................T..................
Peziza badia	...C.......AC...................................?....?..G.CTCC...........G.......AA.................
Plectania nigrella	.T.C....CC.?T.......A...........C...............?....?..G...C............G.......AA.................
Pleospora herbarum	.T.C.......??.......A...G...............................G..T.............G.......TA..G.......A......
Renispora flavissima	...........??...G..TCC......................................
Sclerotinia sclerotiorum	...........???.G..G...CC...................AA.................
Sordaria fimicola	...C.......??......................T....................?..TCC...........G.......C..........C.......
Spathularia flavida	...C......T??..................................?........G...CC...................AA.................
Talaromyces flavum	...C.......??...G...C.......A............CA.................
Trichophyton rubrum	...........??......................................C....G....C...................C..................
Tuber cf rapaeodorum	.T.C....CC.?T.......A..........................G?....?..C...CC...........G.......AA.................
Uncinocarpus reesii	...........??...G...C.......................................
* Hymenoscyphus ericae	...C.......??...G...C....................AA.................
* Oidiodendron maius	...C.......??...................A.......................G...C....................AA.A..............?
* Myxotrichum arcticum	G..........??........C..................................G....C...................AA.................
* Phialocephala fortinii	...........??...G...C....................AA.................
* Variable White Taxon	GG.C.......??.......TC..................................G...C....................AA.................
* Uncinocarpus uncinocarpus	...........??...G...C....................C..................
* Gymnoascus reessii	...........??...G...C....................C..................
Saccharomyces cerevisiae	.T......CCTTT.....A.A....................T.....?T....?..A.......A................T..................

Appendix 7. Continued

Taxon	201 ... 300
Auxarthron zuffianum	CGCCGGCGATGGTTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACCATGGTGGCAACGGGTAACGGGGAATTAGGGTTCGAT
Chaetomium elatum	....C..CT..G.CT.....AG..G.....A.............A.GG......C....C
Ctenomyces serratus	....
Cudonia confusa	...?..T..TT..........................T..
Eupenicillium javanicum	....
Gymnoascoideus petalosporus	....
Gyromitra esculenta	T..TT..........................T..
Leotia lubrica	....TT..........................T..
Leptosphaeria doliolum	...T.....C.......................................A.G..T....T.........TT.............................
Morchella elata	T..TT..........................T..
Onygena equina	....
Peziza badia	T..TT..........................T..
Plectania nigrella	T..TT..........................T..
Pleospora herbarum	...T.....C.......................................A.G..T....T.........TT.............................
Renispora flavissima	....
Sclerotinia sclerotiorum	T..G...TT..........................T..
Sordaria fimicola	....C..CT..G.CT.....AG........A.............A.GG......C....C
Spathularia flavida	....TT..........................T..
Talaromyces flavum	....
Trichophyton rubrum	....
Tuber cf rapaeodorum	....TT..........................T..
Uncinocarpus reesii	T..T..
* Hymenoscyphus ericae	T..TT..........................T..
* Oidiodendron maius	T......A..................................R..T...A.G.CT....T...A.....TT..........................T..
* Myxotrichum arcticum	T..T...A.G.CT....T...A.....TT..........................T..
* Phialocephala fortinii	T..T....AG......T....A.....TT..........................T..
* Variable White Taxon	T..TT..........................T..
* Uncinocarpus uncinocarpus	....
* Gymnoascus reessii	....
Saccharomyces cerevisiae	T..T...TT..................A..........

Appendix 7. Continued

```
                              301                                                                                                400
Auxarthron zuffianum          TCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACT
Chaetomium elatum             C.......A....................T.....C................................................................
Ctenomyces serratus           ...................................C.....................................T..........................
Cudonia confusa               ...................................C..................................?................T...........A
Eupenicillium javanicum       ...................................C.....................................T..........................
Gymnoascoideus petalosporus   ...................................C.....................................T..........................
Gyromitra esculenta           ...................................C..................................?.............................
Leotia lubrica                ...................................C................................................................
Leptosphaeria doliolum        ...................................C................................................................
Morchella elata               ...................................C................................................................
Onygena equina                ...................................C.....................................TC.........................
Peziza badia                  ...................................C........................................?.......................
Plectania nigrella            ...................................C................................................................
Pleospora herbarum            ...................................C................................................................
Renispora flavissima          ...................................C...................................A............................
Sclerotinia sclerotiorum      ........A....................T.....C................................................................
Sordaria fimicola             C.......A....................T.....C................................................................
Spathularia flavida           ...................................C................................................................
Talaromyces flavum            ...................................C.....................................T..........................
Trichophyton rubrum           ...................................C.....................................T..........................
Tuber cf rapaeodorum          ...................................C................................................................
Uncinocarpus reesii           ...................................C...................................A............................
* Hymenoscyphus ericae        ...................................C................................................................
* Oidiodendron maius          .......R...........................C................................................................
* Myxotrichum arcticum        ................................TA.C................................................................
* Phialocephala fortinii      ...................................C................................................................
* Variable White Taxon        ...................................C................................................................
* Uncinocarpus uncinocarpus   ...................................C..r......................M.......Y...............T..............
* Gymnoascus reessii          ...................................C.....................................T..........................
Saccharomyces cerevisiae      ...................................C..................................TA.TT.A.....................AC
```

Appendix 7. Continued

```
                                401                                                                                                 500
Auxarthron zuffianum            GATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGAACAATTTAAATCCCTTAACGAGGAA?CAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGT
Chaetomium elatum               ..............C.....................T.....................?.....................................
Ctenomyces serratus             ..............C.....................T.........C...........?.....................................
Cudonia confusa                 .....................................T.....................?.....................S.........
Eupenicillium javanicum         .....G..............C.....................................?.....................................
Gymnoascoideus petalosporus     ..........C....C.....................................?.....................................
Gyromitra esculenta             .....................................T.....??????????????????..?.....................................
Leotia lubrica                  ....TT...G.....A..C...AA.............T.....................?.....................................
Leptosphaeria doliolum          .....................................T.........C.T...........?.....................................
Morchella elata                 .....................................T.....................?.....................................
Onygena equina                  ..............C.............C........T.....................?.....................................
Peziza badia                    A........G.T..A..CC..................T...........T...........?.....................................
Plectania nigrella              ..........CT..C?....................T....A..................?.....................................
Pleospora herbarum              .....................................T.........C.T...........?.....................................
Renispora flavissima            ..............C.....................................A.....?.....................................
Sclerotinia sclerotiorum        ................A....................T...........A.....?.....................................
Sordaria fimicola               .....................................T.....................T....?.....................................
Spathularia flavida             .....................................T.....................?.....................................
Talaromyces flavum              ..........................................C..................?.....................................
Trichophyton rubrum             ..............A.....................T.........C.............?.....................................
Tuber cf rapaeodorum            .....................................T.....................?.....................................
Uncinocarpus reesii             A.............C.....................T.....................?.....................................
* Hymenoscyphus ericae          .....................................T.....................?.....................................
* Oidiodendron maius            .....................................T.....................?.....................................
* Myxotrichum arcticum          .....................................T.....................?.....................................
* Phialocephala fortinii        .....................................T.....................?.....................................
* Variable White Taxon          .....................................T.....................?.....................................
* Uncinocarpus uncinocarpus     ..............C.....................T.....................?.....................................
* Gymnoascus reessii            ..............C.....................T.....................A.....................................
Saccharomyces cerevisiae        ..........C.A..C.....................T.....G.....A.....????.....................................
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Appendix 7. Continued

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                                 501                                                                                                  600
Auxarthron zuffianum             AATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGGCTGGCCGGTCCGCTTCGCCGCGTGCACTGG
Chaetomium elatum                .....................................AG.....................................C..A..C..........C..A.............
Ctenomyces serratus              ..........................................................................C.................C..A.G...........
Cudonia confusa                  ..........................................................................C....T....?.......C..A.............
Eupenicillium javanicum          ..................................................................................................C..A.....A.T.....
Gymnoascoideus petalosporus      ..................................................................................................C..A.G...........
Gyromitra esculenta              ..................................................................................................C..A.............
Leotia lubrica                   ..........................................................C...C.................C..A.............
Leptosphaeria doliolum           ..................................................A.................G.......C..A.......T...T.
Morchella elata                  ..........................................................................C........T......T.C..A...A........A
Onygena equina                   ..................................................................................................G...........
Peziza badia                     ..................................................................AC...C.CAA......TA.C..A...TA........
Plectania nigrella               ..........................................................................C.........?.....A.C..A...T.A.A.....
Pleospora herbarum               ..................................................A......C.........G.......C..A...........C.
Renispora flavissima             ..................................................................................................G...........
Sclerotinia sclerotiorum         ..........................................................................C....T...........C..A.............
Sordaria fimicola                .....................................AG.....................................C.CA..C...........C..A.............
Spathularia flavida              ..........................................................................C....T...........C..A.............
Talaromyces flavum               ..........................................................................C.C.T.CT.........C..A.....A.T.....
Trichophyton rubrum              ..........................................................................C.................TC..A.G...........
Tuber cf rapaeodorum             ..................................................................................................C..A.............
Uncinocarpus reesii              ..................................................................................................A.G...........
* Hymenoscyphus ericae           ..........................................................................C.................C..A.............
* Oidiodendron maius             ..........................................................................C.................C..A.............
* Myxotrichum arcticum           ..........................................................................C.................C..A.............
* Phialocephala fortinii         ..........................................................................C.................C..A.............
* Variable White Taxon           ..........................................................................C.................C..A.............
* Uncinocarpus uncinocarpus      ..........................................................................C.................C..A.G...........
* Gymnoascus reessii             ..........................................................................C.................CG.AAG...........
Saccharomyces cerevisiae         ..................................................T.....C.C..T..........A..TTTTCGTGTACTG.A
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Appendix 7. Continued

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                              601                                                                                              700
Auxarthron zuffianum          ??TCCGGCTGGCCTTTCCTTCTGGGGATTCC?CATGGCCTTCACTGGCTGTGG?GGGGAACCAGGACTTTTACTGTGAAAAAATTAGAGTGTTCAAAGC
Chaetomium elatum             ??CT........T............A..AC.T?....C..........G...AC.....................C...C........TC.C.T....A
Ctenomyces serratus           ??..........................ACGT?....................C?............................................
Cudonia confusa               ??....A.C..............A....GC.?G....C......T...G...TT.......T.............T.......................
Eupenicillium javanicum       ??.........A................AC.T?.....................?............................................
Gymnoascoideus petalosporus   ??.........AT...............GC..?.................C...?............................................
Gyromitra esculenta           ??......C..?T............CT.GC.?G....C....GT....G...TC.....................T....................???
Leotia lubrica                ??......C...................CC.?G....CA....G....TG..CT?...G................T?......................
Leptosphaeria doliolum        ??......C.......?........A..AC.T?....C....T.....GC..TT?...G................T.......................
Morchella elata               ??......C..A.............CT.GC.?G....C..........G...TC.....................T.......................
Onygena equina                ??.........A................AC..?T..................A.?............................................
Peziza badia                  ??.TT..TC...T............CA.ACT?G....C..........G...TTT....................T.......................
Plectania nigrella            ??......C...T............CT.AC.T?....C..........G...TT.....................T.......................
Pleospora herbarum            ??......C.......?.......AA..AC.T?....C..........GC..TT.......T.............T.......................
Renispora flavissima          ??......C..A................A...?.................C...?............................................
Sclerotinia sclerotiorum      ??....A.C...T...............GC.?G....C..........G...TC.....................T.......................
Sordaria fimicola             ??CT...T.................A..AC.?G....C..........G...TC.....................C...C........TC.C.T....A
Spathularia flavida           ??....A.C..............A....GC.?G....C......T...G...TC.......T.............T.......................
Talaromyces flavum            ??.....A..........T.........A...?....................C.............................................
Trichophyton rubrum           ??..................CT......AC..?................C...GC?.........G.................................
Tuber cf rapaeodorum          ??......C..AT............CT.GC.?G....C..........G...TC.....................T.......................
Uncinocarpus reesii           ??.........A................GC..?T..................A.?............................................
* Hymenoscyphus ericae        ??......C................A..GC.?G....C..........G...TC.....................T.......................
* Oidiodendron maius          ??......C...........C.......GC.?G....C..........G...TC.....................T.......................
* Myxotrichum arcticum        ??......C...........C.......GC.?G....C..........G...CC.....................T.......................
* Phialocephala fortinii      ??......C...T............A..GC.?G....C..........G...TC.......T.............T.......................
* Variable White Taxon        ??......C...................GC.?G....C..........G...TC.....................T.......................
* Uncinocarpus uncinocarpus   ??......C...T...............GC..?.........T...........?............................................
* Gymnoascus reessii          ??..........T...............GC.T?.....................?............................................
Saccharomyces cerevisiae      TT...AA.G................CT.AC.TTG.G?T....?G?.....C.??T..C.................T.......................
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Appendix 7. Continued

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                              701                                                                                                800
Auxarthron zuffianum          AGGCCT?TTGCTCGGATACATTAGCATGGAATAATAGAATAGGACGTGTGGTTCTATTTTGTTGGTTTCTAGGACCGCCGTAATGATTAATAGGGATAGT
Chaetomium elatum             ......A?......A.................................................................................C...
Ctenomyces serratus           ......?.............................A...............................................................
Cudonia confusa               ......A?......A.....................................................................................
Eupenicillium javanicum       ......?.......A.................................C...................................................
Gymnoascoideus petalosporus   ......?.........................................C................................................G..
Gyromitra esculenta           ?????????????????...............................C...................................................
Leotia lubrica                ......A?......A.....................................................................................
Leptosphaeria doliolum        ......?.......A....G............................C...C...........................................C...
Morchella elata               ....A.?.......A.................................C...................................................
Onygena equina                ......?.....................................T.......................................................
Peziza badia                  ....A.?.A.....A.............................................................A.......................
Plectania nigrella            ....A.?.......A.................................C...................................................
Pleospora herbarum            ......?.......A....G................A......G....C.T.....................AGA.....C.........C...A.C...
Renispora flavissima          ......?.........................................C...................................................
Sclerotinia sclerotiorum      ......A?......A.....................................................................................
Sordaria fimicola             ......A?......A.................................................................................C...
Spathularia flavida           ......A?......A.....................................................................................
Talaromyces flavum            ......?.........................................C...................................................
Trichophyton rubrum           ......?.............................................................................................
Tuber cf rapaeodorum          ....A.A?......A.................................C...................................................
Uncinocarpus reesii           ......?.............................................................................................
* Hymenoscyphus ericae        ......A?......A.....................................................................................
* Oidiodendron maius          ......A?......A.....................................................................................
* Myxotrichum arcticum        ......A?......A.....................................................................................
* Phialocephala fortinii      ......A?......A.............................................................................?.......
* Variable White Taxon        ......A?......A.....................................................................................
* Uncinocarpus uncinocarpus   ......?.........................................C................................................G..
* Gymnoascus reessii          ......?..........................................................................................G..
Saccharomyces cerevisiae      ....G.A.......A...T............................T............................AT..................CG..
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Appendix 7. Continued

	801–900
Auxarthron zuffianum	CGGGGGCGTCAGTATTCGGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGTG
Chaetomium elatum	.......A.........AAT.........................AT..........................T........................?.
Ctenomyces serratus	....
Cudonia confusa	......T.........GC.T.........................A.GC.........................A.........?...............
Eupenicillium javanicum	.................A..G.
Gymnoascoideus petalosporus	....C..G.
Gyromitra esculenta	.......A.........AAT.........................AT..........................T..........................
Leotia lubrica	.......A.........AAT.........................AT..........................T..........................
Leptosphaeria doliolum	.......A.........AAT.........................AT..........................T..........................
Morchella elata	.......A.........AAT.........................AT..........................T..........................
Onygena equina	....
Peziza badia	.......A.........AATC......................CGAT..........................T........................G.
Plectania nigrella	.......A..C......AAT.........................AT.......G..................T........................?.
Pleospora herbarum	.......A.........A.T.........................A...........................T..........................
Renispora flavissima	....A..
Sclerotinia sclerotiorum	.......A.........AAT.........................AT..........................T..........................
Sordaria fimicola	.......A.........AAT.........................AT..........................T........................?.
Spathularia flavida	......T.........GC.T.........................A.GC..................???....A.........................
Talaromyces flavum	.................A..G.
Trichophyton rubrum	....C..
Tuber cf rapaeodorum	.......A.........AAT.........................AT..........................T..........................
Uncinocarpus reesii	....
* Hymenoscyphus ericae	.......A.........AAT.........................AT..........................T..........................
* Oidiodendron maius	.......A.........AAT.........................AT..........................T..........................
* Myxotrichum arcticum	.......A.........AAT.........................AT..........................T..........................
* Phialocephala fortinii	.......A.........AAT.........................AT..........................T..........................
* Variable White Taxon	.......A.........AAT.........................AT..........................T..........................
* Uncinocarpus uncinocarpus	....C..
* Gymnoascus reessii	...........................A...................C....?...............................?.............G.
Saccharomyces cerevisiae	.......A..G......AAT...?.....................AT..........................T........C..............A?.

Appendix 7. Continued

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                                901                                                                                               1000
Auxarthron zuffianum            AACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCC?GACTAGGGATCGGACGGGCAACTTTTAATAACCCGTT
Chaetomium elatum               ..............................................................?..T..............CGTTA....T?.G.......
Ctenomyces serratus             ..............................................................?..................GTTTC...T..G.......
Cudonia confusa                 ..............................................................?...........A.G..ATGTTA.C..TT.G..T..C.
Eupenicillium javanicum         ..............................................................?..................A?TTC.A.G..G.......
Gymnoascoideus petalosporus     ..............................................................?.................TGTTTC.A....G.......
Gyromitra esculenta             ..............................................................?.............GG.ATGCTTAC.AG..GG.T.CC.
Leotia lubrica                  ..............................................................?.............G..ATGTTA.C..TT.G..T..C.
Leptosphaeria doliolum          ..................................................G...........?.............G..ATGTT.....TC.G..T..C.
Morchella elata                 ..............................................................?.............TG.ATGCTTAC.AG..GG.T.CA.
Onygena equina                  ..............................................................?.........................G...........
Peziza badia                    ..............................................................?..........T..G..ATGTT.....T?.G..T..C.
Plectania nigrella              ..........GA.................................C................?.............G..ATGT......CG.G..T..C.
Pleospora herbarum              ..................................................G...........?.............G..ATGTT.....TC.G..T..C.
Renispora flavissima            ..............................................................?........................A............
Sclerotinia sclerotiorum        ..............................................................?.............G..ATGTTA.C..TT.G..T..C.
Sordaria fimicola               ..............................................................?..T.............ATGTTA....T?.G..T....
Spathularia flavida             ..............................................................?...........A.G..ATGTTA.C..TT.G..T..C.
Talaromyces flavum              ..............................................................?.............G....GTTTC.A.G..G.....C.
Trichophyton rubrum             ..............................................................?..................GTTTC...T..G.......
Tuber cf rapaeodorum            ..............................................................?.............G..ATGTT.....TC.G..T..C.
Uncinocarpus reesii             ..............................................................?.........................G...........
* Hymenoscyphus ericae          ..............................................................?.............G?.ATGTTA.C..TT.G..T..C.
* Oidiodendron maius            ..............................................................?.............GG.ATGTTA.?..TT.G..T?.C.
* Myxotrichum arcticum          ..............................................................?.............G..ATGTTA.C..TT.G..T..C.
* Phialocephala fortinii        ..............................................................?.............G..ATGTTA.C..TT.G..T..C.
* Variable White Taxon          ..............................................................?.............G..ATGTTA.C..TT.G..T..C.
* Uncinocarpus uncinocarpus     ..............................................................?.................TGTTTA.A.G..G.......
* Gymnoascus reessii            ........?.....................................................C.............G...TG?TTA.A.G..G.....C.
Saccharomyces cerevisiae        .......................T....T.G...............................?.....??......GT..TGTTT.......G....AC.
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Appendix 7. Continued

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                                1001                                                                                                1100
Auxarthron zuffianum            CGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGT?GG
Chaetomium elatum               .............T........A.G......C..CT.....................................................G..?..
Ctenomyces serratus             ..........................................................................................?..
Cudonia confusa                 T.......................C.....................................................................A..?..
Eupenicillium javanicum         ..........................................................................................?..
Gymnoascoideus petalosporus     ..........................................................................................?..
Gyromitra esculenta             ........................C.....................................G.....................A..?..
Leotia lubrica                  .........G...............C.....................................................................A..?..
Leptosphaeria doliolum          ..........................................................................................?..
Morchella elata                 ........................C.....................................G.....................A..?..
Onygena equina                  ..........................................................................................?..
Peziza badia                    .A......................C.....................................G.....................A..?..
Plectania nigrella              ........................C.....................................G.....................A..?..
Pleospora herbarum              ...........................................T.....................................T.....?..
Renispora flavissima            .........G................................................................................?..
Sclerotinia sclerotiorum        ........C...............C.....................................................A.....?..
Sordaria fimicola               .............T........A.G......C..CT.....................................................G..?..
Spathularia flavida             T.......................C.....................................................................A..?..
Talaromyces flavum              ..........................................................................................?..
Trichophyton rubrum             ..........................................................................................?..
Tuber cf rapaeodorum            ........................C.....................................G.....................A..?..
Uncinocarpus reesii             ..........................................................................................?..
* Hymenoscyphus ericae          ........................?........?.....................................................A..?..
* Oidiodendron maius            ..........?.......?.....?........?.........?.....?............?.....................A..?..
* Myxotrichum arcticum          ........................C.....................................................................A..?..
* Phialocephala fortinii        ........................C.....................................................................A..?CA
* Variable White Taxon          ........................C.....................................................................A..?..
* Uncinocarpus uncinocarpus     ..........................................................................................?..
* Gymnoascus reessii            ..............................C...........?.....................................................T..
Saccharomyces cerevisiae        ...T.....................C.....................................G.....................T...A..?..
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Appendix 7. Continued

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                                1101                                                                                                1200
Auxarthron zuffianum            AGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTG
Chaetomium elatum               ....................................................CG..G.....................................T.CG...G.....
Ctenomyces serratus             ....................................................................................................
Cudonia confusa                 .??G................................................C.....................??.............T..G..?G.....
Eupenicillium javanicum         ....................................................................................................
Gymnoascoideus petalosporus     ....................................................................................................
Gyromitra esculenta             ....................................................C.T.....................................A.G...G.....
Leotia lubrica                  ....................................................C..A.....................................G...G.....
Leptosphaeria doliolum          ..................................................TG.....................................T...CA.G.....
Morchella elata                 ....................................................C.T.....................................A.G...G.....
Onygena equina                  T...................................................................................................
Peziza badia                    ...TGCG.?...........................................C.T.....................................TA.G...G.....
Plectania nigrella              ....................................................C.T.....................................A.G...G.....
Pleospora herbarum              ..................................................TG.....................................T...CA.G.....
Renispora flavissima            ....................................................................................................
Sclerotinia sclerotiorum        .??.................................................C.....................................T..G...G.....
Sordaria fimicola               ....................................................CG..G.....................................T.CG...G.....
Spathularia flavida             .???................................................C.....................................T..G...G.....
Talaromyces flavum              ....................................................................................................
Trichophyton rubrum             ....................................................................................................
Tuber cf rapaeodorum            ....................................................C.T.....................................A.G...G.....
Uncinocarpus reesii             ....................................................................................................
* Hymenoscyphus ericae          ....................................................C.....................................T..G...G.....
* Oidiodendron maius            ....................?..?...............................C.....................................T..G...G.....
* Myxotrichum arcticum          ....................................................C.....................................T..G...G.....
* Phialocephala fortinii        ..AG.T.T............................................C.....................................T..G...G.....
* Variable White Taxon          ....................................................C.....................................T..G...G.....
* Uncinocarpus uncinocarpus     ....................................................................................................
* Gymnoascus reessii            ....................................................................................................
Saccharomyces cerevisiae        ...........?........................................C.....................................T..G...G.....
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Appendix 7. Continued

	1201 1300
Auxarthron zuffianum	GTGCATGGCCGTT?CTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTTAACCTGCTAAATAGCCCGGTCCACGTTT?GTGGG
Chaetomium elatum	.............?..TATTG.?...G.CA.T
Ctenomyces serratus	.............?...GG.?...G.CC..
Cudonia confusa	.............?..A..CTAG.?...G.CT..
Eupenicillium javanicum	.............?...CGG..C?T...............G.A...?.C?..
Gymnoascoideus petalosporus	.............?........................A........T....................................AC..G...C.?.C...
Gyromitra esculenta	.............?...C.?G.?..CT.C...
Leotia lubrica	.............?..A..CTAG.?...G.CT..
Leptosphaeria doliolum	.............?..A..CTAG.?...G.CT..
Morchella elata	.............?...CG.G.?...T.C.C.
Onygena equina	.............?..AC........?.....
Peziza badia	.............?..T.A..C.AG.?..CG.CT..
Plectania nigrella	.............?...C..G.?...T.C.A.
Pleospora herbarum	.............?.......C....G....C........................G..........CT.............A..CTAG.?...G.CT..
Renispora flavissima	.............?...C.G......?..C..
Sclerotinia sclerotiorum	.............?...CTAG.?...G.CT..
Sordaria fimicola	.............?..TATTG.?...G.CA.T
Spathularia flavida	.............?..A..CTAG.?...G.CT..
Talaromyces flavum	.............?...CGG..C?T...............G.....?.C?..
Trichophyton rubrum	.............?...GG...CC?.CC..
Tuber cf rapaeodorum	.............?...C..G.A.C.C.C...
Uncinocarpus reesii	.............?..AC........?.....
* Hymenoscyphus ericae	.............?..A..CTAG.?...G.CT..
* Oidiodendron maius	.............?..A..CTAG.?...G.CT..
* Myxotrichum arcticum	.............?..A..CTAG.?...G.CT..
* Phialocephala fortinii	.............?..A..CTAG.?...G.CT..
* Variable White Taxon	.............?..A..CTAG.?...G.CT..
* Uncinocarpus uncinocarpus	.............?...C..G.....?.C...
* Gymnoascus reessii	.............?..AC..G.....?.C...
Saccharomyces cerevisiae	.............T..C..A........TGGT.CTAG.A...?.CT..

Appendix 7. Continued

	1301 1400
Auxarthron zuffianum	CCGCTGGCTTCTTAGAGGA?CTATCGGCT??CAAGCCCGATGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGA?TGTTCTGGGCCGCACGCGC
Chaetomium elatum	A................GA..........??......?..?...................
Ctenomyces serratus	.................GA.......A..??....T.?..?...................
Cudonia confusa	T..C.............GA...???...??....?????......................???????????????????????????????????????
Eupenicillium javanicum	..............G..GA..........??......?........GC.C..............................?...................
Gymnoascoideus petalosporus	.................GA........T.??......?..?...................
Gyromitra esculenta	T................GA..........??......?..?...................
Leotia lubrica	T..C.............GA..........??......?..?...................
Leptosphaeria doliolum	T..TC............GA..........??......?..?...................
Morchella elata	T................GA..........??......?..?...................
Onygena equina	.................GA..........??......?..?...................
Peziza badia	TT..A............GA..........T?......?..?...................
Plectania nigrella	T................GA........A.TT....A.?..?...................
Pleospora herbarum	T..C............AGA......AA..??....TT?..?...................
Renispora flavissima	.................GA..........T?......?..?...................
Sclerotinia sclerotiorum	T................GA..........??......?..?...................
Sordaria fimicola	A................GA..........??......?..?...................
Spathularia flavida	T..C.............GA..........??......?..?...................
Talaromyces flavum	..............G..GA..........??......?........AC.T..............................?...................
Trichophyton rubrum	.................GA.......A..??....T.?..?...................
Tuber cf rapaeodorum	..TT.............GA..........??......?..?...................
Uncinocarpus reesii	.................GA..........??......?..?...................
* Hymenoscyphus ericae	T..C.............GA..........??......?..?...................
* Oidiodendron maius	T..C.............GA..........??......?..?...................
* Myxotrichum arcticum	T..C.............GA..........??......?..?...................
* Phialocephala fortinii	T..C.............GA..........??......?..?...................
* Variable White Taxon	T..C.............GA..........??......?..?...................
* Uncinocarpus uncinocarpus	.................GA..........??......?..?...................
* Gymnoascus reessii	.................G?..........??......?..?...................
Saccharomyces cerevisiae	TTATCCA..........GA........?.TT......?..AC..................

Appendix 7. Continued

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                                1401                                                                                                1500
Auxarthron zuffianum            GCTACACTGACAGGGCCAGCGAGTACATCACCTTGGCCGAGAGGTCCGGGTAAACTTGTTAAACCCTGTCGTGCTGGGGATAGAGCATTGCAATTATTGC
Chaetomium elatum               .............A............TC??.........GA...C........T..........T.....................................
Ctenomyces serratus             ........................T.....................T......T.....................................
Cudonia confusa                 ??????G......A....A.....T.........A.........?.TT......T..........T.....................................
Eupenicillium javanicum         ..............................................T......T.....................................
Gymnoascoideus petalosporus     ........................TT..TT................T......T.....................................
Gyromitra esculenta             .............A....A.....................GA.....T......T..........T.....................................
Leotia lubrica                  .............A....A.....T..............A...C.T......T..........T.....................................
Leptosphaeria doliolum          .............A....A.....T.T........A....A.....T......T..........T.....................................
Morchella elata                 .............A....A...............AA...GA.....T......T..........T.....................................
Onygena equina                  ........................T.....................T......T.....................................
Peziza badia                    .............A....A.....CT..?...........A.....T......T.....G....T.....................................
Plectania nigrella              .............A....A.....................GA.....T......T..........T.....................................
Pleospora herbarum              .............A....A.....T.T.............A.....T......T..........T..................A..................
Renispora flavissima            ..............................................................T.....................................
Sclerotinia sclerotiorum        .............A....A.....TTT..T.....A....A.....T......T..........T.....................................
Sordaria fimicola               ............CA............TC??.........GA............T..........TG....................................
Spathularia flavida             .............A....A.....T.........A.....A....TT......T..........T.....................................
Talaromyces flavum              ........................T.....................T......T.....................................
Trichophyton rubrum             ........................C.....................C........T.....................................
Tuber cf rapaeodorum            .............A....A.....T.........T....GA.....T......T..........T.....................................
Uncinocarpus reesii             ..............................................T......T.....................................
* Hymenoscyphus ericae          .............A....A.?...T...T?.....T....A.....T......T..........T.....................................
* Oidiodendron maius            .............A....A.....T..........T....A.....T......T..........T.....................................
* Myxotrichum arcticum          .............A....A.....T..........T....A.....T......T..........T.....................................
* Phialocephala fortinii        .............A....A.....T.C.T?.....A....A.....T......T..........T.....................................
* Variable White Taxon          .............A....A.....T.C.T?.....A....A.....T......T..........T.....................................
* Uncinocarpus uncinocarpus     ........................TT..TT................T......T.....................................
* Gymnoascus reessii            ........................TT..TT................T......T.....................................
Saccharomyces cerevisiae        ...........G.A..........CT.??.................TT.....T.....G....T.C.....................T.........
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Appendix 7. Continued

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                               1501                                                                                              1600
Auxarthron zuffianum           TCTTCAACGAGGAATGCCTAGTAGGCACAAGTCATCAGCTTGTGCCGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGG
Chaetomium elatum              ...............C.......A..G...............C.TT......................................................
Ctenomyces serratus            ....................................................................................................
Cudonia confusa                ...............T.......A..G...............???T......................................................
Eupenicillium javanicum        ............................G...........C...........................................................
Gymnoascoideus petalosporus    ....................................................................................................
Gyromitra esculenta            ...............T.......A..G...............C.TT......................................................
Leotia lubrica                 ...............T..........G.....................C...................................................
Leptosphaeria doliolum         .......................A..G.GT.........A..C.TT......................................................
Morchella elata                ...............T.......A..GT..............C.TT......................................................
Onygena equina                 ....................................................................................................
Peziza badia                   ...............T.......A..G...............C.TT..C...................................................
Plectania nigrella             ...............T.......A..G...............C.TT......................................................
Pleospora herbarum             .T.....................A..G.GT.........A..C.TT.............A........................................
Renispora flavissima           .........................T..........................................................................
Sclerotinia sclerotiorum       .......................A..G...............C.TT...............T......................................
Sordaria fimicola              ...............C.......A..G...............C.TT......................................................
Spathularia flavida            ...............T.......A..G...............C..T......................................................
Talaromyces flavum             ............................G...........C...........................................................
Trichophyton rubrum            ....................................................................................................
Tuber cf rapaeodorum           ...............T.......A..G...............C.TT......................................................
Uncinocarpus reesii            ....................................................................................................
* Hymenoscyphus ericae         ...............T.......A..G...............C..T......................................................
* Oidiodendron maius           ...............T.......A..G...............C..T......................................................
* Myxotrichum arcticum         ...............T.......A..G...............C..T...................................................C..
* Phialocephala fortinii       ...............T.......A..G...............C..T......................................................
* Variable White Taxon         ...............T.......A..G...............C..T......................................................
* Uncinocarpus uncinocarpus    ..........................G.........................................................................
* Gymnoascus reessii           ....................................................................................................
Saccharomyces cerevisiae       ...............T.......A..G...............C.TT.......................................G..............
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Appendix 7. Continued

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                                1601                                                                                1683
Auxarthron zuffianum            CTCAGTGAGGCCTTCGGACTGGCTCATGGGGGTTGGCAA?CGACCGCCAAGGGCCGG?AAAGTTGGTCAAACTTGGTCATTTA
Chaetomium elatum                ..........T.C.........C..GA.A...C.....?.....A.TC........?....C.ATC......C.........
Ctenomyces serratus              ......................C..GA.T.........?.......TC........?..............C.........
Cudonia confusa                  ..A........T.............A.CA.A.......?...T.AG.CC.A..T..?.......TC................
Eupenicillium javanicum          ........................G.A..........?.....C..C..A.....?..............C.........
Gymnoascoideus petalosporus      ............C.........C..G...........?........C........?....C.....................
Gyromitra esculenta              G.....................C??G.?A.A.C.....?...T.A..???????????....?.....................
Leotia lubrica                   ..........T.CG.AC.....CAGG.A...?C.....??????????????????????????????????????????
Leptosphaeria doliolum           ............C..........TGGA.A.....C...?.....A.TCT.A.....?......C.C......C.........
Morchella elata                  G..........?.......G...AGG...A.A.C..A..?...T.A..GCCA...T.?.............???????????
Onygena equina                   ......................T.G..A.........?.....A..C..A.....?.........................
Peziza badia                     ..................C..TC..G.AAT..C.....?...T.??????????????????????????????????????
Plectania nigrella               ..T...................C.GA..A...C.....?.....A..TT..?....?.........................
Pleospora herbarum               ..........G............GG..A.........?.....A..CCAA.....?......C.C......C.........
Renispora flavissima             ........................G..A.........?.....A..C..A.....?.........................
Sclerotinia sclerotiorum         ..A........T.........C..T.G..A..G......?.AC..A..C..A.G...?....??????????????????????
Sordaria fimicola                ..........T.C.........C..G..A...C.....?.....A..C........?....C.ATC......C.........
Spathularia flavida              ..A........T...A........T.A.CA.A.......?...T.AG.CC.A..T..?.......TC................
Talaromyces flavum               ........................GA...........?.......TC..A.....?.........................
Trichophyton rubrum              ......................C..G..A......A..?........C........?..............C.........
Tuber cf rapaeodorum             ..................G..C.CG....A...C.....?..??????????????????????????????????????
Uncinocarpus reesii              ........................G..A.........?.....A..CC.A.....?.........................
* Hymenoscyphus ericae           ..........T...........C..G..A.AG......?.ACT.A..C........?.......T................
* Oidiodendron maius             ..........T...........CT.G..A.AG......?.ACT.A..C........?.......TC...............
* Myxotrichum arcticum           .C.........T...........CT.G..A.AG......?.ACT.A..C........?.......TC...........G....
* Phialocephala fortinii         ..........T.............G..T.CGC.....?..C.T...C..A.....?.......T................
* Variable White Taxon           ..........T...........C..G..A.AG......?.ACT.A..C........?.......T................
* Uncinocarpus uncinocarpus      ............C.........C..GAA..........?.......TC........?....C...................
* Gymnoascus reessii             ............C.........C..GAA.......G..A.?.....TC........G....C...................
Saccharomyces cerevisiae         ..T..........CA...TCT...T.GA.AA.GG.....?.TC.AT.TC..A..?..AG..T....A..............
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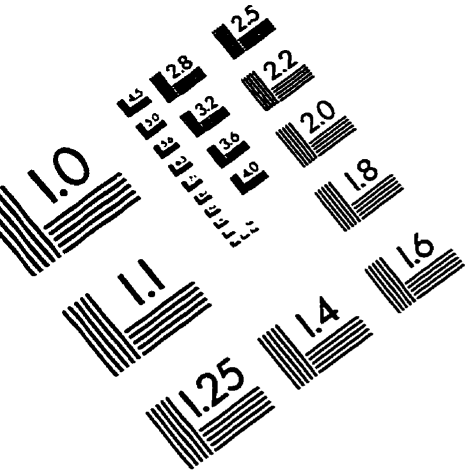
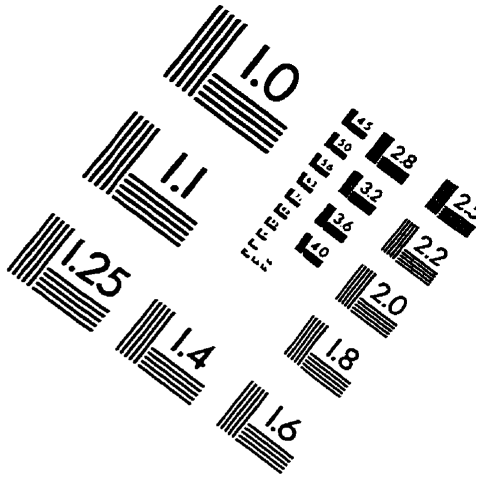
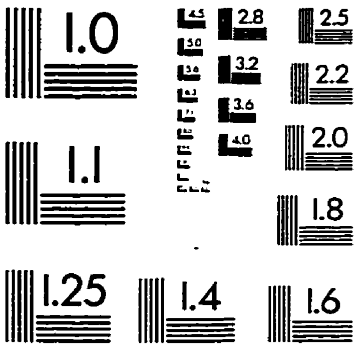
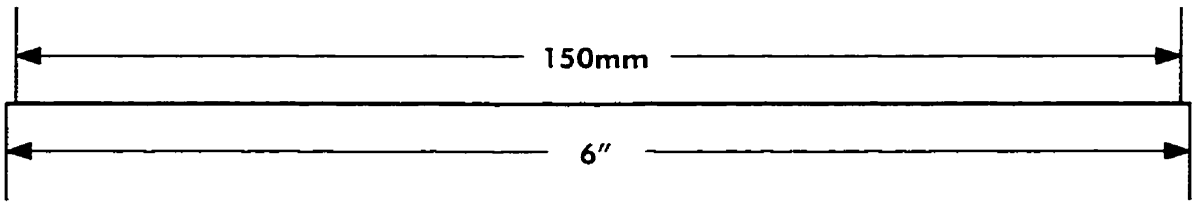
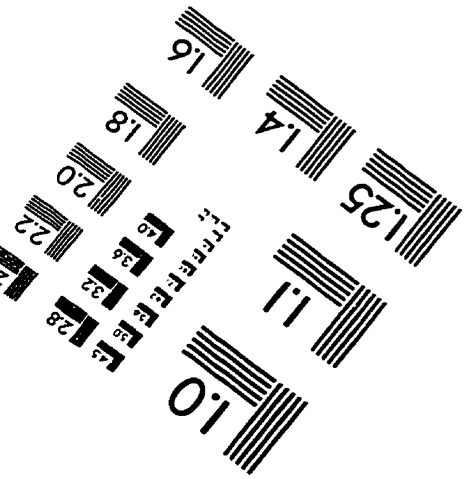
IMAGE EVALUATION
TEST TARGET (QA-3)

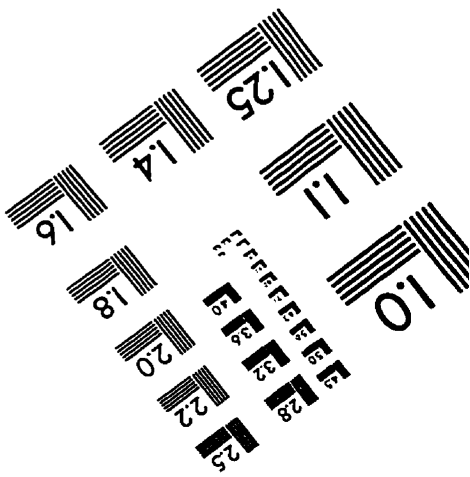












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