

# *Meliniomyces*, a new anamorph genus for root-associated fungi with phylogenetic affinities to *Rhizoscyphus ericae* ( $\equiv$ *Hymenoscyphus ericae*), *Leotiomyces*

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**Abstract:** Sterile fungi isolated from surface-sterilized roots of the *Ericaceae*, and hypothesized to be conspecific based primarily on restriction fragment length polymorphisms, were provisionally named as Variable White Taxon (VWT). In preliminary resynthesis trials with *Vaccinium myrtilloides* or *V. vitis-idaea*, isolates did not form typical ericoid mycorrhizas. Additional isolates obtained from roots of the *Orchidaceae*, *Pinaceae*, *Betulaceae* and *Salicaceae*, and given informal names such as Sterile white 1 (SW1), were thought to represent the same taxon based on cultural similarities. To evaluate conspecificity and infer phylogenetic affinities, partial nuclear ribosomal DNA sequences were determined. Parsimony analyses supported a species level distinction for VWT/SW1 and indicated that the fungus is placed within the species complex referred to as the “*Hymenoscyphus ericae* aggregate” which includes *H. ericae* (*Leotiomyces*), many unnamed taxa and *Cadophora finlandica*. The new genus and species *Meliniomyces variabilis* is proposed to accommodate this root-associated fungus to facilitate discussion and information retrieval, and to provide a foundation for additional experimental work. Although isolates are sterile in culture, they can be identified by morphological characters in conjunction with ribosomal internal transcribed spacer (ITS) sequence data. The mycorrhizal status of *M. variabilis* is not yet clear. In prior published results, strains demonstrated no or some colonization with ericoid and ectomycorrhizal hosts but did not form true ectomycorrhizas. ITS analyses indicated that the “*H. ericae* aggregate” includes several other well-supported clades putatively named as *Meliniomyces* species. Representative strains were examined morphologically for two of these species, described as *M. vraolstadae* and *M. bicolor*. Both include ectomycorrhizal mycobionts of the “*Piceirhiza bicolorata*” morphotype. *Rhizoscyphus ericae* is accepted as the appropriate name for *H. ericae*.

**Taxonomic novelties:** *Meliniomyces* Hambleton & Sigler anam. gen. nov., *Meliniomyces variabilis* Hambleton & Sigler sp. nov., *Meliniomyces bicolor* Hambleton & Sigler sp. nov., *Meliniomyces vraolstadae* Hambleton & Sigler sp. nov.

**Key words:** *Cadophora finlandica*, epacrid, ericoid, *Mycelia sterilia*, mycorrhizas, *Neocudoniella radicea*, *Piceirhiza bicolorata*, rDNA, *Scytalidium*.

## INTRODUCTION

Traditional approaches to the assessment of mycorrhizal diversity have included a range of techniques such as collecting sporocarps or other disseminative structures associated with mycorrhizas, characterizing differences in root morphology, and isolating fungi from roots into pure culture. All of these approaches have limitations but one fundamental difficulty of the latter has been the identification and classification of isolates that do not form mito- or meio- spores in culture and have not been linked in other ways to collections of known fungi. Commonly, studies focussing on the isolation of endophytes, particularly from ericoid but also from ectomycorrhizal roots, list a significant number of sterile isolates grouped primarily on the basis of cultural morphology (e.g. Pearson & Read 1973, Singh 1974, Reed 1989, Steinke *et al.* 1996, Schild *et al.* 1988, Perotto *et al.* 1996, Summerbell 1989, Johansson 2001).

Increasingly, molecular-based methods are being used to help resolve such difficult taxonomic problems. Isolates from ericoid mycorrhizas have been characterized and grouped according to genotype, using isozyme data (Hutton *et al.* 1994), restriction fragment length polymorphism (RFLP) analysis (Perotto *et al.* 1996, Hambleton & Currah 1997, Monreal *et al.* 1999), PCR-primed microsatellite fingerprint patterns (Liu *et al.* 1998) or, more recently, comparisons of DNA sequence data for the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA gene (rDNA) (e.g. McLean *et al.* 1999, Bergero *et al.* 2000, Sharples *et al.* 2000, Chambers *et al.* 2000, Vrålstad *et al.* 2000, 2002a, Allen *et al.* 2003). The latter approach has facilitated the comparison of cultural morphotypes described in various studies, and provided evidence for the evaluation of phylogenetic affinities and the taxonomic significance of characters such as cultural morphology and hyphal configurations in and on roots. The range of phylogenetic diversity revealed has been

extensive and, despite all of these efforts, very few isolates have been identified, and inferred linkages to known species have been limited.

Analyses of sequences generated from cloned PCR products amplified from DNA extracted directly from roots illustrate the deficiencies in culture-based methods for surveying the diversity of fungi that colonize ericoid mycorrhizas. Culture methods tended to favour the recovery of ascomycetes in general, and in particular those ascomycetes that can become established readily on agar media. Allen *et al.* (2003) demonstrated that sebacinoid basidiomycetes predominated in DNA-based detection of endophytes in ericoid roots but never were isolated from surface-sterilized roots. The corresponding limitation of direct DNA sampling from roots, soil and other substrates is that the sequences generated, besides being unidentified, have no associated specimen or culture for study. Identification to a group of known fungi depends entirely on the phylogenetic breadth and taxonomic accuracy of the sequence databases available, and further experimental studies are precluded.

Hambleton & Currah (1997) reported that culturally similar and sterile fungal isolates obtained from roots of *Ericaceae* collected in Alberta, Canada could be differentiated into two taxa on the basis of different RFLP patterns. One was identified as *Hymenoscyphus ericae* (Read) Korf & Kernan after the production of the anamorph, *Scytalidium vaccinii* Dalpé, Sigler & Litten, and the eventual formation of apothecia by an isolate in culture (Hambleton *et al.* 1999). The second taxon was provisionally called "Variable White Taxon" (VWT). Isolates remained sterile under all conditions tested and, though predominantly creamy-white in culture, some developed grey patches or were uniformly grey.

Restriction fragment length polymorphism data indicated that VWT was distinct from *H. ericae*, but the taxonomic significance of the data was difficult to evaluate. The distinction also raised questions about ecological function. In preliminary resynthesis trials pairing VWT with seedlings of *Vaccinium myrtilloides* or *V. vitis-idaea*, few hyphal complexes typical of the ericoid type were observed in root cells (Hambleton, unpub. data). In contrast, when an isolate of *H. ericae* or *Oidiodendron maius* Barron was used, two species known as ericoid mycorrhizal endophytes (Pearson & Read 1973, Couture *et al.* 1983, Read & Bajwa 1985, Hambleton *et al.* 1998), root cells were well-colonized. This was an indication that VWT played a different role in the rhizosphere.

During the seven years since the RFLP characterization of VWT, we recognized other groups of isolates that demonstrated cultural and/or genetic similarities. In some cases these strains were deposited in the University of Alberta Microfungus

Collection and Herbarium, Edmonton, Alberta, Canada (UAMH). Summerbell (1989) described a sterile fungus, designated as "Sterile white 1" (SW1), from serially washed mycorrhizas of *Picea mariana* (P. Mill.) B.S.P. and deposited several isolates, including isolates from an Irish study done by Schild *et al.* (1988). Isolates resembling VWT were obtained from orchid mycorrhizas collected in Ontario, Canada by C. Zelmer. Vrålstad *et al.* (2000) described 15 partial ITS genotypes comprising four cultural groups among mycobionts derived from roots of conifers and hardwoods demonstrating the ectomycorrhizal morphotype informally named "*Piceirhiza bicolorata*" (see Agerer 1997–2002). The published descriptions and illustrations of "morphology group 2" showing white colonies with radial folds and fascicles of hyphae, and the close ITS sequence relationship with *H. ericae* suggested a possible affinity with VWT. Additional research by Vrålstad *et al.* (2002a) resulted in completed ITS sequences for those genotypes and some representative cultures were deposited in UAMH. If all these groups represent the same taxon, this suggests that the distribution of VWT may be cosmopolitan and its ecological role may be cryptic. Our continued monitoring of sequences publicly available retrieved other similar ITS sequences, including some derived from DNA sampled directly from roots.

The objectives of this study were to: 1. assess the conspecificity of isolates suspected of being VWT based on cultural features and characters of the mycelium, by comparing rDNA sequences for a selection of strains isolated from the roots of the *Betulaceae*, *Ericaceae*, *Orchidaceae*, *Pinaceae* and *Salicaceae*; 2. evaluate the phylogenetic relationship of VWT to *H. ericae* in particular, and to other ascomycetes, including *Neocudoniella radicella* Kohn, Summerbell & Malloch, an inoperculate discomycete associated with rootlets from boreal forest sites. Sequences were also compared with those retrieved from GenBank of unidentified root endophytes. Based on phylogenetic and cultural analyses, we describe *Meliniomyces* Hambleton & Sigler anam. gen. nov. for species allied to *H. ericae* and lacking conidia. The type species, *M. variabilis* Hambleton & Sigler, includes strains known previously as VWT or SW1, and the mycorrhizal association of this species is reviewed based on previously published results of resynthesis experiments for specific strains. Analyses of ITS sequence data support the recognition of several other *Meliniomyces* species but we describe only two that we have examined in culture, *M. bicolor* and *M. vraolstadiiae*.

During the final preparation of this manuscript, the new genus *Rhizoscyphus* W.Y. Zhuang & Korf was established to accommodate *H. ericae* and the less well known root-associated inoperculate discomycete,

*H. monotropae* Kernan & Finocchio (Zhang & Zhuang 2004). We acknowledge the rationale for, and evidence supporting, the transfer of *H. ericae* from the genus typified by *H. fructigenus* (Bull. : Fr.) Gray and have used the new combination *Rhizoscyphus ericae* (D.J. Read) W.Y. Zhuang & Korf in the remainder of the paper. We have, though, retained the use of “*H. ericae* aggregate” to avoid confusion regarding this informal designation. It will take some time for the impact of the name change to be fully realized because of the extensive literature and database accessions associated with *H. ericae* and the ecological importance of this species.

## MATERIALS AND METHODS

Twenty strains of fungi suspected of being VWT were examined for morphological characters (Table 1). Colony morphology was characterized on potato-dextrose agar (PDA: Difco Laboratories, Detroit, MI, U.S.A.) and cornmeal agar (CMA: Difco Laboratories, Detroit, MI, U.S.A.). Fungi were grown on these media for a minimum of 3 wk in the dark at room temperature (RT; 20–22°C). Hyphal observations and measurements were made from cereal agar (CER) in slide culture mounts (Sigler & Flis 1998). Eleven of these strains were processed to obtain rDNA sequences; ITS data were publicly available in GenBank for the nine additional strains as indicated in Table 1. Mycelium scraped from the surface of PDA plates or in plugs cut from thin plates of E-strain agar (Egger & Fortin 1990) was used for DNA extraction.

DNA extraction methods, PCR reaction parameters and automated sequencing protocols were as outlined in Hambleton *et al.* (2005–this volume). The sequences determined for six of the 11 strains matched those derived two years previously using methods outlined in Hambleton *et al.* (1998). Primers NS1 and ITS4 (White *et al.* 1990) were used to amplify over 1700 nucleotides (nt) of the small subunit (SSU) as well as the complete ITS region, including ITS1, 5.8S and ITS2. Sequencing primers were selected from the range of SSU and ITS primers given in White *et al.* (1990) and Landvik *et al.* (1997) and consensus sequences were determined from overlapping sequence data for both DNA strands, unless indicated otherwise, using the software Sequencher™ (Gene Codes Corp., Ann Arbor MI). Sequences were similarly determined for *Neocudoniella radicella* UAMH 5794 (ex-paratype strain), *Scytalidium lignicola* Pesante & Peyronel UAMH 1502 (ex-type strain), and three strains of *R. ericae*, UAMH 6735 (ex-type strain) and UAMH 8680, two strains that formed apothecia and ascospores in pot or agar culture, and UAMH 8873 (Table 1).

Data matrices were largely composed of sequences retrieved from GenBank and were deposited in TreeBASE (<http://www.treebase.org/treebase/>; Study Accession No. S1393). Gapped BLAST (Altschul *et al.* 1997) searches using the SSU and ITS sequence data for the strain designated as ex-type of *M. variabilis* (UAMH 8861) were performed initially to determine the most similar sequence matches. The SSU data matrix comprised sequences representing the diversity of inoperculate discomycetes, and anamorphs and other teleomorphs allied to the *Leotiomycetes*, for which complete or nearly complete (>1600 bases) SSU data were available with selected *Pezizomyces* used as the outgroup. The only exception was a partial SSU sequence for *H. fructigenus* (U67430; 959 bases), included to assess the relationship of *R. ericae* to the type species of the genus *Hymenoscyphus* Gray. Two separate ITS alignments were constructed. ITS-A comprised our new sequences for *M. variabilis* and *R. ericae*, previously published data for *M. variabilis* (as unnamed fungi) and authentic cultures of *R. ericae*, and representative sequences for other fungi within the “*H. ericae* aggregate” of Vrålstad *et al.* (2000, 2002a). The ITS-B data matrix comprised the fungi sequenced in this study and GenBank sequences for fungi identified by BLAST as being closely related. These included only a few named fungi of which none were teleomorphs. Data for a range of inoperculate discomycete species in the *Hyaloscyphaceae*, *Dermateaceae* and *Helotiaceae* were initially included in an attempt to find the closest teleomorphic relatives to *M. variabilis* and *R. ericae* but the sequences were difficult to align and none grouped with the ingroup, the “*H. ericae* aggregate” (data not shown). For the final alignment, outgroup taxa were selected from among those species of *Leotiomycetes* included in the SSU analysis for which ITS data were available.

The SSU and ITS-A alignments were visually aligned in Se-Al v1.d1 (Rambaut 1996). The ITS-B data matrix was computer-generated using ClustalW v. 1.83 (Thompson *et al.* 1994) and minor adjustments were made by eye, but only for the ingroup taxa because the outgroup sequences were too divergent for a confident assessment of positional homology. To infer the phylogenetic affinity and taxonomic status of *M. variabilis*, parsimony analyses using the heuristic search algorithm (SSU and ITS-A and ITS-B) and UPGMA analyses (ITS-B) were performed using PAUP\* v. 4.0b10 (Swofford 1999) on the aligned data matrices. The level of support for individual clades was determined from 1000 replicates of either the full heuristic search option or the “fast” stepwise-addition bootstrap option.

4 **Table 1.** Source and accession numbers for strains examined.

Species	UAMH (CBS) No. <sup>a</sup>	Original Name	Source		SSU intron sites <sup>b</sup>	Sequences determined in this study	GenBank No. <sup>4</sup>
			Plant Host	Provenance			
<i>Meliniomyces variabilis</i>	5900 <sup>1</sup>	Sterile white 1	<i>Picea mariana</i> (P. Mill.) B.S.P.	Burt Lake, Burt Twp., Timiskaming Dist., ON, Canada. R. Summerbell (RCS W369), 1982.		ITS	AY838783
	5979 <sup>1</sup>	Sterile white 1	<i>Picea mariana</i> (P. Mill.) B.S.P.	Burt Lake, Burt Twp., Timiskaming Dist., ON, Canada. R. Summerbell (RCS W611), 1982.		ITS	AY838784
	6826 <sup>2</sup>	Sterile white 1	<i>Picea sitchensis</i> (Bong.) Carr.	sitka spruce forest, Ireland. M. Stuart (BB 996). from R. Summerbell.	1506	SSU-ITS	AY838785
	6827 <sup>2</sup>	Sterile white 1	<i>Picea sitchensis</i> (Bong.) Carr.	sitka spruce forest, Ireland. M. Stuart (BB 997). from R. Summerbell.		ITS	AY838786
	8861 <sup>3T</sup> (CBS 116124)	VWT	<i>Rhododendron albiflorum</i> Hook.	alpine heathland, Outpost Lake, Jasper National Park, AB, Canada. S. Hambleton (S-70Ac), 1994.	none	SSU-ITS	AY762619
	8862 <sup>3</sup>	VWT	<i>Vaccinium membranaceum</i> Dougl. ex Torr.	alpine heathland, Outpost Lake, Jasper National Park, AB, Canada. S. Hambleton (S-71Aa), 1994.		ITS	AY838787
	8863 <sup>3</sup>	VWT	<i>Phyllodoce empetriformis</i> (sm.) D. Don	alpine heathland, Outpost Lake, Jasper National Park, AB, Canada. S. Hambleton (S-77Ac), 1994.		ITS	AY838788
	8864 <sup>3</sup> (CBS 116125)	VWT	<i>Empetrum nigrum</i> L.	alpine heathland, Outpost Lake, Jasper National Park, AB, Canada. S. Hambleton (S-86Ac), 1994.	1506	SSU-ITS	AY838789
	10022	VWT	<i>Cypripedium acaule</i> Ait.	red pine plantation, St. Williams, ON, Canada. C. Zelmer (Z 236-1c.3), 1997.		ITS	AY838790
	10028	VWT	<i>Cypripedium acaule</i> Ait.	red pine plantation, St. Williams, ON, Canada. C. Zelmer (Z 238-2b.1), 1997.		ITS	AY838791
	10029	VWT	<i>Cypripedium acaule</i> Ait.	red pine plantation, St. Williams, ON, Canada. C. Zelmer (Z 258#4), 1997.	1506 <sup>c</sup>	SSU ITS	AY838792 AY838793
	10109 <sup>d</sup>	<i>Piceirhiza bicolorata</i>	<i>Pinus sylvestris</i> L.	edge of a coppermine spoil, Follidal, Oppland, Norway. T. Vrålstad (ARON 2879.S; G5), 1998.		–	AJ292201 [AJ243551]

Table 1. (Continued).

Species	UAMH (CBS) No. <sup>a</sup>	Original Name	Source		SSU intron sites <sup>b</sup>	Sequences determined in this study	GenBank No. <sup>4</sup>
			Plant Host	Provenance			
	10110 <sup>4</sup>	<i>Piceirhiza bicolorata</i>	<i>Pinus sylvestris</i> L.	dry, boreal oak-pine forest, Kragero, Telemark, Norway. T. Vrålstad (ARON 2900.S; G3), 1998.	–	–	[=AJ243549]
	10113 <sup>4</sup>	<i>Piceirhiza bicolorata</i>	<i>Populus tremuloides</i> Michx.	dry, boreal oak-pine forest, Kragero, Telemark, Norway. T. Vrålstad (ARON 2903.S; G3), 1998.	–	–	AJ308338
	10114 <sup>4</sup>	<i>Piceirhiza bicolorata</i>	<i>Quercus robur</i> L.	dry, boreal oak-pine forest, Kragero, Telemark, Norway. T. Vrålstad (ARON 2894.S; G4), 1998.	–	–	AJ308339 [AJ243550]
	10115 <sup>4</sup>	<i>Piceirhiza bicolorata</i>	<i>Picea abies</i> (L.) Karst.	5 yr clear-cut spruce forest, Eidsvoll, Akershus, Norway. T. Vrålstad (ARON 2919.S; G3), 1998.	–	–	AJ430148 [AJ243549]
	10380	<i>Hemlock mycorrhiza</i>	<i>Tsuga heterophylla</i> (Raf.) Sarg.	N. Vancouver Is., BC, Canada. M. L. Berbee (pkc09), 2002.	–	–	AY394898
	10381	<i>Hemlock mycorrhiza</i>	<i>Tsuga heterophylla</i> (Raf.) Sarg.	N. Vancouver Is., BC, Canada. M. L. Berbee (pkc32), 2002.	–	–	AY394917
	10382	<i>Hemlock mycorrhiza</i>	<i>Tsuga heterophylla</i> (Raf.) Sarg.	N. Vancouver Is., BC, Canada. M. L. Berbee (pkc21), 2002.	–	–	AY394902
	10420 <sup>5</sup>	<i>Salal mycorrhiza</i>	<i>Gaultheria shallon</i> Pursh	N Vancouver Is., BC, Canada. M. L. Berbee (UBCtra 323), 1998.	–	–	AF149083
<i>Meliniomyces bicolor</i>	10107 <sup>4T</sup> (CBS 116122)	<i>Piceirhiza bicolorata</i>	<i>Nothofagus procera</i> (Poep. & Endl.) Oerst.	North Yorkshire, U.K. A. Taylor (AT67), 1998, from T. Vrålstad (ARON 2805.S; G9)	–	–	AJ430147 [AJ243555]
	10108 <sup>4</sup> (CBS 116123)	<i>Piceirhiza bicolorata</i>	<i>Quercus robur</i> L.	dry, boreal oak-pine forest, Kragero, Telemark, Norway. T. Vrålstad (ARON 2893.S; G11), 1998	–	–	AJ292203
	10356	<i>Cadophora finlandia</i>	<i>Tsuga heterophylla</i> (Raf.) Sarg.	N Vancouver Is., BC, P. Kroeger, from M.L. Berbee (pkc34)	–	–	AY394885
<i>Meliniomyces vraolstadiae</i>	10111 <sup>4T</sup> (CBS 116126)	<i>Piceirhiza bicolorata</i>	<i>Betula pubescens</i> Ehrh.	clear-cut spruce forest, Eidsvoll, Akershus, Norway. T. Vrålstad (ARON 2916.S; G1), 1998	–	–	AJ292199 [AJ243547]
	10112 <sup>4</sup> (CBS 116127)	<i>Piceirhiza bicolorata</i>	<i>Betula pubescens</i> Ehrh.	clear-cut spruce forest, Eidsvoll, Akershus, Norway. T. Vrålstad (ARON 2917.S; G2), 1998	–	–	AJ292200 [AJ243548]

Table 1. (Continued).

Species	UAMH (CBS) Original Name No. <sup>a</sup>	Source		SSU intron sites <sup>b</sup>	Sequences determined in this study	GenBank No. <sup>4</sup>
		Plant Host	Provenance			
<i>Rhizoscyphus ericae</i>	6735 <sup>T</sup>	<i>Pezizella ericae</i>	Calluna vulgaris (L.) Hull United Kingdom. IMI 182065	516, 943	SSU-ITS	AY762620
	8680 <sup>3</sup>	<i>Hymenoscyphus ericae</i>	<i>Ledum groenlandicum</i> Athabasca, AB, Canada.	789, 943, 1506 <sup>c</sup>	SSU ITS	AY762621 AY762622
	8873 <sup>3</sup>	<i>Scytalidium vaccinii</i>	<i>Chamaedaphne calyculata</i> Athabasca, AB, Canada. (L.) Moench	1506 <sup>c</sup>	SSU	AY524847
<i>Neocudoniella radicella</i>	5794 <sup>PT</sup>		mass ascospore isolate from apothecium on <i>Picea</i> rootlets, ON, Canada. D.M. Malloch (DM 359)	943	SSU-ITS	AY524843
<i>Scytalidium lignicola</i>	1502 <sup>T</sup>		<i>Platanus</i> wood, Italy. IMI 62532	none	SSU-ITS	AY762623

<sup>a</sup>Numbers unless otherwise noted UAMH, University of Albert Microfungus Herbarium and Collection, Edmonton, AB, Canada; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. All strains isolated from ecto- or ericoid mycorrhizal roots except *N. radicella* and *S. lignicola*. For isolation methods for strains of *M. variabilis* see <sup>1</sup>Summerbell 1987a, <sup>2</sup>Schild *et al.* 1988, <sup>3</sup>Hambleton & Currah 1997, <sup>4</sup>Vrålstad *et al.* 2002a, <sup>5</sup>Allen *et al.* 2003.

<sup>b</sup>Intron insertion site is defined by the number of the nucleotide located on the five-prime side of its insertion position in the SSU rDNA sequence of *Escherichia coli* Migula (J01695).

<sup>c</sup>SSU intron sequence was not determined in full and was not deposited in GenBank.

<sup>d</sup>GenBa □

<sup>T</sup>Ex-type culture.

<sup>PT</sup>Ex-paratype culture.

## RESULTS

Nearly complete SSU sequences and complete ITS1/5.8S/ITS2 sequences were obtained and deposited in GenBank for nine (SSU) and 15 (ITS) strains in three species (Table 1). Small subunit sequences completed to the three-prime end were deposited with the corresponding ITS sequence as single GenBank accessions. The NS1-ITS4 PCR amplicons varied in size (data not shown) among the fungi sequenced, suggesting the presence of small subunit insertions and this was confirmed for some strains by sequencing. Group I introns were observed in all but two of the SSU sequences generated and were removed prior to phylogenetic analysis. In some cases, the size and position of the insertions hampered sequencing efforts to obtain data for both DNA strands and only the edited SSU sequences were deposited (Table 1). The introns were located by comparing the sequences with a range of SSU sequences using the large gap alignment function of Sequencher™ and then delimited based on the typical Group I splice junctions (Holst-Jensen *et al.* 1999) observed at the insertion sites. The position of each intron site is given below and in Table 1 and is defined by the number of the nucleotide located on the five-prime side of its insertion position in the SSU rDNA sequence of *Escherichia coli* Migula (J01695), as used previously to define intron insertion sites of lichen-forming fungi by Gargas *et al.* (1995).

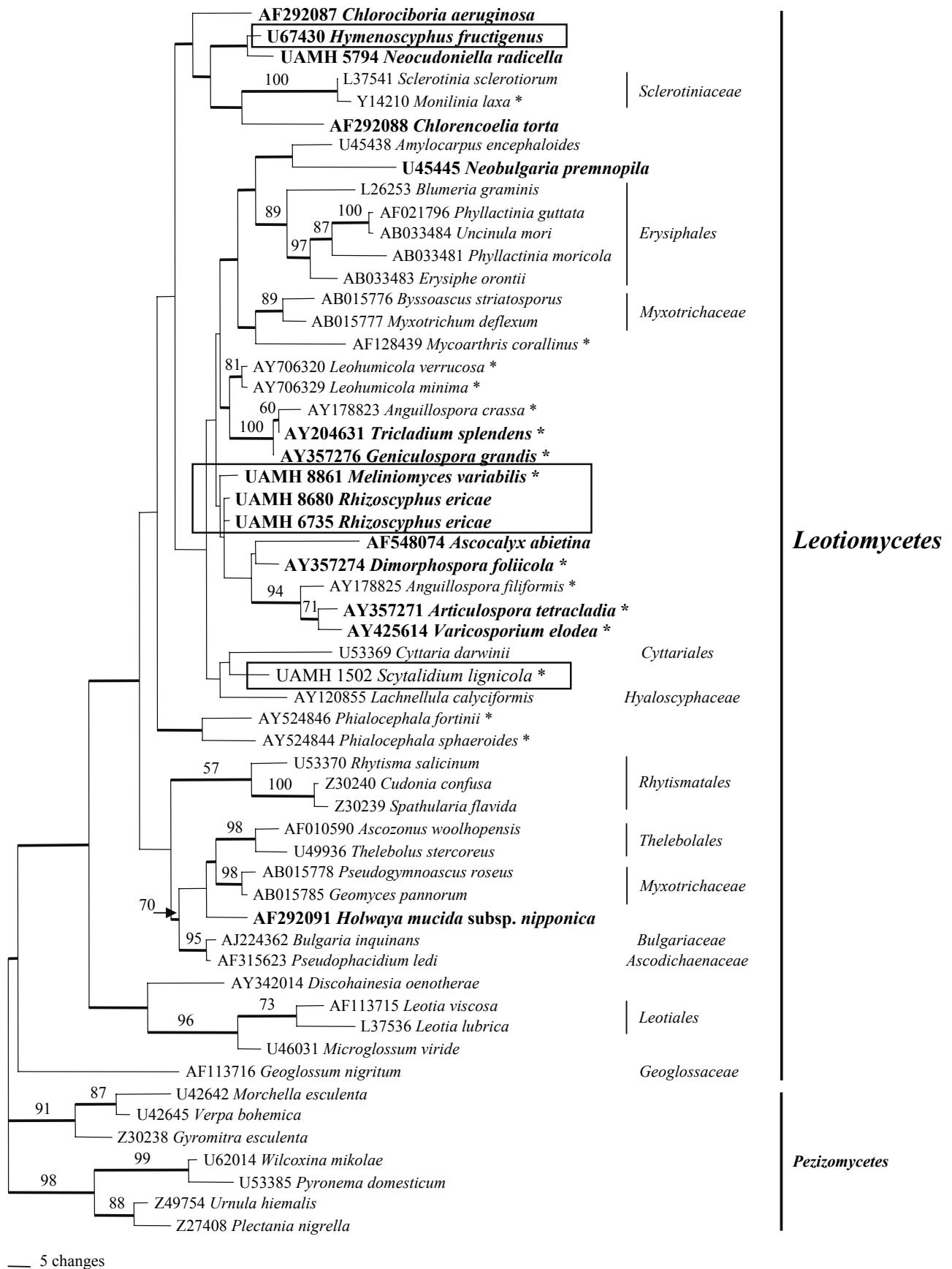
For three of the four strains of *M. variabilis* sampled for SSU sequence data, a Group I intron was observed at site #1506 located immediately upstream to the ITS1 priming site (White *et al.* 1990). UAMH 8861, ex-type strain, had no SSU insertions. The four edited SSU sequences (introns removed) determined for *M. variabilis* were identical. For UAMH 6826 and 8864, the intron sequences themselves differed at one position (length of 337 nt). In addition, these data were nearly identical, differing by at most 2 nt, to SSU intron data published previously for three strains included in our analyses and identified here as *M. variabilis*, pkc15 (AY394887), UAMH 10380 (AY394898) and UAMH 10382 (AY394902). The SSU intron of UAMH 10029 differed substantially but was too long to be sequenced in its entirety using our available primers.

The edited SSU sequences of the ex-type strains of *M. variabilis* and *R. ericae* differed at seven positions. Those of *R. ericae* UAMH 8873 and 8680 were identical and they differed from UAMH 6735 at two positions. *Rhizoscyphus ericae* UAMH 8873 had one SSU Group I intron at site #1506 (not sequenced in full), UAMH 6735 had two, site #516 (291 nt) and #943 (873 nt) and UAMH 8680 had three, site #789 (553 nt), #943 (873 nt) and #1506 (not sequenced in full). The introns for UAMH 6735 and 8680 at site #943 were nearly identical for most of the length except for

a 60 nt stretch near the middle. The *R. ericae* intron sequences closely matched previously published data in GenBank for intron sequences from these same intron sites for other strains of *R. ericae*. *Scytalidium lignicola* had no SSU introns, and *N. radicella* had one, at site #943 (329 nt), but a BLAST search did not retrieve any homologous matches from GenBank.

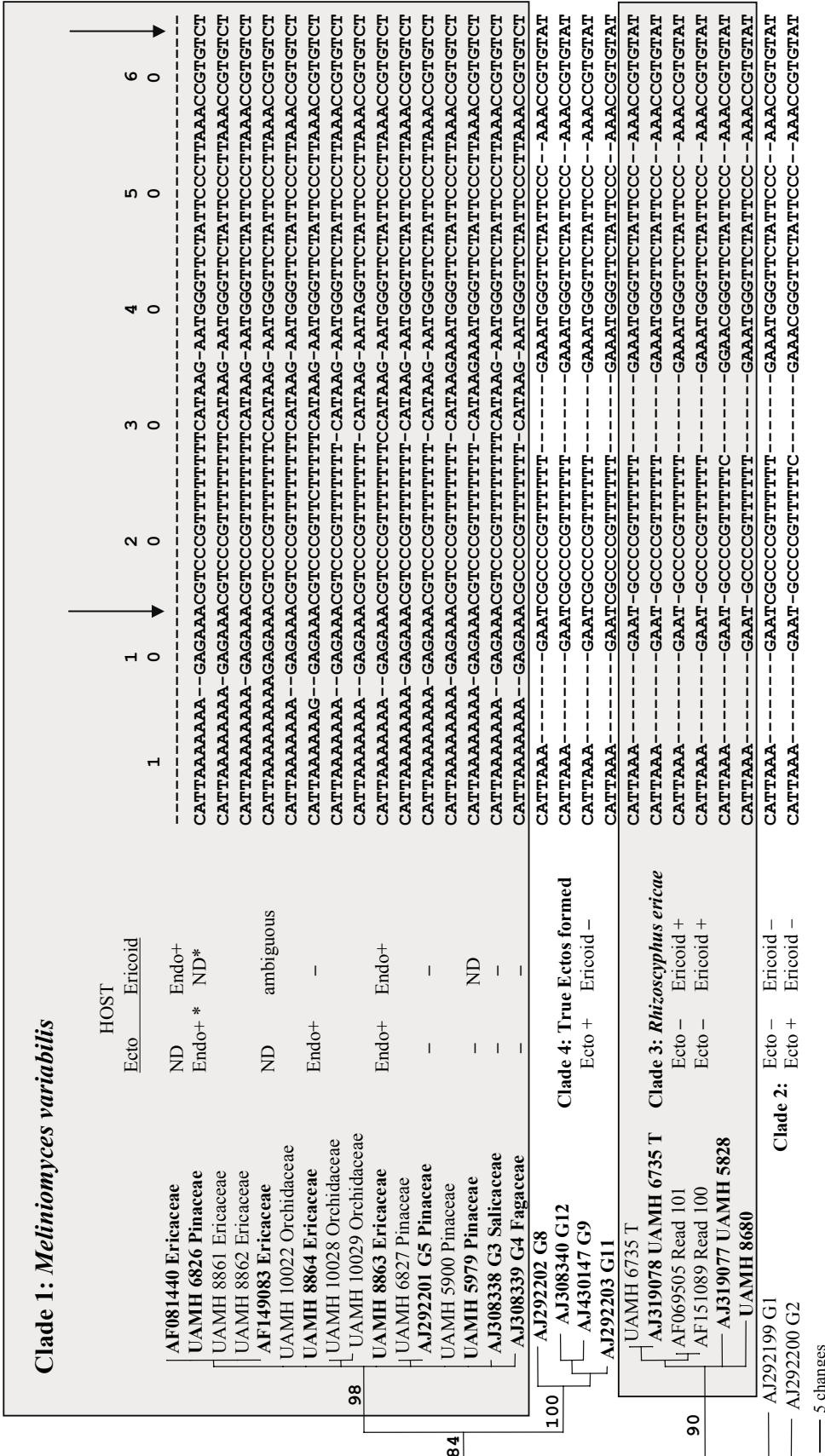
The complete SSU data matrix comprised 56 taxa and 1712 aligned characters. Of these, 1241 were constant and 237 were parsimony-informative. Parsimony analysis of the informative characters alone, using simple stepwise addition with *Plectania nigrella* Z27408 as the reference taxon, yielded 20 most-parsimonious trees (MPTs) of 774 steps, with a consistency index (CI) of 0.447 and a retention index (RI) of 0.647. Results of a bootstrap analysis using the full heuristic search option are shown on one MPT (Fig. 1). The results indicated that *Meliniomyces variabilis* is phylogenetically a member of the *Leotiomycetes* and suggested a close relationship to *R. ericae*, a result predicted by the close sequence similarity of the two species. Relationships among taxa were mostly unresolved. A clade comprising the strongly supported *Leotiales* [with *Microglossum viride* (Schrader) Gillet U46031] and *Discohainesia oenotherae* (Cooke & Ellis) Nannf. (AY342014) was sister to the rest of the *Leotiomycetes*, which formed a large polytomy in the strict consensus of all MPTs. Some familial and ordinal groups received bootstrap support and these clades were also retained in the strict consensus, but the *Helotiaceae* was polyphyletic (Fig. 1, relevant taxon names indicated in bold type), and terminal branch lengths were long. *Rhizoscyphus ericae* did not group with the type species of the genus *Hymenoscyphus*, *H. fructigenus*. Preliminary analyses of a larger data matrix, which included the partial SSU sequences (approximately 1000 nt in length) published by Gernandt *et al.* (2001) for a wider range of inoperculate discomycetes, were performed with and without that portion of the matrix for which these taxa had missing data. The positions of the fungi under study here were not resolved any further, nor were the overall results in conflict (data not shown).

The 11 ITS sequences determined for *M. variabilis* ranged from 480 to 483 nt in length and compiled with 98 % similarity in Sequencher™; the two for *R. ericae* were 463 nt and compiled at 98 % similarity, differing at eight positions, and those of *N. radicella* and *S. lignicola* were 461 and 517 nt, respectively. The ex-type strains of *M. variabilis* and *R. ericae* compiled at 89 % similarity. The ITS-A data matrix comprised 28 taxa and 504 aligned characters. Of these, 52 ambiguously aligned characters were excluded and, of those remaining, 389 were constant and 41 were parsimony-informative. Parsimony analysis yielded 44 MPTs of 84 steps with CI = 0.869 and RI = 0.940.



**Fig. 1.** Results of a parsimony analysis of the small subunit (SSU) ribosomal DNA (rDNA) data matrix showing the placement of *Meliniomyces variabilis* within the *Leotiomycetes*. Inoperculate discomycetes and anamorphs classified in the *Helotiaceae* are indicated in bold type. Anamorphs are indicated with asterisks. Branches in bold were retained in the strict consensus of all 20 most-parsimonious trees (MPTs) and bootstrap values above 50 % are given adjacent to the relevant node.





**Fig. 2.** One of 44 MPITs from a parsimony analysis of the internal transcribed spacer (ITS)-A rDNA data matrix, with bootstrap values for the major nodes indicated. Results of resynthesis trials with host plants, from five prior studies, are indicated adjacent to the appropriate strain. A portion of ITS1 alignment is shown, starting at the five-prime end and numbered from 1 starting after the last five SSU nucleotides (nt) (CATT), illustrating three multinucleotide insertions, nt 5-10, 30-34, 53-54, one transition at nt 14 and one transversion at nt 64 (arrows), that distinguish (in part) Clade 1 isolates. The sequence for AF081440, represented by dashes, does not include data for the ITS1 spacer region. Clade 1. *Meliniomyces variabilis* isolates from ericoid, orchid and ecto- mycorrhizal roots. Tested isolates vary in their ability to form associations with ericoid and ecto-mycorrhizal hosts. See Table 2 for details. The asterisk indicates that although UAMH 6826 and 6827 are both representative of the unspecified isolate used by Schild *et al.* (1988) for resynthesis trials, the results are noted only once. Clade 2. Isolates from ectomycorrhizal root tips ("*Piceihiza bicolorata*" morphotype). Isolates were not ericoid mycorrhizal but varied in their ability to form ectomycorrhizas (Vrålstad *et al.* 2002b). See taxonomy comments for *Meliniomyces vraolstadiae*. Clade 3. *Rhizoscyphus ericae* and its anamorph, *Scytalidium vaccinii*. Isolates were ericoid mycorrhizal in roots of *Vaccinium* and *Rhododendron* but not endophytic in *Picea*, *Pinus* or *Betula* (Piercey *et al.* 2002, Vrålstad *et al.* 2002b). Clade 4. Isolates from ectomycorrhizal root tips ("*P. bicolorata*" morphotype). Isolates were ectomycorrhizal in roots of at least one of *Picea*, *Pinus* and *Betula* but not endophytic in *Vaccinium* (Vrålstad *et al.* 2002b). See taxonomy comments for *Meliniomyces bicolor*

Results of a bootstrap analysis using the full heuristic search option are shown on one unrooted MPT (Fig. 2). Three strongly supported clades were resolved, corresponding to *M. variabilis* (Clade 1), *R. ericae* (Clade 3) and a group of four isolates that form ectomycorrhizal associations (Clade 4), while two other sequences of ectomycorrhizal isolates were excluded (Clade 2). A portion of the ITS-A alignment is shown adjacent to the cladogram in Fig. 2, showing three multinucleotide insertions, nt 5–10, 30–34, 53–54, one transition at nt 14 and one transversion at nt 64, that distinguish (in part) *M. variabilis* from the other taxa in the alignment.

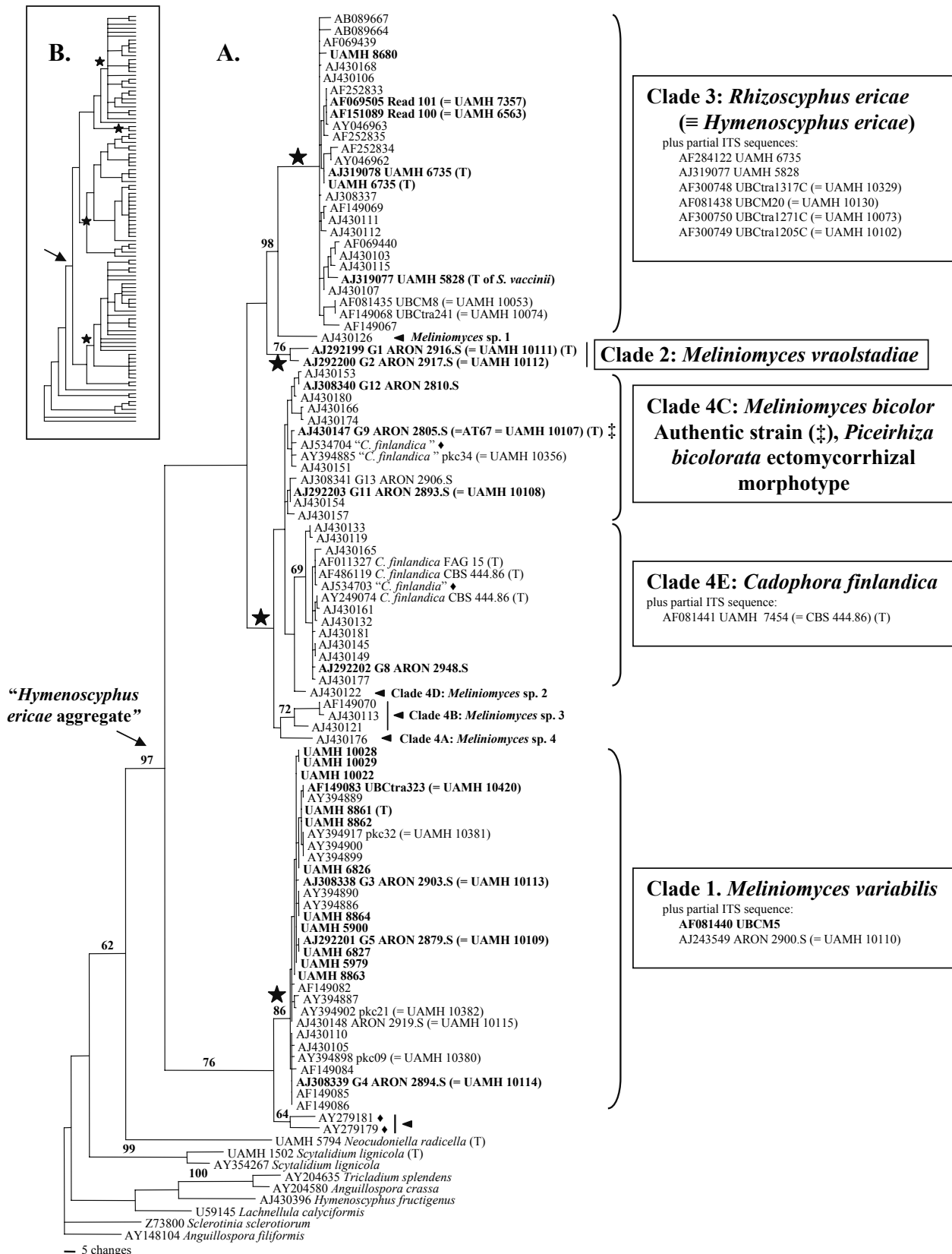
The ITS-B data matrix comprised 104 taxa and 569 characters. A parsimony analysis of the 182 parsimony-informative characters yielded MPTs of 678 steps, with CI = 0.466 and RI = 0.829. Maxtrees was set to 5000 after initial searches indicated that the large number of identical and nearly identical sequences being analysed resulted in an apparently unlimited number of MPTs possible. The intent was to identify groups of closely similar sequences among a taxon set compiled based on high BLAST scores and E values, and whose ITS sequences were alignable by eye. UPGMA analysis of all 569 characters yielded a tree with a very similar topology, finding the same three large clades discussed below but the phylogram was chosen as the best way to illustrate the sequence variation within each major clade, as visualised by the terminal branch lengths. Results of a parsimony bootstrap analysis using the “fast” stepwise-addition bootstrap option are shown on one MPT (Fig. 3A). Sequences based on cultures that were subsequently deposited in UAMH and examined by us are indicated. The ITS-B data matrix comprised many of the same sequences as the data set of Vrålstad *et al.* (2002a). The clade structure of our MPT shown in Fig. 3A is very similar to their Fig. 2 (p 142); to facilitate later discussions, we have assigned clade numbers in our Figs 2 and 3 to correspond with those in their Fig. 2, including the sub-clades A–E of Clade 4.

The large clade receiving high bootstrap support (97 %; Fig. 3A and B, arrow) corresponded to the “*H. ericae* aggregate” of Vrålstad *et al.* (2000, 2002a). Most of the ingroup taxa were resolved into the same four groups as shown in their Fig. 2, that is, two well-supported clades corresponding to *M. variabilis*, and *R. ericae* (Clades 1 and 3), a moderately well-supported group comprising two isolates from ectomycorrhizal root tips (Clade 2), described here as *Meliniomyces vraelstadiae*, and a fourth group including isolates also primarily from ectomycorrhizas (Clade 4). Bootstrap support for the latter clade was lacking with the broader sampling of taxa when compared with results from our ITS-A analysis. The four groups were retained in the strict consensus of all 5000 MPTs (Fig. 3B). For Clades 1 and 3 there was some evidence of sub-clade structure that could not be evaluated further using the data

presented here but the reference and examined strains included for each species encompassed the range of sequence variation shown. Information on strains and sequences assigned to *M. variabilis* and *R. ericae*, based on ITS sequence data and not listed in Table 1, are presented in Appendices A and B, respectively.

Clade 4 comprised several subclades. Clade 4E (bootstrap support 69 %) corresponded to *Cadophora finlandica* (Wang & Wilcox) Harrington & McNew (specific epithet corrected from “*finlandia*”; see Index Fungorum entry for *Phialophora finlandia* on-line at <http://www.indexfungorum.org/Names/namesrecord.asp?RecordID=104628>). It included four GenBank ITS sequences, independently determined and differing by up to 3 nt, for the ex-type strain (FAG 15 = CBS 444.86 = UAMH 7454). The clade included a selection of ectomycorrhizal isolates centered around the G8 genotype of Vrålstad *et al.* (2000) and one isolate from ericoid mycorrhizas (AJ430119). Clade 4C included the G9, G11, G12 and G13 genotypes of Vrålstad *et al.* and several other ectomycorrhizal isolates. The G9 genotype is based on a culture (ARON 2805.S = AT67 = UAMH 10107), authenticated as representing the mycobiont of the ectomycorrhizal morphotype known as “*P. bicolorata*”. *Meliniomyces bicolor* is described for the strains in this clade. It includes two strains named *C. finlandica* by the original depositors of the sequences, identifications that were based on the results of BLAST searches of the ITS sequences and not on conidial characters (AJ534704, Tedersoo *et al.* 2003 and AY394885, pers. comm. M. Berbee).

Five additional putative species are indicated on Fig. 3A (arrowheads). Four are named as *Meliniomyces* sp. based on the supposition that, for those sequences derived from cultures, the isolates were sterile. No information to the contrary was provided by the original researchers (Vrålstad *et al.* 2000, 2002a, Allen *et al.* 2003). The species are not formally described because they each comprise only a few isolates, none of which has been seen by us. A fifth potential species forms a sister clade to *M. variabilis* and comprises two sequences, AY279181 and AY279179, of uncultured fungi from epacrid roots from Australia, but no further published information could be found regarding their provenance. They were the only ITS sequences that grouped within the *R. ericae* species complex from among the many deposited in GenBank for root endophytes from epacrid and ericoid hosts from the Southern Hemisphere. Additional sequences that grouped in Clade 4 in preliminary analyses, but were excluded to facilitate presentation of the results, included 16 derived from cultured fungi, each one identical to a sequence that was included, and 39 sequences based on uncultured fungi. Source information for fungi not examined by us but assigned to *C. finlandica*, *M. bicolor* and *Meliniomyces* sp. 1 through 4 based on our ITS results is provided in Appendix C.



**Fig. 3.** A. One of 5000 MPTs from an aborted parsimony analysis of the ITS-B data matrix. Sequences included in the ITS-A analysis (Fig. 2) are indicated in bold type. Sequences AJ534704 and AY394885 were deposited under the name “*Cadophora finlandica*” (as “*C. finlandica*”) based on ITS sequence similarity scores (see text for further discussion). Arrowheads indicate additional species that could be described if isolates are available for examination. All ingroup sequences were derived from cultures isolated from roots, except for four (♦) derived from DNA extracted directly from roots. A majority of ingroup taxa were resolved into four clades, indicated by stars adjacent to the relevant nodes, corresponding to *Meliniomyces variabilis*, *Meliniomyces vraolstadae*, *Cadophora finlandica*/*Meliniomyces bicolor*, and *Rhizoscyphus ericae*. B. Strict consensus of all 5000 MPTs with taxon names removed. Stars indicate the same four clades as in A.

## Taxonomy

**Meliniomyces** Hambleton & Sigler, **anam. gen. nov.**  
MycoBank MB500244

*Etymology:* *Melinio-* (G) for Elias Melin, who initiated studies of the diversity of sterile root endophytes of conifers with a description of *Mycelium radialis atrovirens* (Melin 1922); *-myces* (G) for fungus.

Coloniae in agar albae vel griseae, tarde crescentes, glabrae, velutinae vel fasciculateae; hyphae septatae, hyalinae, leves vel asperulatae, laqueos vel fila formantes, 1.5–5 µm. Mycelia sterilia; conidia (mitosporae) et chlamydo-spores absunt. Teleomorphosis ignota. *Meliniomyces* adfinis *Rhizoscypho* et *Leotiomyce*ti; species generis *Meliniomyces* partim per ITS cognitae.

*Species typica:* *Meliniomyces variabilis* Hambleton & Sigler

Colonies on all media white to grey, slow growing, smooth, velvety or fasciculate; hyphae septate, hyaline, smooth to finely asperulate, uniform to irregularly swollen, sometimes forming swollen terminal cells, often forming loops and aggregating into strands, 1.5–5 µm diam. Conidia and chlamydo-spores lacking. Teleomorph unknown. Affinity to *Rhizoscyphus* and *Leotiomyces*; genus recognised partially through ITS.

***Meliniomyces variabilis*** Hambleton & Sigler, **sp. nov.**  
MycoBank MB500245, Figs 4A–H, 5–11.

*Etymology:* *variabilis* (L) referring to variability in culture pigmentation.

Coloniae in agar tarde crescentes, mycelium aerium tenue; in PDA albae vel luteae, deinde pallide-griseae vel atro-griseae ad centrum vel ad marginem; interdum zonatae; planae vel leviter elevatae ad centrum et radialiter sulcatae; glabrae, fasciculateae ad centrum; fasciculi interdum spiraliter compositi. In CMA, coloniae restrictae, albae vel griseae, immersae. Hyphae septatae, hyalinae, leves, 1.5–2.5 µm latae; in CER laqueos vel fila formantes; in CMA cellulas inflatas formantes.

*Typus:* Canada: Alberta, Jasper National Park, Outpost Lake, ex radice *Rhododendron albiflorum* Hook., S. Hambleton (S-70Ac), 1994. Colonia exsiccata et cultura viva, UAMH 8861.

Colonies slow-growing at RT and producing thin aerial mycelium on all media. Colonies 4–5.5 cm diam on PDA and 4.5–6 (7) cm diam on CER, after 35 d; mostly creamy-white to yellowish white, sometimes becoming pale to dark grey centrally or near the periphery, sometimes zonate; flat to slightly raised centrally and then radially furrowed, glabrous, hairy or, especially in the centre, fasciculate; fascicles sometimes oriented in a spiral pattern. On CMA, colonies very restricted, creamy white to greyish; hyphae completely

submerged. Hyphae in slide culture preparations on CER septate, hyaline, smooth, 1.5–2.5 µm wide, often forming loops and aggregating into strands. Hyphae in CER slide culture, or on CMA, short, swollen and often lobed. Conidia and chlamydo-spores lacking, even after prolonged incubation at RT or 4 °C. Teleomorph unknown.

*Distribution:* Canada (British Columbia, Alberta, Ontario), Norway, Ireland.

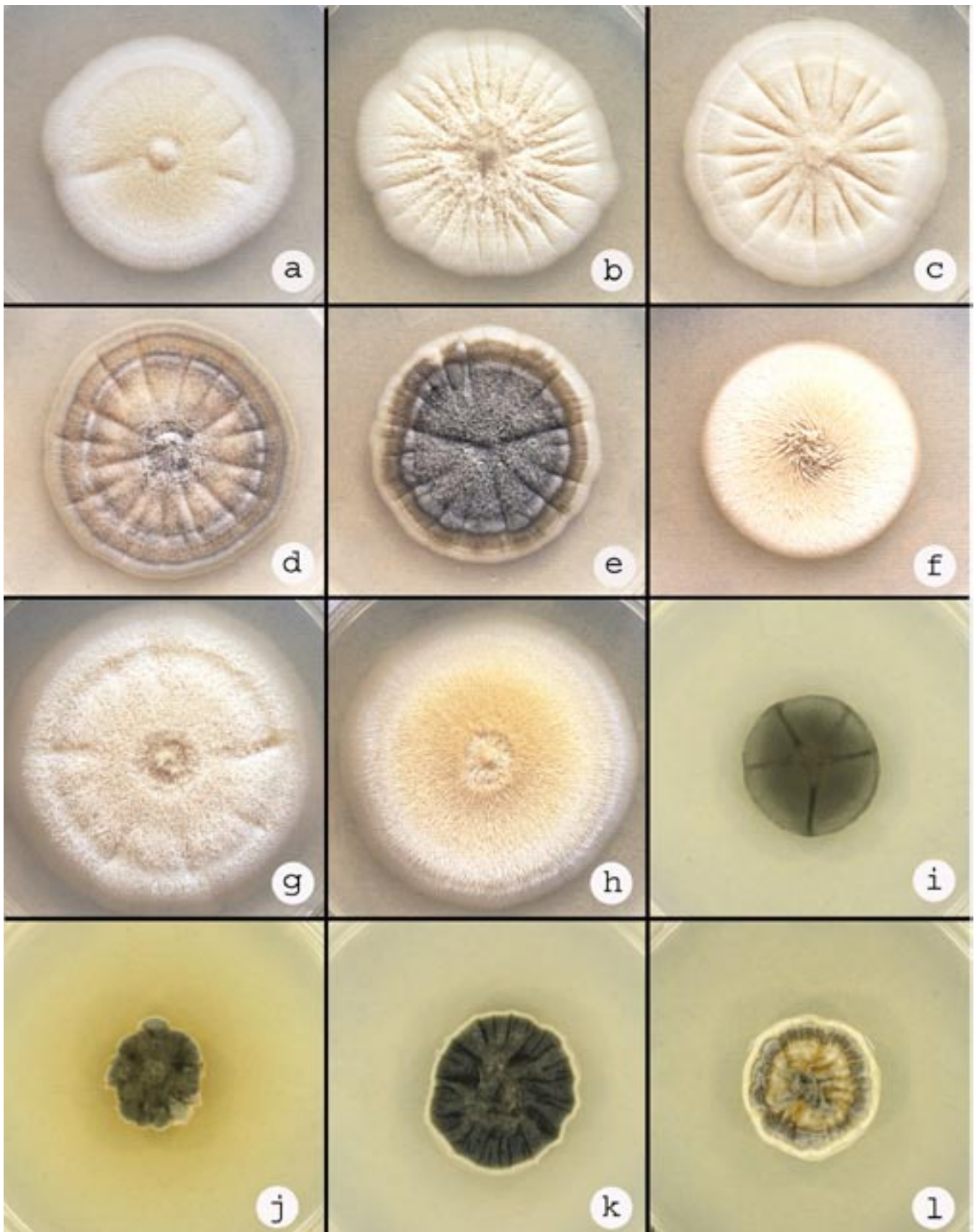
*Material examined:* Table 1 lists the provenance of the 20 living cultures examined.

*Molecular characters:* The ITS sequences for the eleven strains of *M. variabilis* sequenced in this study compiled at 98 % similarity. The consensus sequence is given in the following format: [three-prime 18S]ITS1\*5.8S\*ITS2[five-prime 28S]. Nt in bold indicate differences among the sequences: nt underlined are changes, either transitions or transversions; nt in brackets are indels (nt present in some but not all of the sequences).

[CATT(A)AAAAARGAGAAACGTCCCG(T)TY  
TTTTYCATAAG(A)AATRGGTCTATTCCCTTAA  
ACCGTGTCTACATACCTTTGTTGCTTTGGC(A)G  
GCCGCCTTCGGGCGTTCGGCTCCGGCTGATAGT  
GYCTGCCAGAGGACCCAACTCTATGTTTGTAGT  
GATGTCTGAGTACTATATAATATTTA\*AACTTT  
CAACAACGGATCTCTTGGTTCTGGCATCGATG  
AAGAACGCAGCGAAATGCGATAAGTAATGTG  
AATTGCAGAATTCAGTGAATCATCGAATCTTT  
GAACGCACATTGCGCCCCTTGGTATTCCGAGG  
GGCATGCCTGTTTCGAGCGTCATT\*(TT)ATAAC  
CACTCAAGCCTGGCTTGGTATTGGGGYTTTCG  
CGTGTTTCGCGGCCCTTAAATCAGTGGCGGTG  
CCGTCTGGCTCTAAGCGTAGTAATTTCTCTCG  
CTATAGGGTTCYGGTGGTTACTTGCCAAAACC  
CCCTATTTTTYCTA[GGTTGACCTCGGA]

RFLP DATA previously published (Hambleton & Currah 1997) for UAMH 8861, 8862, 8863, 8864 are given as fragment lengths, in base pairs, visualised after digests of total genomic DNA using four restriction endonucleases: *AluI* 642, 147, 109; *HhaI* 560, 333, 87; *HinfI* 455, 308, 170; *RsaI* 560, 194, 179.

*Additional information:* Strains we have not seen, but have assigned to this species based on ITS sequences with 97 % or greater similarity, are listed in Appendix A. In Hambleton & Currah (1997), cultures of UAMH 8861 (Fig. 10), 8863 (Fig. 11; incorrectly attributed to strain S-77Aa rather than S-77Ac) and 8864 (Fig. 12) are compared with cultures of *R. ericae* (Figs 1–9). In Vrålstad *et al.* (2000) cultural and/or micro-morphology are shown for ARON 2900.S (UAMH 10110) in Fig. 3E and F, for ARON 2879.S (UAMH 10109) in Fig. 3G, H and I, and ARON 2903.S (UAMH 10113) in Fig. 4F, G, N and O. The results



**Fig. 4.** Colony morphology of selected UAMH strains on PDA after 42 d, except where noted. A–H. *Meliniomyces variabilis*. A. 8861. B. 8862. C. 5900. D. 8863. E. 8864. F. 10029. G. 6827. H. 10022. I–J. *Meliniomyces bicolor* after 35 d. I. 10107. J. 10108. K–L. *Meliniomyces vraolstadae*. K. 10111. L. 10112. Differences in overall hue between A–H and I–L are a result of the images being taken with different cameras and under different copy stand conditions.

of physiological tests using three unspecified strains of SW1 included positive activity for pectinase and urease but an absence of activity for lipase, gelatinase and cellulase (Summerbell & Malloch 1988).

*Comments:* *Meliniomyces variabilis* strains are identified using a suite of morphological characters in conjunction with ITS sequence data. A measurable length difference in the sequence is diagnostic for the differentiation of *M. variabilis* from its closest known relatives and specific character state differences for *M. variabilis* are consistent for the data available (Fig. 2). The fungus has been isolated from the roots of a range of plant species in six families, *Ericaceae*, *Fagaceae*, *Orchidaceae*, *Pinaceae*, *Cornaceae* and *Salicaceae*, and from disparate geographic locations across Canada and in Europe (Table 1). Based on present information, the species appears to be restricted to cold-temperate soils of the Northern Hemisphere. Only two of the many sequences available for endophytes from Southern Hemisphere were similar, forming a sister group to *M. variabilis* in our analyses (Fig. 3A).

*Meliniomyces variabilis* is similar to *R. ericae* in having slow growth and restricted colony size on agar media, colonies with fasciculate strands and radial furrows, and smooth, narrow hyphae often forming loops and aggregating into strands. *Meliniomyces variabilis* differs from *R. ericae* in having thin glabrous to hairy colonies that are white to yellowish white and lacking arthroconidia or any other types of conidia. However, strains of *M. variabilis* that form darker grey colonies are more difficult to distinguish from *R. ericae*, except that colonies are generally thinner. On PDA or BAF medium (Summerbell, pers. comm.; for recipe see [www.dsmz.de/media/med392.htm](http://www.dsmz.de/media/med392.htm)), the hyphae of some strains of *M. variabilis* grow in a distinctive pinwheel or spiral pattern (Fig. 9). On rich media, a high proportion of hyphae will grow on top of the agar surface and, because hyphal tips actually rotate as they grow, those hyphae roll a bit and tend to grow on a curve in the direction of their tip rotation (Madelin *et al.* 1978). Although this characteristic is not unique to the species, it was not observed by us in strains of *R. ericae*.

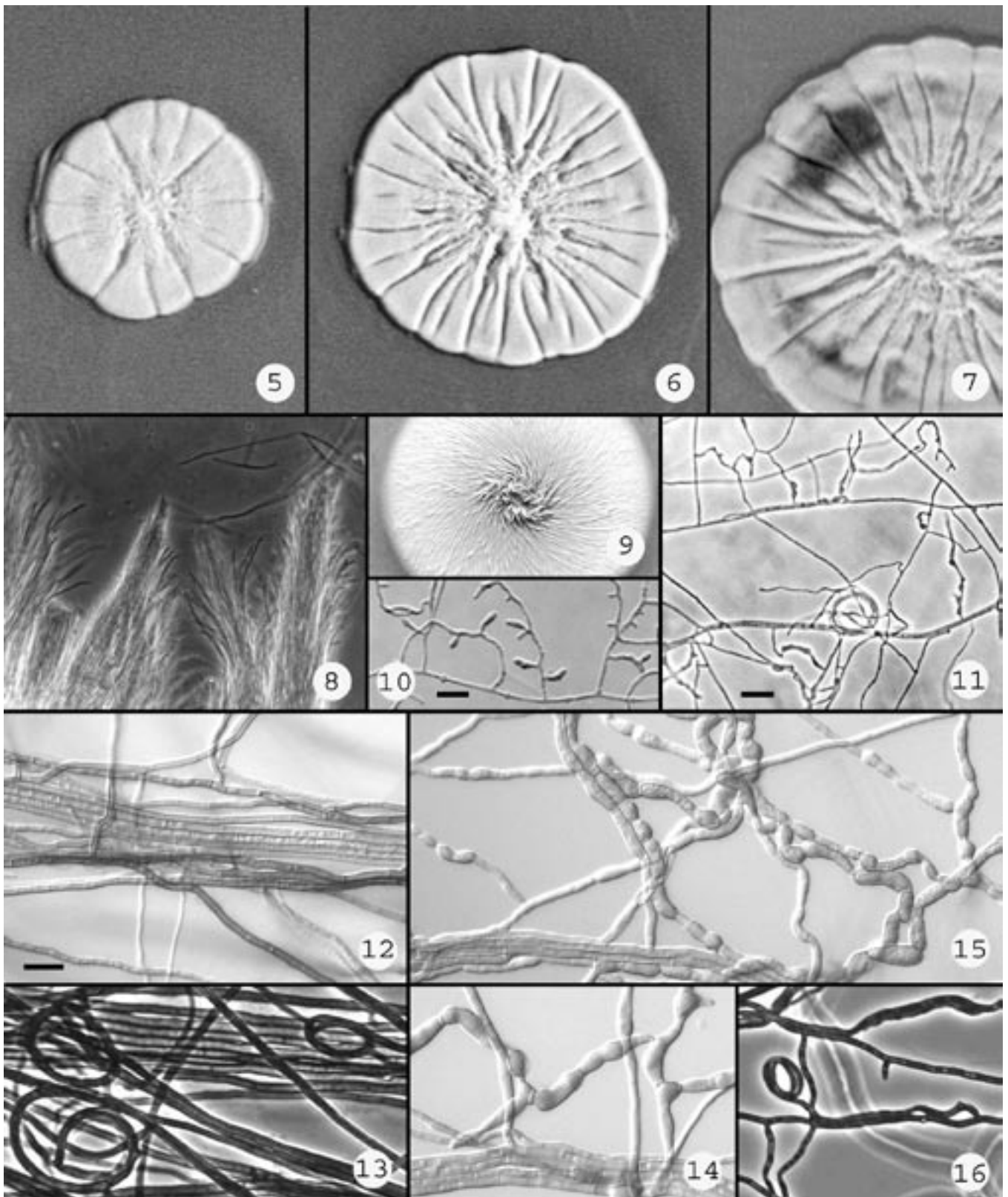
The detailed observations and colony characteristics published for strains from ectomycorrhizas by Schild *et al.* (1988) as D. S. type 2, Summerbell (1989) as SW1 and Vrålstad *et al.* (2000) as “morphology group 2”, were distinctive enough, given their origin in roots, to suggest a close relationship to our ericoid isolates and to illustrate that *M. variabilis* is recognisable without the aid of molecular characters. Those isolates of Schild *et al.* (1988, see Fig.1B), represented by UAMH 6826 and 6827, were described as “slower growing, lighter coloured isolates with fine septate hyphae, characterized by their production of upright

fascicles of sterile hyphae. The majority of such fasciculate isolates, designated D.S. type 2, initially produced a white to cream coloured colony which, on ageing, developed zones or patches of darker grey mycelium.” D.S. type 2 accounted for 32 % and 5 % (April collections), and 11 % and 31 % (November collections) of isolates from 2 sites in Ireland sampled over four years, and 64 % from an additional site sampled in November only. Percentages indicate the number of surface sterilized mycorrhizal segments sampled that yielded a culture.

A wide range in the frequency of isolation depending on site was also reported by Hambleton & Currah (1997), for whom the recognition of *M. variabilis* was aided by RFLP analysis. Surface sterilized roots of 19 species of *Ericaceae* were collected at three sites – a bog, an alpine heathland and a stable sand dune – over three years. Frequency of occurrence, expressed as the percentage of collections per site yielding a culture of *M. variabilis*, was 68 %, 13 % and 28 % respectively. The results of both studies suggest that the species is abundant in the rhizosphere but that there are edaphic factors influencing its distribution and relative frequency. Alternatively, the differences may be a result of factors related to sampling methods or to the range of endophytes colonizing roots in a specific habitat. Although there was a higher incidence of *M. variabilis* in roots collected from the bog, the faster growing dematiaceous fungus *Phialocephala fortinii* Wang & Wilcox was absent. The latter fungus, so prevalent as a root endophyte in the other two sites, may have out-competed *M. variabilis* as a root colonizer or masked its presence on the culture plate during isolation.

Isolates obtained by Summerbell (1989) and assigned here to *M. variabilis* were frequently obtained from ectomycorrhizas and were also relatively common from the assimilative roots of a co-occurring herbaceous endomycorrhizal species, *Cornus canadensis* L. Of 24 cultural groups of nonsporulating isolates distinguished, Summerbell noted that SW1 (represented by UAMH 5900 and 5979) and “Sterile dark 1” were strongly suspected of being conspecific, observing that “dark colonies occasionally gave rise to white sectors and vice-versa”. Serially washed as well as washed and surface-disinfected mycorrhizal roots were sampled but also feeder-root bark and the wash water from the serial dilutions. SW1 accounted for 11.2 %, 15.4 %, 2.6 %, and 1.0 % of isolates from each of those treatments, indicating that it was “abundant on mycorrhizae, very uncommon on bark and rarely isolated from wash water”, all observations consistent with a nonsporulating fungus exhibiting an endophytic life-strategy.

The basis of this endophytism in roots is unknown and results from resynthesis trials by other authors (see Table 2 and Fig. 2), conducted using varied



**Figs 5–16.** 5–11. *Meliniomyces variabilis*. Figs 5–7. UAMH 8861 ex-type strain on PDA shown at 3, 5 and 10 wk demonstrating the gradual darkening of colony pigmentation with prolonged incubation. 8. Fasciculate hyphae of UAMH 5979. 9. Close-up of UAMH 6826 on PDA at 35 d showing the spiral orientation of fascicles and surface hyphae. 10. Light micrograph of UAMH 8864 from slide culture at 51 d showing hyphae and hyphal loops. 11. Light micrograph of S-39a from slide culture at 35 d showing short swollen hyphal tips but no true conidia (previously printed in Hambleton & Currah 1997). 12–13. *Meliniomyces bicolor*. Light micrographs from slide cultures showing hyphal characteristics. Note the formation of hyphal strands and loops. 12. UAMH 10107, 28d. 13. UAMH 10108, 48d. 14–16. *Meliniomyces vraolstadiae*. Light micrographs from slide cultures showing hyphal characteristics. Note development of doliform or irregular swellings, hyphal loops, the aggregation of hyphae into strands. 14. UAMH 10112, 28d. 15. UAMH 10111, 35d. 16. UAMH 10111, 20 d. Scale bars = 10  $\mu$ m. Bar for 12–16 shown in 12.

experimental methods, are difficult to interpret. Some strains colonized roots of ectomycorrhizal host plants, forming intra- or inter-cellular structures but lacking a distinct mantle; others showed no colonization. There were no reports of a true ectomycorrhizal association being formed. UAMH 8863 and UBCM5 (AF081440) both colonized roots of an ericoid host. Only UAMH 8863 formed endomycorrhizal structures in roots of both host types. Several failed to colonize roots of either host type. Results were inconsistent for isolates from the same type of mycorrhizas. These inconsistent results are in contrast to those from the same studies for *R. ericae*, which was consistently ericoid mycorrhizal, and for Clade 4 isolates (Fig. 2) which were all ectomycorrhizal. Perhaps the ability to colonize plant roots is strain-specific, but this has not been rigorously tested. Perhaps an endophytic life-strategy is only one of several adopted by *M. variabilis*. At least some of the variation in response was probably due to suboptimal experimental conditions that failed to induce an expression of endophytic capability.

Standard (or optimal) conditions for ericoid mycorrhizal synthesis have not been established. While the methods for the containerised growth of ecto-forming host plants were relatively similar among the studies cited (Table 2), possibly based on experience gained from research aimed at the silviculture industry, conditions for ericoid-forming plants differed in temperature, light and other factors. The fungus was grown in the container before the seedling was added, or vice-versa, or both were added at the same time. Piercey *et al.* (2002) used the same conditions for both plant partners but their conditions differed from all other studies for both hosts. They used sphagnum as a growth medium in tube culture, rather than a petri dish chamber with agar, for resynthesis with ericoid hosts. Only one experiment cited has included the step of recovering the inoculated fungus from the *in vitro* colonized root, thus the fourth step of Koch's postulates has rarely been proven. Berch *et al.* (2002) reisolated the colonizing fungus and retested the mycorrhizal synthesis, but they did not resequence the isolated fungus to confirm its identity. A thorough assessment of experimental conditions is needed to develop a more uniform protocol for ericoid mycorrhizal synthesis experiments. The protocol should incorporate a reisolation / identification step to ensure no external or cross contamination escapes detection, though the risk is lower when the inoculant is sterile. Confirming the identity of a sterile inoculant would formerly have been a challenging task but the task is easier with molecular tools. Sampling from untreated and surface-sterilized inoculated roots, as well as the surrounding soil medium, would also provide an assessment of the vigour and survival of the fungal partner used.

The Group I introns identified in this study occurred at insertion sites previously reported in ascomycetes (Gargas *et al.* 1995, Gargas & DePriest 1996, Perotto *et al.* 2000, Hambleton *et al.* 2005–this volume). The introns themselves were not analysed further because this was not a focus of our study and, in some cases, would have necessitated the development of additional sequencing primers. The presence of introns at specific SSU rDNA sites was not conserved among strains of *R. ericae* (or among strains of *M. variabilis*), a result that concurs with those of a previous study by Egger and Sigler (1993), in which the presence or absence of an intron at site #1506, located immediately upstream from the universally conserved ITS1 priming site, was identified for multiple strains of *R. ericae*. Both studies showed that an intron was lacking at that location in the ex-type strain UAMH 6735 but was present in some of the other strains examined. When present, the intron sequences themselves demonstrated some usefulness for identification purposes. BLAST searches retrieved species-specific matches to intron sequences for specific insertion sites for both *M. variabilis* and *R. ericae*. For UAMH 10029, though, a substantially longer intron than those seen in *R. ericae* and other *M. variabilis* strains was observed at site #1506. Partial sequencing indicated that it differed substantially in length and in base composition from intron sequences at that site in other strains of *M. variabilis*.

***Meliniomyces bicolor*** Hambleton & Sigler, **sp. nov.**  
Mycobank MB500246, Figs 4I–J, 12, 13.

*Etymology*: *bicolor* (L) for mycobionts from the ectomycorrhizal morphotype "*Piceirhiza bicolorata*".

Similar *Meliniomyces variabilis*; coloniae in agaro velutinae, griseae; hyphae leves vel asperulatae, 1.5–3 µm latae.

*Typus*: UK: North Yorkshire, ex radice Nothofagus procera, A. Taylor (AT67), 1998. Colonia exsiccata et cultura viva, UAMH 10107.

Colonies on PDA and CER at 22 °C, slow growing (2.2–2.8 cm diam after 35 d); on PDA dark grey, felty to velvety, elevated with radial folds, margin entire or irregular; yellow diffusible pigment present in UAMH 10108. On CER, thin, flat, yellowish-white with streaks of grey. Hyphae in slide culture preparations on CER, septate, uniform in diameter with occasional swellings, 1.5–3 µm wide, smooth to slightly asperulate, subhyaline to brown, often aggregating into strands, forming occasional loops.

*Distribution*: Norway, Canada (British Columbia).

*Material examined*: Table 1 lists the provenance of the three living cultures examined.

*Molecular characters*: The ITS sequences for the strains of *M. bicolor* examined in this study



**Table 2.** Summary of previously published resynthesis experiments with isolates of *Meliniomyces variabilis*.

Reference; Isolates Used	Host Used	Resynthesis Methods	Resynthesis Results
Schild <i>et al.</i> 1988; D.S. type 2 (strain not specified; represented in this study by UAMH 6826, 6827) isolated from ecto host	ectomycorrhizal: <i>Picea sitchensis</i>	germinated seedlings and 3–4 wk old fungus inoculum added at same time to large glass test tubes using 1:12 peat moss-vermiculite mixture saturated with MMN <sup>1</sup> . 15h light / 9h dark; tubes partly sub- merged in water bath at 16 °C	definite intracellular invasion of cortical cells; seedlings remained healthy
Summerbell 1987; UAMH 5979 isolated from ecto host	ectomycorrhizal: <i>Picea mariana</i>	seedlings established in flask culture using vermiculite/peat moss soil mixture moistened with MMN (sugars removed); inoculated with blocks of mycelium, after first true leaves formed at 3 wk. 18h light / 6h dark; 18 °C	no fungal colonization formed in roots, no mantle formed but restricted areas of a thin structure resembling a mantle were observed
Piercey <i>et al.</i> 2002; UAMH 8863 UAMH 8864 isolated from ericoid hosts	ectomycorrhizal: <i>Picea mariana</i>	fungus established in pyrex tube culture on base of MMN agar overlaid with soil layer of sphag- num moss; after 10 d, 6–8 wk seedlings added. 18h light 23 °C / 6h dark 18 °C	ecto host roots: intra and intercellular hyphae in the epidermal and outer cortical cell layers, hyphae associated with root surface but no true mantle
	ericoid: <i>Ledum groenlandi- cum</i>		ericoid host roots: typical ericoid hyphal com- plexes formed in root cells by 8863 but not by 8864
Vrålstad <i>et al.</i> 2002b; ARON 2903.S AJ308338, UAMH 10113) ARON 2894.S (AJ308339, UAMH 10114) ARON 2879.S (AJ292201, UAMH 10115) isolated from ecto hosts	ectomycorrhizal: <i>Betula pubescens</i> <i>Picea abies</i> <i>Pinus sylvestris</i>	ecto hosts: 4 wk seedlings germinated on water agar added to flasks with vermi- culite / peat moss soil mixture moistened with MMN (sugars reduced) at same time as plugs of mycelium from 4 wk cultures natural light; max. 18 °C	no fungal colonization formed in roots of any host, no hy- phae associated with root surface
	ericoid: <i>Vaccinium vitis-idaea</i>	ericoid host: petri dish growth chambers used, fungus inoculated on carbon filters overlaid on low nutrient agar (MMN); after 1 mo at 25 °C, 2–3 wk seedlings added natural light; max. 18 °C	
Berch <i>et al.</i> 2002; UBCM5 (AF081440) UBCtra 323 (AF149083, UAMH 10420) isolated from ericoid hosts	ericoid: <i>Gaultheria shallon</i>	petri dish growth chambers used, seedlings established on low nu- trient agar (MMN); inoculated with fungus after real leaves emerged 18h light / 6h dark, 25 °C	UBCM5 formed typical ericoid hyphal complexes in root cells  UBCtra 323 ambiguous

<sup>1</sup> MMN – Modified Melin-Norkrans' nutrient solution

compiled at 98 % similarity. The consensus sequence is given in the following format: [three-prime 18S]ITS1\*5.8S\*ITS2[five-prime 28S]. NT in bold indicate differences among the sequences: nt underlined are changes, either transitions or transversions; nt in brackets are indels (nt present in some but not all of the sequences).

[CATT]AAGAATCGCCCCGTTTTTTGAAATGG  
GTTCTATTCCCAAACCGTGTATACATACCTTTG  
TTGCTTTGGCAGGCCGCCTTTTAGGGCGTCGGC  
TYCGGCTGACTGCGYCTGCCAGAGGACCCAA  
ACTCGTTTGTAGTGTCTGAGTACTATRT  
AATAGTTA\*AACTTTCAACAACGGATCTCTT  
GGTTCTGGCATCGATGAAGAACGCAGCGAAA  
TGCGATAAGTAATGTGAATTGCAGAATTCAGT  
GAATCATCGAATCTTTGAACGCAYATTGCGCC  
CCTTGGTATTCCGAGGGGCATGCCTGTTTCGAG  
CGTCATT\*ATAACCACTCAAGCCTCGGCTTGG  
TCTTGGGGTTCGCGGTCTCGCGKCCCTTAAAA  
TCAGTRGCGGTGCCGTCTGGCTCTAAGCGTAG  
TAATTCTCTCGCTATAGRGTCCTCCGGYGGYTRC  
CTGCCAGAA(C)CCCCCATTTTTTTAC[GTTGA].

*Additional information:* In Vrålstad et al. (2000) cultural and /or micro- morphology are shown after 1 and 8 wk on PDA for ARON 2805.S (UAMH 10107) in Fig. 3A and B, and for ARON 2893.S (UAMH 10108) in Fig. 3C and D.

*Comments:* The species is ectomycorrhizal with hardwood and conifer tree species. In Vrålstad et al. (2000), the ex-type strain UAMH 10107 (AT67; G9) is listed as an authentic culture isolate of “*P. bicolorata*” that “has previously been used to synthesise typical *P. bicolorata* ectomycorrhizae with Norway spruce” (p. 552). Resynthesis experiments for UAMH 10108 (G11) were reported in detail in Vrålstad et al. (2002b); it formed ectomycorrhizas with *Betula pubescens*, *Picea abies* and *Pinus sylvestris* but did not form ericoid mycorrhizas with *V. vitis-idaea*. Additional strains we have not seen, but assigned to this species based on ITS sequences with 97 % or greater similarity, are listed in Appendix C. Among these are two additional strains that have been tested for mycorrhizal capability, ARON 2906.S (G13) and ARON 2810.S (G12) (Vrålstad et al. 2002b). The initial identification provided on the GenBank accession record for UAMH 10356 (pkc34) as *C. finlandica* was not based on conidial characters but rather on BLAST matches and on a rough alignment and phylogeny (M. Berbee, pers. comm.).

Colonies of *M. bicolor* (Fig. 4I–J) are uniformly dark grey and felty to velvety, whereas those of the two other species treated here are white to greyish white or grayish black and glabrous to fasciculate. Hyphal swellings and loops are uncommon. No conidia are formed on any medium, including cereal agar.

*Meliniomyces vraolstadiæ* Hambleton & Sigler, **sp. nov.** MycoBank MB500247, Figs 4K–L, 14–16.

*Etymology:* *vraolstadiæ* (L) in honor of Dr. Trude Vrålstad.

Similis *Meliniomyces variabilis*; coloniae in agaroleves, albae vel griseae; hyphae leves, 1.5–3.5 µm latae, saepe cum tumoribus doliformibus vel irregularibus.

*Typus:* **Norway:** Akershus, Eidsvoll, ex radice *Betula pubescens*, T. T. Vrålstad (ARON 2916.S; G1), 1998. Colonia exsiccata et cultura viva, UAMH 10111.

Colonies on PDA and CER at 22 °C, slow growing (2.5–3.5 cm diam after 35 d); on PDA, elevated, furrowed, felty, slightly zonate, greyish black with narrow white margin (UAMH 10111, Fig. 4K), or glabrous with fascicles of hyphae, slightly zonate at the periphery, creamy-white to grey (10112, Fig. 4L). On CER, flatter with radial folds, felty, pale greyish brown with orange-grey margin (10111) or dark grey centrally with white margin (10112). Hyphae in slide culture preparations on CER, septate, subhyaline to brown, aggregating into strands and developing loops, 1.5–3.5 µm wide, often developing doliform or irregular swellings up to 5.5 µm wide.

*Distribution:* Norway.

*Material examined:* Table 1 lists the provenance of the two living cultures examined.

*Molecular characters:* The ITS sequences for *M. vraolstadiæ* compiled at 95 % similarity. The consensus sequence is given in the following format: [three-prime 18S]ITS1\*5.8S\*ITS2[five-prime 28S]. NT in bold indicate differences among the sequences: nt underlined are changes, either transitions or transversions; nt in brackets are indels (nt present in one but not both of the sequences).

[CATT]AAGAAT(C)GCCCCGTTTTTYGAAAYG  
GGTTCTATTCCCAAACCGTGTATACATACCTTT  
GTTGCTTTGGCGGGCCGCCTTCGGGCGTTGGC  
(T)TCYRGCTGAYTGCGCCCGCCAGAGGACCC  
AACTCGTTTGTTTARTGATGTCTGAGTACYA  
TATAATAG(T)TTA\*AACTTTCAACAACGGATC  
TCTTGGTTCTGGCATCGATGAAGAACGCAGCG  
AAATGCGATAAGTAATGTGAATTGCAGAATTC  
AGTGAATCATCGAATCTTTGAACGCACATTGC  
GCCCYTGGTATTCCGAGGGGCATGCCTGTTTC  
GAGCGTCATT\*ATAACCACTCAAGCCTTGGCT  
TGGTATTGGGG(T)TTCGCGGTTYCGCGGCYCT  
TAAAATCAGTGCGGTTGCCGTCTGGCTCTAAG  
CGTAGTAA(T)TTYTCTCGCTATAGGGTCCCGG  
YGGTGGCTTGCCARAACCCCC(T)TATTTTYC  
TAY[GTTGA].

*Additional information:* In Vrålstad et al. (2000) cultural and / or micro- morphology is shown after 1

and 8 wk on PDA for ARON 2916.S (UAMH 10111) in Fig. 4A–C and E, and for ARON 2917.S (UAMH 10112) in Fig. 4D.

*Comments:* Parsimony analysis clearly supports formal recognition of this taxon, although the degree of ITS sequence difference between the two strains is greater than that found for the other two described species of *Meliniomyces*. Colonial differences also were observed as noted in the description above. In resynthesis experiments, UAMH 10111 (G1) was nonmycorrhizal and UAMH 10112 (G2) was ectomycorrhizal (Vrålstad *et al.* 2002b). With the discovery of more representatives, additional characters may be found that clarify the genetic, morphological and ecological variation noted.

## DISCUSSION

The impetus for this study was to provide a name for the nonsporulating taxon we recognised based on both molecular and morphological characters, *M. variabilis*, a taxon for which experimental data were steadily accumulating. Parsimony analyses supported the species level distinction suggested by morphological similarities among examined strains, and indicated that the fungus is closely related to, but distinct from, *R. ericae* (*Leotiomyces*). In the process of this investigation, additional species of *Meliniomyces* were recognised, and a number of nomenclatural issues were raised. The molecular analyses presented here, and elsewhere, suggest that the “*H. ericae* aggregate”, as coined by Vrålstad *et al.* (2000, 2002a), is a natural group comprising several species. The group is known only from cultures, the expression of the teleomorph is uncommon, and conidial morphs are lacking or are inconsistently produced. Sterile (*Meliniomyces*), arthroconidial (*Scytalidium*) and phialidic (*Cadophora*) forms are allied to the group. In the context of phylogenetic concepts, *Meliniomyces* spp. would be named more appropriately in the genus *Rhizoscyphus*, but at present, the International Code of Botanical Nomenclature does not allow the application of a teleomorph name in the absence of the teleomorph (Greuter *et al.* 2000).

Should a teleomorph be discovered for *M. variabilis* (or any of the other allied species), we expect it will be an apothecial structure but there is no certainty that one will be found. Ascomata of *R. ericae* have never been recognised or collected from nature, and have been documented only rarely *in vitro*. In the case of the holotype, structures were produced in pot culture (Read 1974) and were not entirely mature (Hambleton *et al.* 1999). Despite reports that several strains have fruited in agar culture (Webster 1976, Vegh 1979;

specimens not preserved in herbaria as far as we are aware), the ex-type culture, UAMH 6735, has not produced ascomata under our conditions despite repeated attempts. UAMH 8680 produced ascomata in agar culture, using the method outlined by Webster (1976) but was the only one of 20 strains assayed that did so. It provided the basis for more detailed observations of ascomatal structure and ascospores (Hambleton *et al.* 1999).

Placing our new species in the anamorph genus *Scytalidium* Pesante was considered because it already includes *S. vaccinii*, the anamorph of *R. ericae* (Dalpé *et al.* 1989, Egger & Sigler 1993). *Scytalidium* was described for *S. lignicola*, which has thin-walled hyaline fission arthroconidia and thick-walled brown pigmented chlamydospores. Following Ellis’s description of the genus as having darkly pigmented conidia (1971), Sigler & Carmichael (1976) included several additional species based on form but these were not necessarily related. This led to a broad concept of the genus which is in need of revision. *Scytalidium vaccinii* is similar to *S. lignicola* in forming narrow hyaline to subhyaline arthroconidia, but differs in lacking chlamydospores. To examine the phylogenetic relationship between these two species, the ex-type strain of *S. lignicola*, was sequenced and included in the analyses (Figs 1, 3). The results were inconclusive, but they provided no strong basis for placing the new taxa in *Scytalidium*, nor did they counterindicate the decision to place the arthroconidial anamorph of *R. ericae* in *Scytalidium*.

A second consideration is that the arthroconidial state of *R. ericae* is not consistently observed. Of the substantial number of preserved *R. ericae*-like cultures, only some have exhibited the arthroconidial state while most are apparently sterile. In part, this may be a result of culture methods. Arthroconidia are often absent on PDA but are usually formed on CER within four to five weeks (Egger & Sigler 1993). Although CER is not available commercially and is not in common use by those studying root-associated fungi, it has proven useful for recognising and promoting sporulation among ericoid mycorrhizal fungi (Sigler & Gibas 2005—this volume). Nonetheless, even with long incubation times and special cultural conditions, attempts to induce the conidial state may be unsuccessful (Hambleton *et al.* 1999). Because molecular characterization now allows for the verification of nonsporulating isolates as *R. ericae*, it has become accepted practice to name both sterile and conidial morphs by the teleomorph name, as for example with UAMH 8873. The anamorph name is rarely applied.

The other anamorph allied to *R. ericae* is *Cadophora finlandica*, a relationship suggested previously by analyses of ITS data (Stoyke *et al.* 1992, Monreal *et al.* 1999, Grünig *et al.* 2002). Vrålstad *et al.* (2002a,

Fig 2, Clade 4E) demonstrated that a sequence for the ex-type strain of *Phialophora finlandica* C.J.K. Wang & H.E. Wilcox (AF011327; specific epithet corrected from “*finlandia*”, see www.MycoBank.org) grouped within the “*H. ericae* aggregate”. Harrington & McNew (2003) transferred the species to *Cadophora* Lagerb. & Melin, which they redefined to comprise *Phialophora*-like anamorphs allied to the *Leotiomycetes*. Their ITS sequence analyses, however, suggest that the genus is not monophyletic because *C. finlandica* grouped with *R. ericae* in a clade that was separate from and basal to the well-supported clade comprising the type species, *C. fastigiata* Lagerb. & Melin. There are now four ITS sequences independently derived from ex-type cultures of *C. finlandica*, and our analyses confirm its placement within the “*H. ericae* aggregate.” The ex-type strain of *C. finlandica* was isolated from mycorrhizal roots of *Pinus sylvestris* and produced conidia only after prolonged incubation in the cold, at 5 °C. It formed ecto- and ectendo- mycorrhizal associations with conifers (Wang & Wilcox 1985, Wilcox & Wang 1987). Cold treatment of the isolates that group with *C. finlandica* (Fig. 3A, Clade 4E) may induce conidiogenesis in some strains, while focused morphological studies could provide a sound basis for identifying strains in the absence of the phialidic state. This has been achieved with the root endophyte *P. fortinii*, another species that forms conidia sporadically and unreliably with cold temperature incubation, but can be identified using vegetative characters in the absence of conidiation (Currah & Tsuneda 1993).

In opting to erect the genus *Meliniomyces* typified by the sterile VWT, we follow in a tradition of naming recognisable and ecologically important species despite a lack of sporulation. Well-known root-associated genera in this category include *Rhizoctonia* DC. *sensu stricto*, considered as anamorphic *Ceratobasidiaceae*, and *Cenococcum* Moug. & Fr., allied to the *Dothideomycetes* (Lobuglio *et al.* 1996). As has been done for the xylariaceous *Muscodor* Worapong, Strobel & W. M. Hess, described in 2001 (Worapong *et al.* 2001) and now comprising three species, we provide molecular characters as part of the description of *Meliniomyces* species, in order to delimit the taxa within a phylogenetic context. Although many isolates of *M. variabilis* were examined, fewer strains were available for the other two species and the range of morphological variation may be greater than described. Several more species could be described if isolates are available for examination (arrowheads, Fig. 3B). If future systematic studies, and analyses of conserved gene regions for more members of the “*H. ericae* aggregate”, support the hypothesis that the group is monophyletic as shown here and by Vrålstad *et al.* (2002a), then it would be logical, from a phylogenetic viewpoint, to transfer both *S. vaccinii* and *C. finlandica*

to *Meliniomyces*, and emend the genus to encompass not only sterile but phialidic and arthroconidial forms. There is support for this on morphological grounds as well, as these fungi have similar colony characteristics and are often sterile.

Two sequences that grouped with *M. variabilis* were derived from fungal DNA extracted directly from roots and more were allied to other species in the “*H. ericae* aggregate”. These were among the large number of ITS sequences for unidentified, presumably sterile, cultures isolated from mycorrhizal roots and found to be allied to the *Leotiomycetes*, and the increasing number of unidentified ITS sequences generated from roots and other environmental sources of DNA, that are deposited in GenBank at various levels of the taxonomic hierarchy (e.g. Vandenkoornhuysen *et al.* 2002, Allen *et al.* 2003, Schadt *et al.* 2003). The number of publicly available ITS sequences is greater and generally more diverse than that of other genes for fungi, such that the ITS sequence has become the molecular datum of choice for identification purposes. While a certain percentage of unnamed sequences will represent members of previously unsequenced lineages, BLAST searches of many will suggest a relationship to a known taxonomic group. The value of making these sequences publicly available for taxonomic and ecological studies is unquestionable but the preponderance of unidentified ITS sequences allied to the *Leotiomycetes* may exceed the number for named inoperculate discomycetes.

Because many sequences are not analysed as part of a phylogenetic analysis and their taxonomic status is not resolved, the GenBank records can indicate classification at almost any level of the taxonomic hierarchy. For example, the sequences attributed here to *M. variabilis* (Table 1 and Appendix A) are presently deposited as *Hymenoscyphus* sp., at the order level as unclassified *Helotiales*, or at the Kingdom level simply as Fungi. Because of the difficulty of evaluating the taxonomic significance of small amounts of sequence divergence, depositors are understandably reluctant to assign provisional names to sterile fungi. Notations on the GenBank record of the closest taxonomic placement possible for an unnamed sequence would allow for more efficient retrieval based on keyword mining of the database. Detailed studies of groups of closely related sterile cultures, such as this one, potentially leading to the discovery of new characters and followed by identifications or descriptions of new taxa, are needed to facilitate information retrieval and discussion, and provide a solid foundation for additional experimental work and analyses. The deposition of representative strains in culture collections and their availability to other researchers is of primary importance. Even at the risk of over-interpreting the molecular data, assigning names will help the dialogue. In the

interests of tracking information and specimens, and to provide our herbaria, culture collections, and database providers with the tools to do this, such studies must be tackled. Representation from lineages comprising these taxa in future multi-gene phylogenetic studies of the *Leotiomyces* may in fact be critical for understanding relationships among the fungi we know as the inoperculate discomycetes.

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## APPENDIX A

Strains not listed in Table 1 and not examined were assigned to *Meliniomyces variabilis* based on ITS parsimony analysis. Sequences grouped in Clade 1, Fig. 3 and compiled in Sequencher<sup>TM</sup> with a minimum 97 % sequence similarity. Two sequences derived from DNA extracted directly from ectomycorrhizal root tips from Sweden, AF476981 and AF481384, also grouped with Clade 1 in preliminary analyses.

GenBank No.	Strain Identifier	Mycorrhizal Root Type Sampled	Host Genus	Country of Origin
AF081440 <sup>1</sup>	UBCM5	ericoid	<i>Gaultheria</i>	BC, Canada
AF149082	UBCtra43	ericoid	<i>Gaultheria</i>	BC, Canada
AF149084 (=AF149085) (=AJ430110)	UBCtra69 (UBCtra56) (ARON 2971.S)	ericoid	<i>Gaultheria</i> ( <i>Gaultheria</i> ) ( <i>Calluna</i> )	BC, Canada (BC, Canada) (Norway)
AF149086	UBCtra51	ericoid	<i>Gaultheria</i>	BC, Canada
AJ430105	ARON 2896.S	ericoid	<i>Calluna</i>	Norway
AY394886 (=AY394890)	pkc12 (pkc22 <sup>2</sup> )	ecto	<i>Tsuga</i>	BC, Canada
AY394887	pkc15	ecto	<i>Tsuga</i>	BC, Canada
AY394889 (=AY394899) (=AY394900)	pkc38 (pkc33) (pkc18)	ecto	<i>Tsuga</i>	BC, Canada

<sup>1</sup>Partial ITS sequence.

<sup>2</sup>Examined at UAMH but not retained due to heavy bacterial contamination.



## APPENDIX B

Source information for complete ITS sequences derived from cultures and assigned to *Rhizoscyphus ericae* based on ITS parsimony analysis. Sequences grouped in Clade 3, Fig. 3 and compiled in Sequencher™ with a minimum 97 % sequence similarity, except as noted. Duplicate genotypes (in brackets) were included in preliminary analyses only. Reference sequences for the species are annotated on Fig. 3A (names are in bold type) and were derived from the ex-type strain UAMH 6735 (AJ319078, AY762620), two additional authentic strains UAMH 6563 (AF151089) and UAMH 7357 (AF069505), ex-type strain of *Scytalidium vaccinii* UAMH 5828 (AJ319077), and UAMH 8680 (AY762621), teleomorphic strain, all isolated from ericoid roots from either the United Kingdom or Canada. Sequences (of which some are partial ITS) for six additional strains isolated from ericoid roots of *Gaultheria* from BC, Canada and deposited at UAMH are annotated on Fig. 3A: UAMH 10053 (AF081435), 10073 (AF300750), 10074 (AF149068), 10102 (AF300749), 10329 (AF300748), 10130 (AF081438). They all formed arthroconidia under our growth conditions.

GenBank No.	Strain Identifier	Mycorrhizal Root Type Sampled	Host Genus	Country of Origin
AB089664 (=AB089665) (=AB089666) (=AB089668)	E98013 (E98014) (E98018) (E98100)	ericoid	<i>Rhododendron</i>	Japan
AB089667	E98053	ericoid	<i>Rhododendron</i>	Japan
AF069439	21	liverwort	<i>Cephaloziella</i>	Eastern Antarctica
AF069440	BH	liverwort	<i>Cephaloziella</i>	Australia
AF149067	UBCtra141	ericoid	<i>Gaultheria</i>	BC, Canada
AF149069	UBCtra274	ericoid	<i>Gaultheria</i>	BC, Canada
AF252833	DGC23	ericoid	<i>Calluna</i>	U.K.
AF252834 (96 %)	GU23	ericoid	<i>Calluna</i>	U.K.
AF252835	GU27	ericoid	<i>Calluna</i>	U.K.
AJ308337 (=AJ430102) (=AJ430104) (=AJ430116) (=AJ430117) (=AJ430118)	ARON 2888.S (ARON 2886.S) (ARON 2884.S) (ARON 2996.S) (ARON 2997.S) (ARON 2998.S)	ericoid	<i>Calluna</i> ( <i>Calluna</i> ) ( <i>Empetrum</i> ) ( <i>Vaccinium</i> ) ( <i>Vaccinium</i> ) ( <i>Vaccinium</i> )	Norway
AJ430103	ARON 2887.S	ericoid	<i>Calluna</i>	Norway
AJ430106 (=AJ430108) (=AJ430109)	ARON 2967.S (ARON 2969.S) (ARON 2970.S)	ericoid	<i>Calluna</i>	Norway
AJ430107	ARON 2968.S	ericoid	<i>Calluna</i>	Norway
AJ430111 (=AY394907)	ARON 2922.S (pkc29)	ericoid	<i>Vaccinium</i> ( <i>Tsuga</i> )	Norway (BC, Canada)
AJ430112	ARON 2961.S	ericoid	<i>Vaccinium</i>	Norway
AJ430115	ARON 2995.S	ericoid	<i>Vaccinium</i>	Norway
AJ430168	ARON 3032.S	ecto	<i>Pinus</i>	Norway
AY046962 (96 %)	not available	ericoid	<i>Calluna</i>	Colorado, U.S.A.
AY046963	not available	ericoid	<i>Rhododendron</i>	Colorado, U.S.A.

Note. Additional duplicate and unique genotypes derived from DNA extracted directly from ectomycorrhizal roots were included in preliminary analyses only: from Norway AJ430130 (*Betula*) and AJ430159, AJ430169 AJ430170, AJ430171 (*Pinus*).

## APPENDIX C

Source information for sequences derived from cultures, included in the ITS-B data matrix (Fig. 3), and assigned to *Cadophora finlandica* (compiled in Sequencher™ with a minimum 98 % sequence similarity), *Meliniomyces bicolor* (97 % except as noted) or *Meliniomyces* sp. 1 through 4 based on parsimony analysis. Duplicate genotypes (in brackets) were included in preliminary analyses only. Reference sequences for *C. finlandica* are AF011327 (FAG 15), AF486119 and AY249074 (CBS 444.86) and AF081441 (UAMH 7454), all based on the ex-type strain isolated from ectendomycorrhizal roots of *Pinus* from Finland. Reference sequences for *M. bicolor* are AJ430147 (UAMH 10107), AJ292203 (UAMH 10108) and AY394885 (UAMH 10356). All sequences were derived from cultures except for two which were derived from DNA extracted directly from roots, AJ534703 and AJ534704.

Species	GenBank No.	Strain Identifier	Mycorrhizal Root Type Sampled	Host Genus	Country of Origin
<i>Cadophora finlandica</i> (Clade 4E)	AJ292202	ARON 2948.S (G8)	ecto	<i>Picea</i>	Sweden
	(=AJ430190)	(ARON 3055.S)			
	(=AJ430191)	(ARON 3057.S)			
	AJ430119	ARON 3010.S	ericoid	<i>Vaccinium</i>	Norway
	(=AJ430150)	(ARON 3015.S)	(ecto)	( <i>Salix</i> )	
	AJ430132	ARON 3041.S	ecto	<i>Betula</i>	Norway
	AJ430133	ARON 3042.S	ecto	<i>Betula</i>	Norway
	AJ430145	ARON 3060.S	ecto	<i>Betula</i>	Norway
	AJ430149	ARON 3008.S	ecto	<i>Populus</i>	Norway
	AJ430161	ARON 2983.S	ecto	<i>Pinus</i>	Norway
	AJ430165	ARON 3006.S	ecto	<i>Pinus</i>	Norway
	(=AJ430163)	(ARON 3004.S)			
	(=AJ430164)	(ARON 3005.S)			
	AJ430177	ARON 3043.S	ecto	<i>Pinus</i>	Norway
AJ430181	ARON 3048.S	ecto	<i>Pinus</i>	Norway	
(=AJ430182)	(ARON 3049.S)				
AJ534703	–		ecto	–	Estonia

Note. Additional duplicate and unique genotypes derived from DNA extracted directly from ectomycorrhizal roots were included in preliminary analyses only: from 1. Norway: AJ430123, AJ430129, AJ430134, AJ430146 (*Betula*); AJ430160, AJ430162, AJ430183, AJ430186, AJ430187, AJ430188, AJ430189 (*Pinus*) 2: Sweden: AF476977, AF481385. 3. U.S.A.: AY310847.

<i>Meliniomyces bicolor</i> (Clade 4C)	AJ308340	ARON 2810.S (G12)	ecto	<i>Picea</i>	Norway
	(=AJ430152)	(ARON 2938.S)			
	AJ308341	ARON 2906.S (G13)	ecto	<i>Populus</i>	Norway
	AJ430151	ARON 2936.S	ecto	<i>Picea</i>	Norway
	AJ430153	ARON 2953.S	ecto	<i>Picea</i>	Norway
	(=AJ430158)	(ARON 3003.S)			
	AJ430154	ARON 2954.S	ecto	<i>Picea</i>	Norway
	(=AJ430155)	(ARON 2955.S)			
	AJ430157	ARON 2958.S	ecto	<i>Picea</i>	Norway
	(=AJ430156)	(ARON 2956.S)			
	AJ430166	ARON 3018.S	ecto	<i>Pinus</i>	Norway
	(=AJ430167)	(ARON 3021.S)			
	AJ430174	ARON 3034.S	ecto	<i>Pinus</i>	Norway
	(=AJ430175)	(ARON 3035.S)			
AJ430180	ARON 3047.S	ecto	<i>Pinus</i>	Norway	
AJ534704	–		ecto	–	Estonia

Note. Additional duplicate and unique genotypes derived from DNA extracted directly from ectomycorrhizal roots were included in preliminary analyses only: from 1. Norway: AJ430124, AJ430125, AJ430131, AJ430135, AJ430136, AJ430137, AJ430138, AJ430139, AJ430140, AJ430141, AJ430142, AJ430143, AJ430144 (*Betula*); AJ430172, AJ430173, AJ430178, AJ430179, AJ430184, AJ430185 (*Pinus*) 2: Sweden: AF476973 (96 %), AF481386, AF481389 (96 %). 3. Finland: AJ633109. 4. Germany: AJ549974 (*Betula*). 5. U.S.A.: AY310848.

## APPENDIX C (Continued).

Species	GenBank No.	Strain Identifier	Mycorrhizal Root Type Sampled	Host Genus	Country of Origin
<i>Meliniomyces</i> sp. 1	AJ430126 (=AJ430127) (=AJ430128)	ARON 3024.S (ARON 3025.S) (ARON 3028.S)	ecto	<i>Betula</i>	Norway
<i>Meliniomyces</i> sp. 2 (Clade 4D)	AJ430122	ARON 2965.S	ecto	<i>Deschampsia</i>	Norway
<i>Meliniomyces</i> sp. 3 (Clade 4B)	AF149070 (=AJ430114)	UBCtra264 (ARON 2963.S)	ericoid	<i>Gaultheria</i> ( <i>Vaccinium</i> )	BC, Canada (Norway)
	AJ430113	ARON 2962.S	ericoid	<i>Vaccinium</i>	Norway
	AJ430121 (=AJ430120)	ARON 3014.S (ARON 3012.S)	ericoid	<i>Vaccinium</i>	Norway
<i>Meliniomyces</i> sp. 4 (Clade 4A)	AJ430176	ARON 3066.S	ecto	<i>Pinus</i>	Norway

