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RELATEDNESS OF THE ERICOID ENDOPHYTES *SCYTALIDIUM VACCINII* AND *HYMENOSCYPHUS ERICAE* INFERRED FROM ANALYSIS OF RIBOSOMAL DNA

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ABSTRACT

Phenotypic similarities between the root endophytes *Scytalidium vaccinii*, a hyphomycete, and *Hymenoscyphus ericae*, an ascomycete, led to speculation that *S. vaccinii* might be the anamorph of *H. ericae*. To test this hypothesis, we used the polymerase chain reaction to amplify portions of the ribosomal DNA from the ex-type cultures of each species and from cultures of several endophytes putatively identified as *Scytalidium* or *H. ericae* based on colonial and morphological similarities. An initial restriction fragment analysis of the amplified rDNA separated the *H. ericae* isolates from England from the ex-type culture of *S. vaccinii* and most isolates tentatively identified as *H. ericae* from North America. However, restriction mapping of the amplified fragment revealed that the apparent differences were an artifact resulting from the presence of one or two insertions in the small and large subunit ribosomal RNA genes of the North American isolates. Sequencing of homologous regions of the small subunit and the 5′ internal transcribed spacer indicated that sequence divergence between pairs of isolates identified as *Scytalidium* and *H. ericae* was low, ranging from 1.2–3.5%, as compared to divergence of up to 24% with *H. monotropae*, another ericoid endophyte. Although there was variation among the isolates, molecular and morphological evidence suggests that *S. vaccinii* and *H. ericae* are anamorph and teleomorph states of a single taxon.

Key Words: ericoid mycorrhizae, molecular systematics, nuclear ribosomal RNA genes, polymerase chain reaction

In 1973 Pearson and Read used a maceration technique which selectively isolated endophytic fungi from roots of ericaceous plants. When roots were macerated rather than directly plated, the majority of isolates were identified as "slow-growing dark mycelia" either with or without "segmenting hyphae." Both cultural types were capable of mycorrhiza formation and they demonstrated little host specificity within the Ericaceae. Similar cultural types recognizable by their hyphal segmentation were also isolated by dilution plating of soil and found to be mycorrhizal, but the authors speculated that nonsegmenting forms were probably underrepresented using this technique. One segmenting isolate was found to produce apothecia in association with roots of *Calluna vulgaris* (L.) Hull. following inoculation of seedlings in pot culture and was named *Pezizella ericae* Read (Read, **1974**). Although a brief cultural description was provided, only the teleomorph structures were illustrated. The hyphal segmentation was illustrated only in the earlier report (Pearson and Read, **1973**; FIG. 8) and was notable for the zig-zag arrangement of the segments or arthroconidia.

Kernan and Finocchio (**1983**) described another small, stipitate discomycete associated with roots of *Monotropa uniflora* L. as *Hymenoscyphus monotropae* Kernan & Finocchio. Together with Korf, they speculated that Read's fungus was closely related and proposed the new combination *H. ericae* (Read) Korf & Kernan. Although *H. monotropae* fruited in pot culture in association with roots, it failed to fruit in axenic culture and its cultural characteristics were not described.

Although *H. ericae* seems to have a fairly widespread distribution in Europe, where both sterile

and fruiting isolates have been found in association with ericaceous plants (Perotto et al., **1990**; Vegh and Gianinazzi-Pearson, **1979**), Litten et al. (**1985**) observed that the inconspicuous apothecia had not been found in North American habitats. An endophyte from cortical cells of native Maine *Vaccinium angustifolium* Ait. was determined as *H. ericae* by Dr. Y. Dalpé (pers. comm.) on the basis of cultural and morphological similarities with an authentic strain and by its formation of typical ericoid mycorrhizae. A second ericoid mycorrhizal fungus isolated from the same host species demonstrated some differences and was described as *Scytalidium vaccinii* Dalpé, Litten & Sigler (Dalpé et al., **1989**). Subsequently, examination by the junior author (LS) of several additional isolates putatively identified as *H. ericae* indicated that these taxa may be more similar than originally thought. The purpose of this study was to test the hypothesis that *S. vaccinii* is the anamorph of *H. ericae*.

MATERIALS AND METHODS

All of the studied strains are maintained in the University of Alberta Microfungus Collection (UAMH) and are listed in TABLE I. Observations on colonial morphology and growth rates were made on 10% Pablum cereal agar (CER) (Padhye et al., **1973**) incubated at 25 C; slide cultures were used to examine microscopic features. Colony color terminology follows Kornerup and Wanscher (**1978**).

For DNA extraction, isolates were grown on CER or E-strain agar (Egger and Fortin, **1990**). Plugs were removed with a cork borer (4 mm diam) from the edge of a growing colony, freeze-dried and stored desiccated at room temperature. Nucleic acids were isolated from approximately 50 mg of the freeze-dried material using the miniprep protocol of Zolan and Pukkila (**1986**). The samples were diluted 1:10 or 1:20 with sterile distilled deionized water, then 1 μl of the dilution was used as a template to amplify a portion of the ribosomal DNA using the polymerase chain reaction (PCR). Reactions were carried out in 100 μl volumes containing 2 units of *Taq* DNA polymerase (Promega Corp.), 50 mM KCl, 10 mM Tris-HCl (pH 9 at 25 C), 1.5 mM $MgCl_2$, 0.01% gelatin (w/v), 0.1% Triton X-100, 50 μM each of dATP, dCTP, dGTP, and dTTP, and 0.4 μM each oligonucleotide primer. Samples were amplified on a DNA Thermal Cycler (Perkin Elmer Cetus) using the following cycle parameters: 94 C for 45 sec, 46 C for 60 sec, and an extension step at 72 C for 120 sec initially and increased by 1 sec per cycle, using the maximum ramp time between each temperature. The total number of cycles was 30 with an initial denaturation step of 2 min and a final extension step of 5 min. Nucleic acids were precipitated by addition of 1/10 volume of 3 M sodium acetate followed by two volumes of cold 95% ethanol and collected by centrifugation (6500 rpm, 3 min, 25 C). The pellet was washed twice with 500 μl of 70% ethanol, then dried overnight in a vacuum desiccator. The pellet was resuspended in 50 μl of TE buffer [10 mM Tris-HCl (pH 8 at 25 C), 1 mM EDTA] and stored at −20 C.

An initial comparison of restriction fragment patterns of the isolates was performed by digesting approximately 1 μg of DNA amplified using primers ITS9mun and NL10mun with the restriction endonucleases *Alu*I, *Cfo*I, *Cla*I, *Eco*RI, *Hae*III, *Hin*fI, *Msp*I, *Nde*II, and *Rsa*I (Boehringer Mannheim) for 3–5 h at 37 C using the buffer provided by the manufacturer. After digestion the samples were loaded on a 2% Nusieve/0.5% Seakem (FMC Bioproducts) agarose gel and fragments separated by electrophoresis in Tris-borate buffer followed by staining with ethidium bromide.

The fragment amplified with primers ITS9mun and ITS10mun was mapped from a subset of isolates representing each unique restriction fragment pattern using single and double digests with the restriction endonucleases *Bam*HI, *Cla*I, *Eco*RI, *Sca*I, and *Xba*I (Boehringer Mannheim). In addition, we amplified a smaller region delineated by the primers ITS9mun and ITS4 and mapped this fragment using the following restriction endonucleases that cleave at 4 or 5 base recognition sites: *Alu*I, *Cfo*I, *Hin*fI, *Msp*I, *Mva*I, and *Rsa*I (Boehringer Mannheim). The amplified fragments were precipitated and subjected to electrophoresis as described above. The ribosomal DNA was also mapped for position of several primer annealing sites within the ribosomal RNA gene repeat by PCR amplification as described above, except the annealing temperature was lowered to 37 C.

Primers ITS9mun and ITS10mun were used to amplify a portion of the ribosomal DNA for sequencing using an Applied Biosystems Model 373A automated DNA sequencer. Fragments were amplified with either a 37 or 42 C annealing temperature and precipitated as previously described. Sequencing reactions were carried out directly from amplified double-stranded DNA template with a *Taq* DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems) using the conditions recommended by the manufacturer. Following cycle sequencing, samples were precipitated by adding a double volume of isopropanol, then centrifuged (13 000 rpm, 15 min, 25 C). The pellet was washed twice with 500 μL of 70% ethanol before loading on a 6% polyacrylamide gel attached to the DNA sequencer.

Primers ITS1, ITS3, ITS4, and ITS5 are from White et al. (**1990**). Sequences of other oligonucleotide primers are: ITS9mun—TGTACACACCGCCCGTCG, ITS10mun—GCTGCGTTCTTCATCGAT, and NL10mun—GGAACCTTTCCCCACTTC (all sequences are written 5′ to 3′). Letter designations follow the convention in White et al. (**1990**): primers with odd numbers anneal to the coding strand; primers with even numbers anneal to the noncoding strand. The label "mun" stands for Memorial University of Newfoundland. Annealing sites are shown in FIG. 15.

RESULTS

Morphology.—Although some differences could be observed between the ex-type cultures of each taxon when examined individually, examination

TABLE I

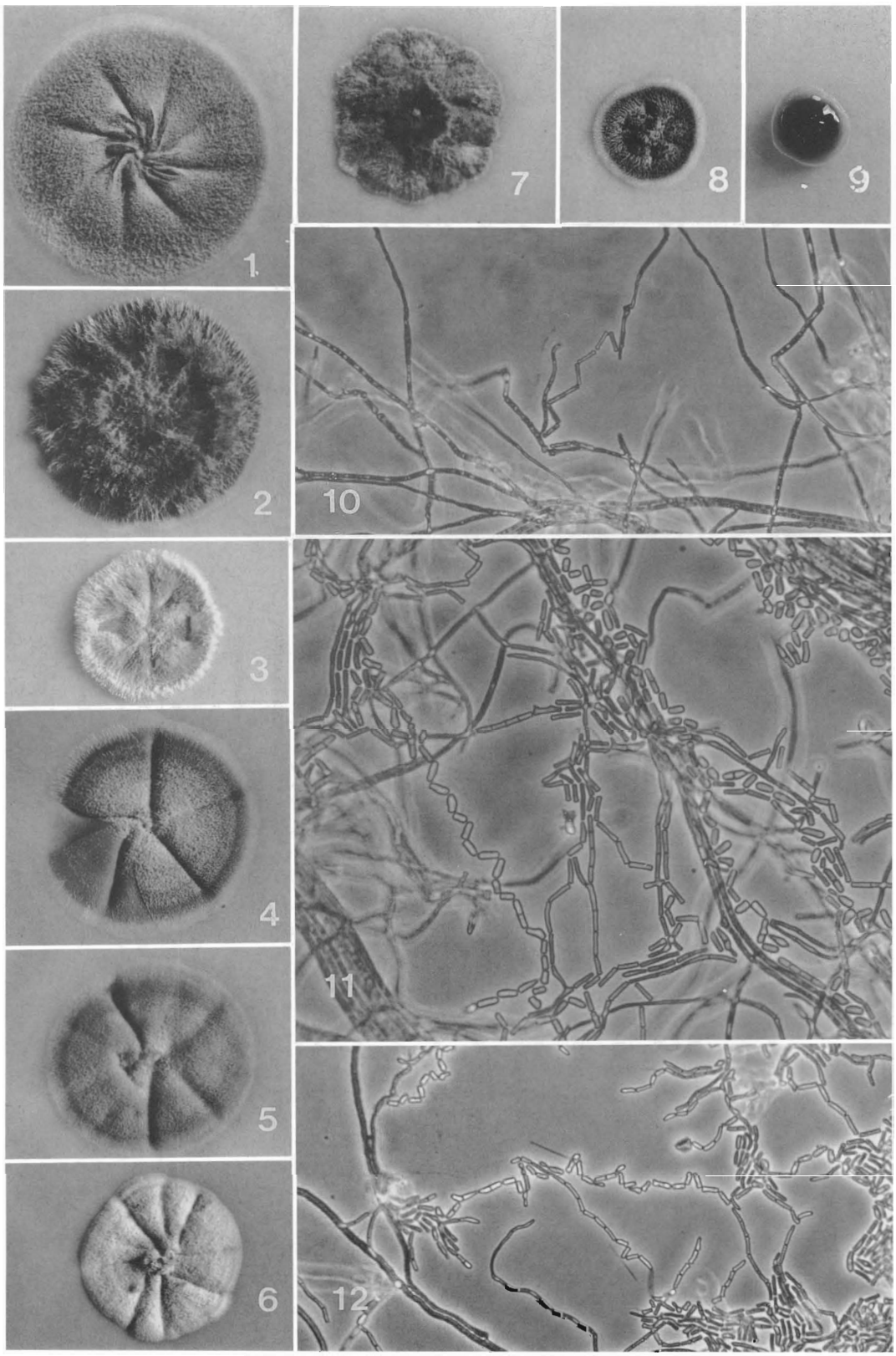
SOURCE AND MORPHOLOGICAL FEATURES OF SPECIES AND ISOLATES STUDIED

UAMH number[a]	Identification	Host and geographic origin	Determiner	Source of isolate[b]	Arthro-conidia	Growth rate CER (mm/da)[c]
5828 (T)	*Scytalidium vaccinii*	*Vaccinium angustifolium*, Washington Co., Maine	Y. Dalpé & L. Sigler	DAOM 196925	+	1.2
6735 (T)	*Hymenoscyphus ericae*	*Calluna vulgaris*, Bolsterstone, Yorkshire, United Kingdom	D. Read	IMI 182065	+	1.4
6513 (A)	*Hymenoscyphus ericae*	?Ericaceae, Sheffield, United Kingdom	D. Read	DAOM 185550	+	1.0
6563 (A)	*Hymenoscyphus ericae*	?unknown	D. Read	Read 100	+	1.4
6561	*Hymenoscyphus ericae*	*Vaccinium angustifolium*, Bar Harbor, Maine	Y. Dalpé	DAOM 195234	+	1.2
6562	*Hymenoscyphus ericae*	*Vaccinium angustifolium*, Bar Harbor, Maine	Y. Dalpé	DAOM 195235	+	1.1
6598	?*Hymenoscyphus ericae*	*Vaccinium angustifolium*, Alma, Quebec	L. Sigler	DAOM 191322 (Couture 98b)	−	1.3
6599	?*Hymenoscyphus ericae*	*Vaccinium angustifolium*, Alma, Quebec	L. Sigler	DAOM 191323 (Couture 98a)	−	1.2
6600	?*Hymenoscyphus ericae*	*Vaccinium angustifolium*, Alma, Quebec	Y. Dalpé	DAOM 191324 (Couture 82)	+	1.0
6663	?*Hymenoscyphus ericae*	*Vaccinium corymbosum*, Alma, Quebec	M. Couture	DAOM 185115 (Couture 2)	+	1.3
6045	*Scytalidium* sp.	Human specimen, Windsor, Ontario	L. Sigler	OMH FR1538.87	+	1.1
6601	?Sterile unidentified	*Vaccinium angustifolium*, ?Quebec		DAOM 191326 (Couture 20)	−	0.6
6650 (T)	*Hymenoscyphus monotropae*	*Monotropa uniflora*, New York	M. Kernan	ATCC 52305	−	ND
1405	*Oidiodendron rhodogenum*	Wood pulp sludge, Norway	H. Robak	DAOM 75838	+	ND

[a] T = culture derived from type specimen; A = authentic, examined by original author.

[b] Cultures obtained from the following collections or individuals: DAOM, Canadian Collection of Fungus Cultures, Ottawa, Canada; ATCC, American Type Culture Collection, Rockville, Maryland; IMI, International Mycological Institute, Kew, United Kingdom; OMH, Dr. R. C. Summerbell, Ontario Ministry of Health, Toronto, Canada; Dr. D. Read, Dept. of Botany, Univ. of Sheffield, Sheffield, United Kingdom.

[c] ND, not determined.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of other isolates determined as *H. ericae* or *Scytalidium* (TABLE I) demonstrated intermediate colonial forms (FIGS. 1–7). All isolates were slow growing. UAMH 6735, ex-type culture of *H. ericae,* grew faster and its colonies remained whitish-grey (7E/F2–4), with a white margin, flat with radiating furrows and a fine nap of aerial hyphae (FIG. 1). In contrast, UAMH 5828, *S. vaccinii,* grew slightly slower and colonies were dark greyish brown (6–7E/F4) with raised fascicles of reddish brown (7E6) hyphae and a white margin (FIG. 2). Two isolates from the United Kingdom, UAMH 6513 and 6563, identified by Read as *H. ericae,* demonstrated colonies somewhat intermediate between these two types: 6513 was among the slower-growing forms and had light grey-brown colonies with tufts of aerial hyphae near the margin (FIG. 3), whereas 6563 was faster-growing and had light grey colonies similar to those of the ex-type of *H. ericae.* Most isolates from North America formed light or dark grey-brown colonies (FIGS. 4–6) often with a light margin and light reverse, but none developed the reddish-brown hyphal fascicles as seen in *S. vaccinii* on CER. Three isolates, UAMH 6598, 6599 and 6601 (Couture et al., **1983**) failed to produce arthroconidia (TABLE I); however, two of them (FIG. 4) formed similar colonies which were light to dark grey brown, flat with few radial folds, and low aerial nap. These features, along with their isolation from roots of ericaceous plants, suggested an affinity with authentic *H. ericae.* In contrast, colonies of 6601 were black with a black reverse and a white glabrous margin (FIGS. 8, 9) and grew more slowly. Droplets of pale brown exudate occurred on the colony surface, and on some media, such as potato dextrose agar (Difco), colonies appeared to be covered with slime (FIG. 9).

Most isolates formed arthroconidia profusely when grown on CER. Vegetative hyphae were subhyaline or pale brown and often aggregated into strands. The fertile hyphae were slightly narrower and they fragmented by schizolytic dehiscence to form cylindrical, hyaline or subhyaline, 0-to-1 septate arthroconidia measuring 3–11 × 1.5–2.5 (−3) μm, commonly 3–7 × 1.5–2.5 μm. The propensity of the arthroconidia to remain connected in zigzag chains (FIGS. 10–14) was a unifying characteristic of all the strains studied. This striking feature was also noticed by Pearson and Read (**1973**) in their first isolations of *H. ericae.*

Molecular analysis.—The length of the fragment amplified by primers ITS9mun and NL10mun ranged from 2.1 to 2.7 kilobases (kb). An initial comparison of isolates based upon comigration of restriction fragments (results not shown) identified two main groups: one contained the authentic *H. ericae* isolates from the United Kingdom (6513, 6563, 6735) plus one isolate from North America (6663); the second contained the ex-type of *S. vaccinii* (5828), an isolate tentatively identified as *Scytalidium* (6045), and several North American isolates putatively identified as *H. ericae* (6561, 6562, 6598, 6599, 6600) or considered of uncertain affinity (6601) (TABLE I). However, restriction mapping revealed that the size difference among isolates was due to the presence of either one or two large insertions (approx. 350 bp each), one in the small subunit rRNA gene exactly 31 base pairs upstream of the 3′ end, and the other in the large subunit rRNA gene about 950 bp downstream of the 5′ end (see FIG. 15). Thirty restriction sites were mapped. Of these, 23 occurred in the homologous regions common to all isolates while six were in the first insert and one was in the second insert. Of the 23 sites in homologous regions common to all isolates, 10 were invariant.

As a further confirmation of similarity among isolates, DNA sequences of the fragment amplified by primers ITS9mun and ITS10mun were aligned (FIG. 16) and compared using sequence editing software (Applied Biosystems). The nucleotide at position 8 was highly ambiguous in all sequencing runs. Since it could not be deter-

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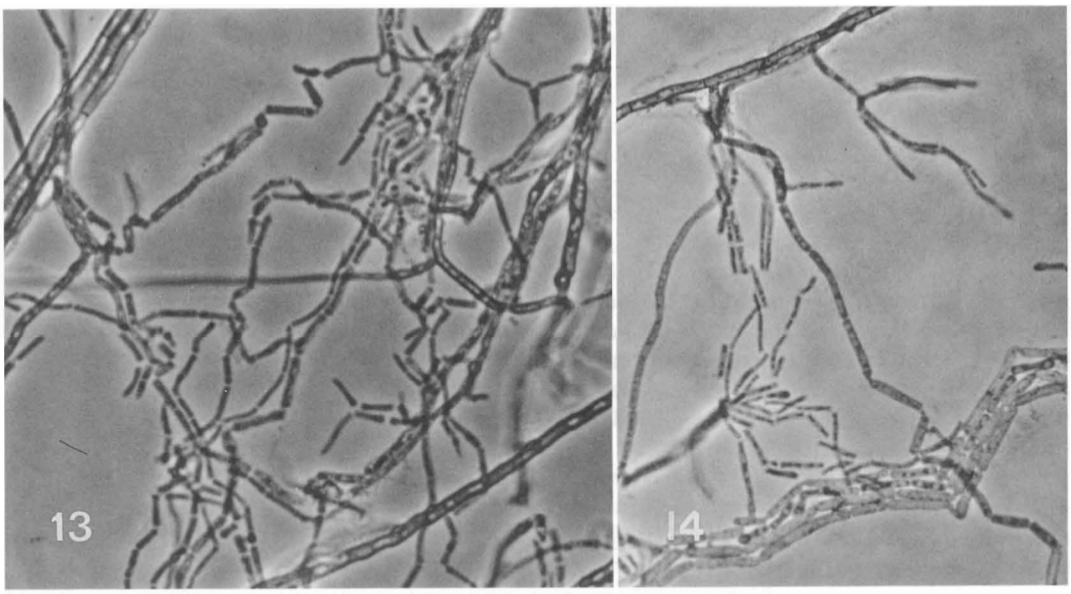
FIGS. 1–12. Colony types and microscopic morphologies of *Hymenoscyphus ericae, Scytalidium vaccinii* and other isolates. 1–9. Colonies on CER after 35 da at 25 C, except where noted. All ×0.8. 1. UAMH 6735, ex-type of *H. ericae.* 2. UAMH 5828, ex-type of *S. vaccinii.* 3. UAMH 6513, authentic isolate of *H. ericae.* 4. UAMH 6598 at 28 da. 5. UAMH 6562. 6. UAMH 6600 at 28 da. 7. UAMH 6045. 8. UAMH 6601 at 28 da. 9. UAMH 6601 at 39 da on potato dextrose agar. 10–12. Narrow, cylindrical arthroconidia forming in zigzag chains. 10. UAMH 6735, ex-type of *H. ericae,* ×460. 11. UAMH 6563, authentic isolate of *H. ericae* from the United Kingdom, ×610. 12. UAMH 6663, isolate from North America, ×460.



FIGS. 13–14. *Scytalidium vaccinii* and *Scytalidium* species. Narrow cylindrical arthroconidia forming by schizolytic dehiscence. Note zigzag chains. 13. UAMH 5828, ex-type of *S. vaccinii*, ×610. 14. UAMH 6045. Isolate from human patient in North America, ×610.

mined reliably, it was coded as "N." The alignment clearly shows the sequence divergence of *H. monotropae*, which differed from the consensus sequence at 44 positions and has several unique insertions/deletions. It also differed in cultural features demonstrating greyish white, flat, woolly colonies and lacking an arthroconidial stage. The remainder of the isolates tested were much more homogeneous, differing at from 1 to 5 positions from the consensus sequence and with all insertions/deletions conserved.

To compare isolates independently of the insertions, a data matrix was produced based upon homologous restriction sites in regions flanking the inserts. A distance matrix based upon estimates of nucleotide substitutions per site was generated from these data by the program REAP (McElroy et al., **1991**) using the formula of Nei and Li (**1979**). A second distance matrix was independently derived from the aligned DNA sequences (minus the insert) by the program REAP using the two-parameter model of Kimura (**1980**). These matrices are shown in TABLE II. The estimates of nucleotide substitution rates derived from the sequence data were higher than those from the restriction site data. This is likely because the ITS9mun/NL10mun fragment contains a higher proportion of conservative gene coding regions, and because the sequence estimates give greater weight to transversions over transitions. (The ratio of transversions to transitions is not known for the restriction map data.) However, the restriction site maps did detect some variation among isolates that was not evident from the sequence data.

In order to incorporate the variation detected by restriction mapping in the analysis, all restriction sites outside of the region sequenced were coded as transitions and added to the sequence data matrix. This combined data matrix was used to generate a distance matrix using the formula of Nei and Li (**1979**), then subjected to UPGMA

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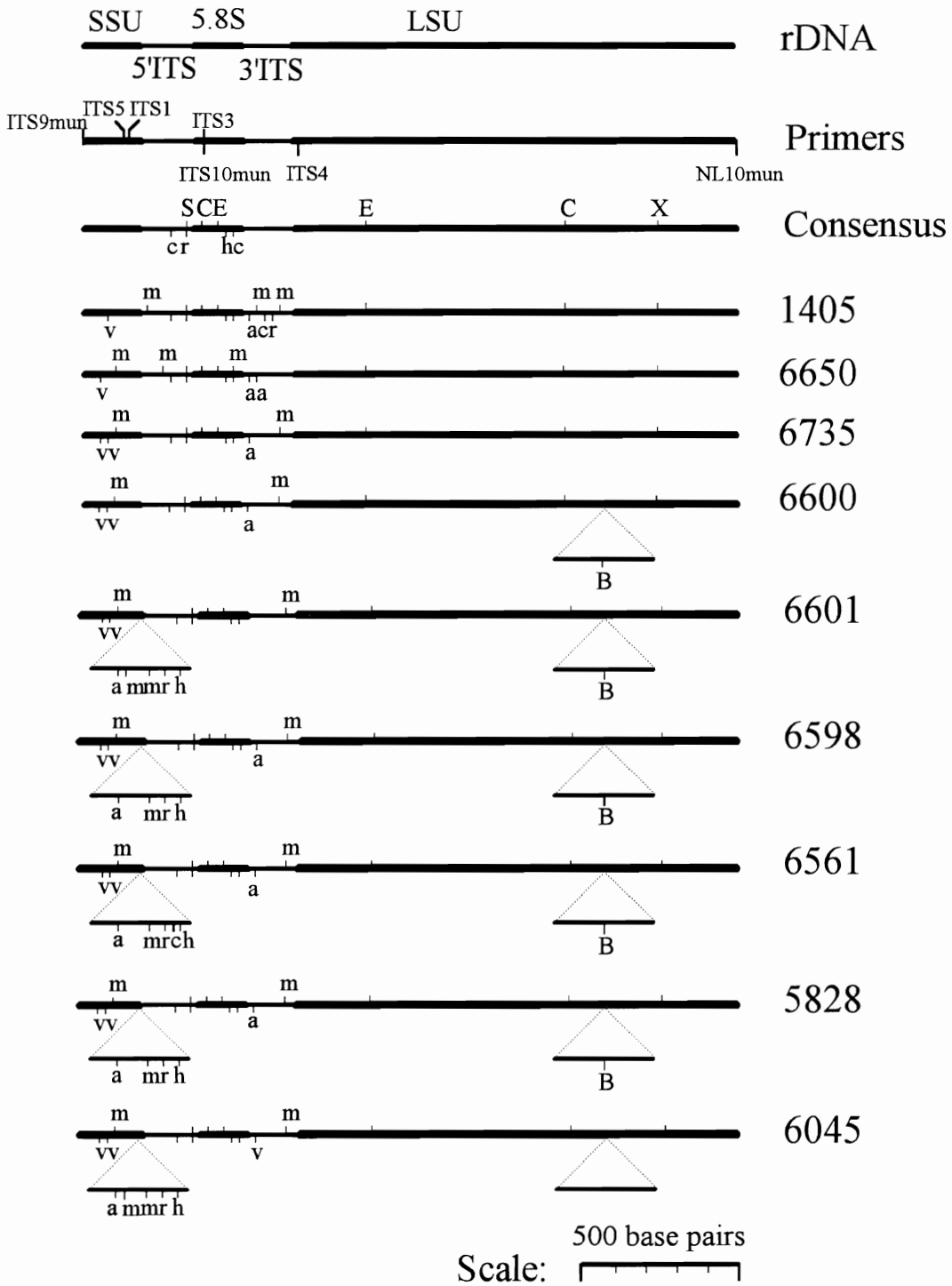
FIG. 15. Restriction site maps for *Scytalidium* and *Hymenoscyphus* isolates. The first map shows the small subunit (SSU), 5.8S subunit, and large subunit (LSU) portions of the ribosomal RNA genes and the 5′ and 3′ internal transcribed spacers (ITS). The second map shows the annealing sites of the primers; primers above the line prime the coding strand, primers below the line prime the noncoding strand. The third map shows position of consensus restriction sites common to homologous regions of all isolates. Individual maps labelled with the



UAMH isolate number show variable sites present in that isolate. The extended portions seen in isolates 6600 to 6045 represent the insertions and the dotted lines indicate their position. Restriction endonucleases used were: B—*Bam*HI, C—*Cla*I, E—*Eco*RI, S—*Sca*I, X—*Xba*I, a—*Alu*I, c—*Cfo*I, h—*Hin*fI, m—*Msp*I, r—*Rsa*I, v—*Mva*I.

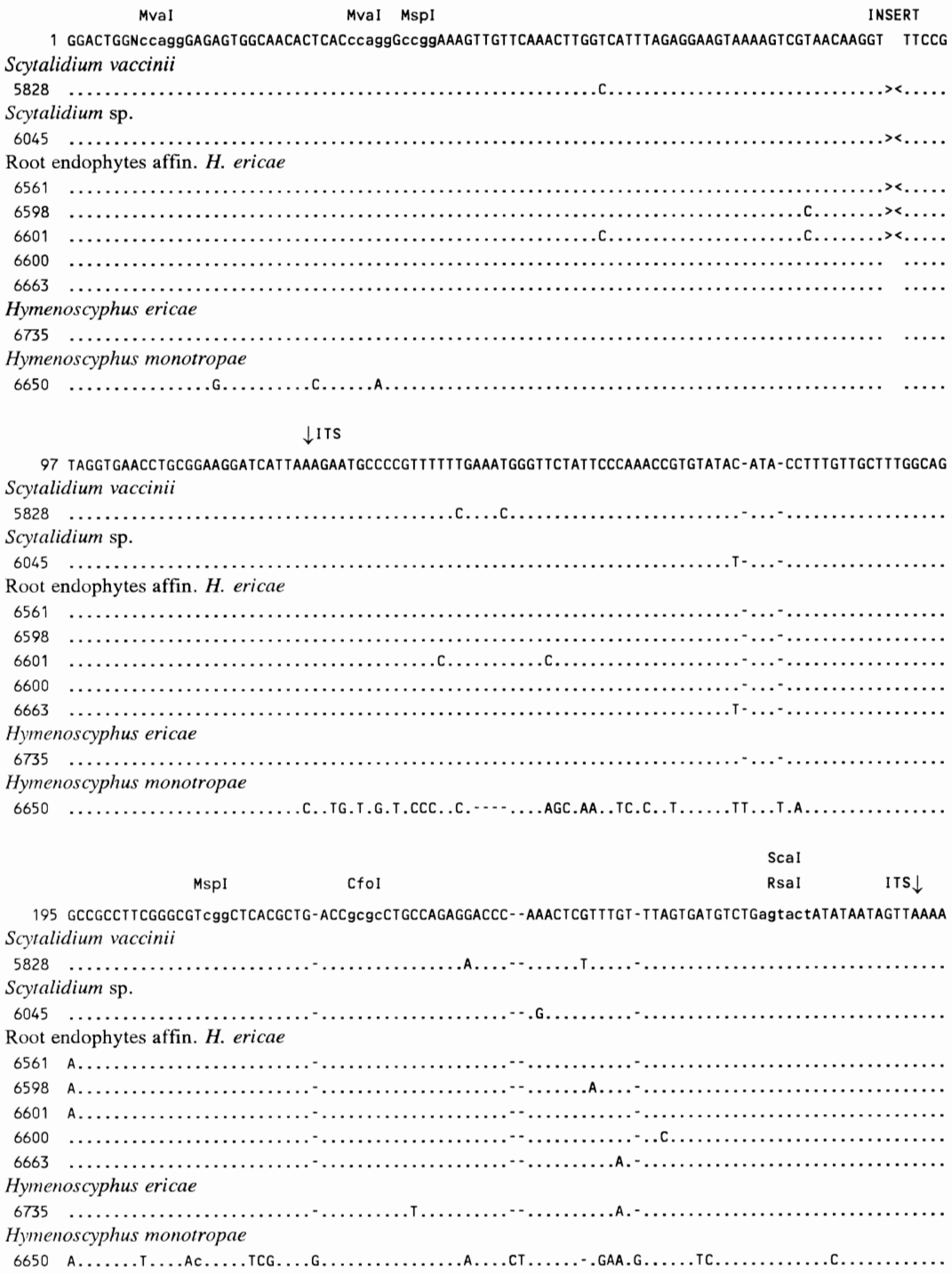


FIG. 16. Aligned sequences of a 292 basepair portion of the small subunit ribosomal RNA gene and the 5′ internal transcribed spacer. The position of the optional insertion and the first and last base in the ITS are indicated. The consensus sequence for all isolates is shown first (the first nucleotide in each row is numbered at the left). Only nucleotides that differ from the consensus sequence are shown for each isolate; identical

TABLE II

EVOLUTIONARY DISTANCE ESTIMATES[a] FROM RIBOSOMAL RNA GENE SEQUENCES

	5828	6045	6561	6598	6600	6601	6663	6735	6650
5828		0.0251	0.0214	0.0287	0.0214	0.0288	0.0251	0.0251	0.1460
6045	0.0192		0.0106	0.0178	0.0106	0.0252	0.0071	0.0142	0.1505
6561	0.0000	0.0192		0.0071	0.0071	0.0142	0.0106	0.0106	0.1460
6598	0.0000	0.0192	0.0000		0.0142	0.0142	0.0178	0.0178	0.1548
6600	0.0000	0.0192	0.0000	0.0000		0.0214	0.0106	0.0106	0.1548
6601	0.0063	0.0131	0.0063	0.0063	0.0063		0.0252	0.0252	0.1596
6663	0.0063	0.0134	0.0063	0.0063	0.0063	0.0130		0.0071	0.1415
6735	0.0063	0.0134	0.0063	0.0063	0.0063	0.0130	0.0000		0.1505
6650	0.0410	0.0539	0.0410	0.0410	0.0410	0.0518	0.0361	0.0361	

[a] The lower-left half of the matrix gives distance estimates (average number of nucleotide substitutions per site) based upon restriction maps; the upper-right half gives distance estimates based upon comparison of aligned DNA sequences.

cluster analysis using the program NTSYS-pc (Rohlf, **1992**) to show groupings among isolates (FIG. 17). The main division was between *H. monotropae* and the remainder of the isolates, with separation occurring at a level of approximately 0.17 nucleotide substitutions per site. The relationships between isolates in the *H. ericae-S. vaccinii* cluster was not as clear, with isolates in this group differing by less than 0.03 nucleotide substitutions per site. The cluster analysis resulted in three tied trees that varied in placement of isolate UAMH 6600 with either the 6561/6598 group or with the 6663/6735 group; only one of these trees is shown in FIG. 17. We caution that the phenogram presented does not necessarily reflect phylogenetic relationships. A cladistic analysis of the sequence data using the program PAUP (results not shown) was unable to resolve the *H. ericae-S. vaccinii* isolates with any confidence, suggesting there were homoplasies and too few phylogenetically informative sites for a meaningful phylogenetic analysis.

DISCUSSION

The internal transcribed spacer (ITS) has been sequenced from other fungal taxa. Lee and Taylor (**1992**) compared intraspecific variability in the ITS among taxa of *Phytophthora*. Several taxa showed no intraspecific variation, but *P. citrophthora* (R. & E. Smith) Leonian exhibited 2.4–4.2% divergence in the 5′ ITS among 3 isolates, and *P. cinnamomi* Rands showed 4.6% divergence (although only 2 of 17 isolates were variable). Baura et al. (**1992**) compared ITS sequences from *Gastrosuillus laricinus* (Singer & Both) Thiers, *Suillus grevillei* (Kl.) Singer, and *S. spraguei* (Berk. & Curt.) Kuntze. They found up to 3% divergence in sequence among three isolates of *S. grevillei* and 12% divergence between *S. grevillei* and *S. spraguei*. *Gasterosuillus laricinus,* although differing considerably in morphology, exhibited no divergence from *S. grevillei*. Gardes et al. (**1991**) sequenced the 3′ ITS from *Laccaria* and *Thelephora terrestris* Ehrhart: Fr. They found that three *L. bicolor* (R. Maire) Orton strains differed by 1–2% in sequence and were divergent from *L. laccata* (Scopoli: Fr.) Berk. & Br. by about 3.5% and from *L. proxima* (Boud.) Pat. by up to 5%. *Thelephora terrestris* was about 32% different from *L. bicolor* and it was difficult to align sequences due to multiple small insertions and deletions. Anderson and Stasovski (**1992**) found extremely low levels of diversity, less than 0.5%, in the ITS region among closely related taxa and intersterility groups in *Armillaria*. However, when the more distantly related *A. mellea* (Vahl: Fr.) Karsten and *A. tabescens* (Scopoli: Fr.) Singer were compared, divergence was greater and similar problems with alignment were reported. We had difficulty aligning the isolates of *H. ericae* and *S. vaccinii* with *H. mono-*

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nucleotides are indicated by a dot. A dash indicates a gap introduced for alignment. Letters in lower case represent nucleotides that form restriction sites in at least one isolate. (The restriction endonuclease is shown above the sequence.) Sequences of selected isolates (6045, 5828, 6735, 6650) are available from Genbank (accession numbers: L06324, L06325, L06430, L06431).

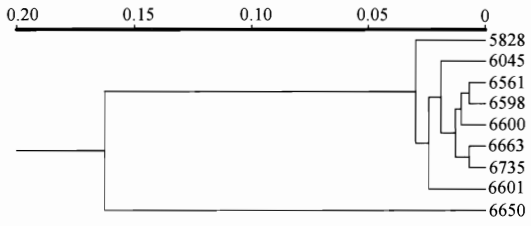

FIG. 17. UPGMA cluster analysis of the nucleotide substitution matrix generated from the combined sequence and restriction site data file. The scale is average number of nucleotide substitutions per site.

tropae, which diverged 24% from the consensus sequence. However, insertions/deletions were conserved among isolates of *H. ericae* and *S. vaccinii* and divergence was only 1.2–3.5%. This is comparable to levels of intraspecific divergence reported in other studies.

The low levels of divergence seen among isolates identified as *H. ericae,* and between this group and the ex-type of *S. vaccinii,* indicates that the taxa are closely related and probably conspecific. Supporting evidence comes from the conserved position of insertions and deletions among isolates. Even so, the two isolates that were most divergent from the ex-type of *H. ericae* (6735) were the unidentified hyphomycete (6601) and the ex-type of *S. vaccinii* (5828). They differed at five nucleotide positions from the consensus sequence and at seven positions from *H. ericae* (6735). The remaining isolates demonstrated degrees of divergence ranging from 0–3 nucleotide substitutions compared to the consensus sequence.

Most of the isolates (TABLE I) that were provisionally identified as *H. ericae* shared morphological similarities with authentic isolates. Even the two isolates lacking typical arthroconidia (6598, 6599) demonstrated similar cultural features (FIG. 4). The ex-type of *S. vaccinii* (5828) differed somewhat from *H. ericae* (6735) in colonial features (FIGS. 1, 2) but they closely resembled each other microscopically (FIGS. 10, 13). The unidentified root endophyte (6601), which was one of the most divergent in molecular characters, was the most morphologically distinct strain (FIGS. 8, 9). It lacked any of the cultural features defined by Read (**1983**) or observed by us as being characteristic of the *H. ericae-S. vaccinii* group. Although 6601 appears to be closely related to this group based on molecular evidence, its relationship may be more complex than indicated.

We define the *H. ericae-S. vaccinii* group as having the following suite of characters (see FIGS. 1–14). Isolates are slow growing (25–40 mm at 4 weeks) on all media, pale grey, greyish brown or reddish brown, characteristically with narrow white margin and have a low velvety nap or develop fascicles of aerial hyphae. They usually form narrow (1.5–2.5 μm wide), 0-to-1 septate arthroconidia which tend to remain connected in zigzag chains. Vegetative hyphae often form aggregates or strands. Isolates which fail to form arthroconidia may be identified as belonging to this group if they share similar colonial features and if they demonstrate mycorrhizal association. However, Pearson and Read (**1973**) assessed the mycorrhizal infectivity of a number of soil isolates of slow growing dark fungi, some of which developed characteristic segmenting hyphae, and found that only some demonstrated mycorrhizal association. The taxon identified as *Scytalidium* sp. (UAMH 6045) appears to be closely allied to other members of this group, despite its unusual substrate. Isolation from a human patient would not preclude it from being a root endophyte, as clinical specimens are often contaminated with soil fungi. Even though earlier studies (Dalpé et al., **1989**) showed that this isolate was not able to colonize seedling roots of *Vaccinium angustifolium,* we have identified it as *S. vaccinii* based on its morphological and genetic similarities.

Polymorphism for presence or absence of the inserts existed within the North American strains and did not appear to be a good predictor of relationship or correlate with the degree of divergence. For example, the ex-type of *S. vaccinii* (5828) differed from the ex-type of *H. ericae* (6735) at seven nucleotide positions despite the presence of two insertions in 5828 and their absence in 6735. Within North America, isolates lacking insertions differed from isolates with insertions at as few as three nucleotide positions. Nonetheless, the isolate most genetically similar to the type of *H. ericae* was 6663, the only North American isolate to lack insertions.

We have evidence that at least one of the insertions is an intron. Sequence analysis of the small subunit insert shows that it has the conserved sequence motifs and secondary structure of a group I intron (Egger, Goodier, and Osmond, unpubl. data), and it occurs in the same

position as a group I intron in *Pneumocystis carinii* Chagas (Sogin and Edman, **1989**). Group I introns have been observed in nuclear ribosomal RNA genes of other organisms (see Burke, **1988**), and intraspecific polymorphism for such introns has been observed in *Physarum* (Muscarella et al., **1990**) and in the mitochondrial large subunit ribosomal RNA gene in *Saccharomyces* (Burke, **1988**). We suspected that the insertion in the large subunit gene is also an intron. Molecular characterization of both inserts is currently underway and will be discussed in a separate paper.

Based on the initial restriction analysis, which was biased by the presence or absence of the insertions, we would have concluded that the data falsified our hypothesis that *S. vaccinii* represented the anamorph of *H. ericae*. However, when homologous sequences were compared independently of the insertions, a close relationship became evident. Our finding points out potential problems for researchers using molecular characters to study anamorph-teleomorph relationships because techniques, such as restriction fragment analysis and "PCR fingerprinting" (Vilgalys and Hester, **1990**), are susceptible to misinterpretation due to length variations. It is important to ensure that fragments amplified are the same size before inferences about relationship are drawn.

We conclude that while there is variation in the ribosomal DNA, it is not sufficient to preclude *S. vaccinii* and *H. ericae* representing an anamorph-teleomorph relationship. The range of variation observed is consistent with expected levels of intraspecific variability in a genetically diverse species. However, in the absence of a direct confirmation through production of a sexual stage in *S. vaccinii,* some doubts remain. We are unable to eliminate the possibility of geographical divergence or population subdivision between North American or European isolates. Little is known about the biology of *H. ericae* and the factors that induce fruiting or the production of an anamorph, but so far the teleomorphic state has not been observed under field conditions in North America (Litten et al., **1985**) and efforts to produce the teleomorphic state in some isolates (5828, 6561, 6562) in synthesis culture have been unsuccessful (Dalpé, pers. comm.). Therefore, it is possible that North American strains reproduce asexually and could be derived from sexual populations in Europe. At present we do not have sufficient numbers of isolates to test this hypothesis. In any event, we feel that the name *S. vaccinii* can be used to apply to arthroconidial ericoid endophytes which fail to demonstrate apothecia characteristic of the teleomorph *H. ericae.*

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