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The genus *Oidiodendron*: species delimitation and phylogenetic relationships based on nuclear ribosomal DNA analysis

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Abstract: Nuclear ribosomal DNA sequences (ITS region) of fifteen species in the hyphomycete genus *Oidiodendron* and ten species from the 4 genera in the Myxotrichaceae, *Byssoascus, Gymnostellatospora, Myxotrichum,* and *Pseudogymnoascus,* were analysed to: (i) reveal the levels of intra- versus interspecific sequence variation within the genus *Oidiodendron,* clarify species delimitation and examine the usefulness of some morphological characters used for identification; (ii) assess the possible conspecificity of documented ericoid mycorrhizal strains of *Oidiodendron*; and (iii) test the hypothesis based on morphological inference that the genus *Oidiodendron* belongs with the genus *Myxotrichum* in the Myxotrichaceae (Onygenales). Comparison of molecular and morphological data for multiple strains of *O. griseum, O. tenuissimum* and *O. maius* revealed that conidiophore length and the production of a diffusing pigment are not reliable key characters for the genus. Several historically important ericoid mycorrhizal strains, documented as *O. griseum,* were reidentified as *O. maius.* Parsimony analyses of 23 *Oidiodendron* strains showed that three highly supported monophyletic groups, each one consisting of a pair of species, are resolved within the genus. A low level of sequence divergence between the species in these pairs suggests conspecificity for each pair. Other interspecific relationships were not well-supported by bootstrap values. Parsimony analysis of a second dataset composed of mitotic and meiotic taxa showed that *Oidiodendron, Myxotrichum* and *Byssoascus* form a well-supported monophyletic group within the Myxotrichaceae, and that this group has diverged significantly from the two other genera in the family. Relationships in the monophyletic group were correlated with anamorph state produced; three *Myxotrichum* spp., and *B. striatosporus,* all with distinct or reported *Oidiodendron* states, nested with anamorphic species, while species of *Myxotrichum* with other anamorph states were excluded. In addition, sequence divergence measures between the meiotic and mitotic species clustered in two monophyletic clades were found to be comparable to the intraspecific levels for *Oidiodendron* spp. These results support the initial hypothesis that *Oidiodendron* is closely related phylogenetically to the genus *Myxotrichum* and suggest that the generic concept of *Byssoascus* needs reexamination.

Key Words: ascomycetes, ericoid mycorrhiza, molecular systematics, Myxotrichaceae, Onygenales

INTRODUCTION

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

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

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as to which characteristics should be emphasized for identification purposes.

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

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

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

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

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

MATERIALS AND METHODS

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

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

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

TABLE I. Name, strain number, origin, the analysis that each strain was included in and GenBank accession number of fungi used for sequence analysis

Species	Strain[a]	Origin[b]	Data matrix[c]	GenBank No.
Oidiodendron Robak				
O. cerealis (Thüm.) Barron	UAMH 1522	peat soil; ON Canada.	Oid, Myx	AF062788
O. chlamydosporicum Morrall	UAMH 6520	T, soil ex boreal forest; SK Canada.	Oid, Myx	AF062789
O. citrinum Barron	UAMH 1525	T, soil ex cedar bog; ON Canada.	Oid, Myx	AF062790
O. echinulatum Barron	IMI 110132 (UAMH 8467)	A, soil ex cedar bog; ON Canada.	Oid, Myx	AF062791
O. flavum Szilvinyi	UAMH 1524	soil ex cedar bog; ON Canada.	Oid, Myx	AF062792
O. griseum Robak	CBS 249.33 (UAMH 1403)	A, wood pulp; Sweden. (Melin & Nannfeldt, 1934)	Oid, Myx	AF062793
	UAMH 1693	Douglas fir timber; BC Canada.	Oid[d]	AF062794
	UAMH 4080	wood chips and bark ex logging truck; AB Canada.	Oid[d]	AF062795
(received as *O. tenuissimum*)	DAOM 51071 (UAMH 8528)	*Pinus contorta*; AB Canada.	Oid	AF062796
	UAMH 8925	ex roots *Vaccinium myrtilloides*; AB Canada. (Hambleton & Currah, 1997)	Oid	AF062797
O. maius Barron	UAMH 1540	T, soil ex cedar bog; ON Canada. (Barron, 1962)	Oid, Myx	AF062798
(received as *O. griseum*)	DAOM 184107 (UAMH 8529)	ex roots *Vaccinium corymbosum*; PQ Canada. (Couture et al., 1983)	Oid[d]	AF062799
	UAMH 8921	ex roots *Vaccinium myrtilloides*; AB Canada. (Hambleton & Currah, 1997)	Oid[d]	AF062800
	UAMH 8922	ex roots *Vaccinium vitis-idaea*; AB Canada. (Hambleton & Currah, 1997)	Oid[d]	AF062801
O. periconioides Morrall	DAOM 197506 (UAMH 8527)	T, soil; SK, Canada.	Oid, Myx	AF062802
O. pilicola Kobayasi	UAMH 7526	forest soil; Sweden.	Oid, Myx	AF062787
O. rhodogenum Robak	UAMH 1405	A, sludge in pulp strainers; Norway.	Oid, Myx	AF062803
O. scytaloides Gams & Söderström	UAMH 6521	T, forest soil; Sweden.	Oid, Myx	AF062804
O. setiferum Udagawa & Toyazaki	UAMH 5715	T, house dust; Japan.	Oid, Myx	AF062805
O. tenuissimum (Peck) Hughes	CBS 238.31 (UAMH 8511)	T of *O. fuscum*, wood pulp; Norway. (Robak, 1932) (=*O. tenuissimum*, Barron, 1962)	Oid, Myx	AF062807
	CBS 920.73 (UAMH 8512)	forest soil; Sweden.	Oid	AF062808
O. truncatum Barron	UAMH 1399	T, soil ex mixed woods; ON Canada.	Oid, Myx	AF062809
O. sp. nov. (received as *O. tenuissimum*)	CBS 315.95 (UAMH 8513)	leaf litter; Canary Islands Spain.	Oid, Myx	AF062806
Myxotrichum Kunze				
M. arcticum Udagawa, Uchiyama & Kamiya[e]	UAMH 7565	T, forest soil; Alaska USA.	Myx	AF062810
M. cancellatum Phillips[e]	UAMH 1911	frozen blueberry pastry; NJ USA.	Myx	AF062811
M. carminoparum Robak	UAMH 1597	T, wood pulp; Norway.	Myx	AF062812
M. chartarum (Nees) Kunze	UAMH 1997	soil; Japan.	Myx	AF062813
M. deflexum Berkeley	UAMH 6365	soil; ON Canada.	Myx	AF062814
M. setosum (Eidam) Orr & Plunkett[e]	UAMH 3835	soil; AB Canada.	Myx	AF062815

were purified using Wizard PCR Preps columns (Promega, Madison, Wisconsin) following the manufacturer's instructions.

Automated DNA sequencing reactions were performed using standardized methods, then processed and analysed on a ABI 373A automatic DNA sequencer (Perkin-Elmer: Applied Biosystems, Foster, California) following the protocols suggested by the manu-

TABLE I. Continued

Species	Strain[a]	Origin[b]	Data matrix[c]	GenBank No.
M. stipitatum (Lindfors) Orr & Kuehn	UAMH 1510	N, sand dune; England.	Myx	AF062816
Byssoascus striatosporus (Barron & Booth) von Arx[e]	UAMH 3572	T, soil; ON Canada.	Myx	AF062817
Gymnostellatospora japonica Udagawa, Uchiyama & Kamiya	UAMH 8899	white spruce log, decay stage 5, fire site, AB, Canada.	Myx	AF062818
Pseudogymnoascus roseus Raillo	UAMH 9163	ex roots *Abies lasiocarpa*; AB Canada.	Oid, Myx	AF062819

[a] Fungi were obtained from culture collections and original isolation work of S.H. Culture collection origin is noted for strains not previously at UAMH. CBS = Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. DAOM = Canadian Collection of Fungal Cultures, Ottawa, Canada. IMI = International Mycological Institute, Egham, United Kingdom. UAMH = University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada.

[b] Ex-type (T), authentic (A) or neotype (N) strains were used as standards for the species wherever possible.

[c] Strains were included in one or both data matrices: Oid = *Oidiodendron*, Myx = Myxotrichaceae.

[d] Strains of *Oidiodendron* excluded from the branch-and-bound analysis of the *Oidiodendron* data matrix.

[e] Teleomorphic species with an *Oidiodendron* state.

facturer. Sequences of complementary strands were determined for those strains serving as the reference standards of the target species as well as any strains whose initial sequence was ambiguous. Consensus sequences were determined for these and used in the analyses. Preliminary alignments of DNA sequences were performed using the automatic DNA sequencer software package SeqEd ver. 1.0. Final alignments were optimized by hand.

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

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

Data matrices were analysed using the maximum parsimony program PAUP 3.1.1 (Swofford, 1993). Parsimony trees were constructed from a bootstrap analysis of each data matrix; 1000 replicate searches were carried out using the heuristic search algorithm, tree bisection-reconnection branch swapping, and random stepwise sequence addition. For the *Oidiodendron* data matrix, a second analysis using the branch and bound search algorithm was performed. To permit timely execution of the data analysis, the dataset was slightly reduced by excluding two strains of *O. griseum* (UAMH 1693, 4080) and three of *O. maius* (UAMH 8529, 8921, 8922).

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

Morphological.—All strains originally received as *Oidiodendron griseum* (UAMH 1403, 1693, 4080, 6514,

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

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

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

[b] This strain was also included in the sequence analysis.

7022, 8507, 8529, 8925), *O. tenuissimum* (UAMH 8511, 8512, 8513, 8528), and *O. maius* (UAMH 1540, 8442, 8921, 8922) (TABLES I, II) were examined for seven morphological characters: colonial morphology; the production of diffusing pigment on agar; the range in length of conidiophores; and size, shape, color and surface texture of the conidia. These data were compared to the original descriptions of these species (TABLE III).

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

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

RESULTS

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

The 50% majority rule consensus tree generated by the bootstrap analysis is presented in FIG. 1 (308 steps). One of three most parsimonious trees (279 steps; consistency index 0.65) generated by the branch and bound search algorithm is shown in FIG. 2. Branch lengths are proportional to the number of nucleotide changes along that branch. The overall topology of the two trees is the same and, while species relationships are clarified in FIG. 2, the lack of resolution in the bootstrap analysis indicates that some of these branches are not well supported.

Although the inferred phylogenies did not resolve all the interspecific relationships in the genus, the ex-type strains of the target species of *O. griseum, O. maius* and *O. tenuissimum* were delimited as distinct in separate monophyletic clades (FIG. 1). Similarly, several pairings of species with high bootstrap support and low interspecific sequence divergence were revealed: *O. griseum*/*O. flavum, O. maius*/*O. citrinum,* and *O. chlamydosporicum*/*O. scytaloides* (FIG. 2).

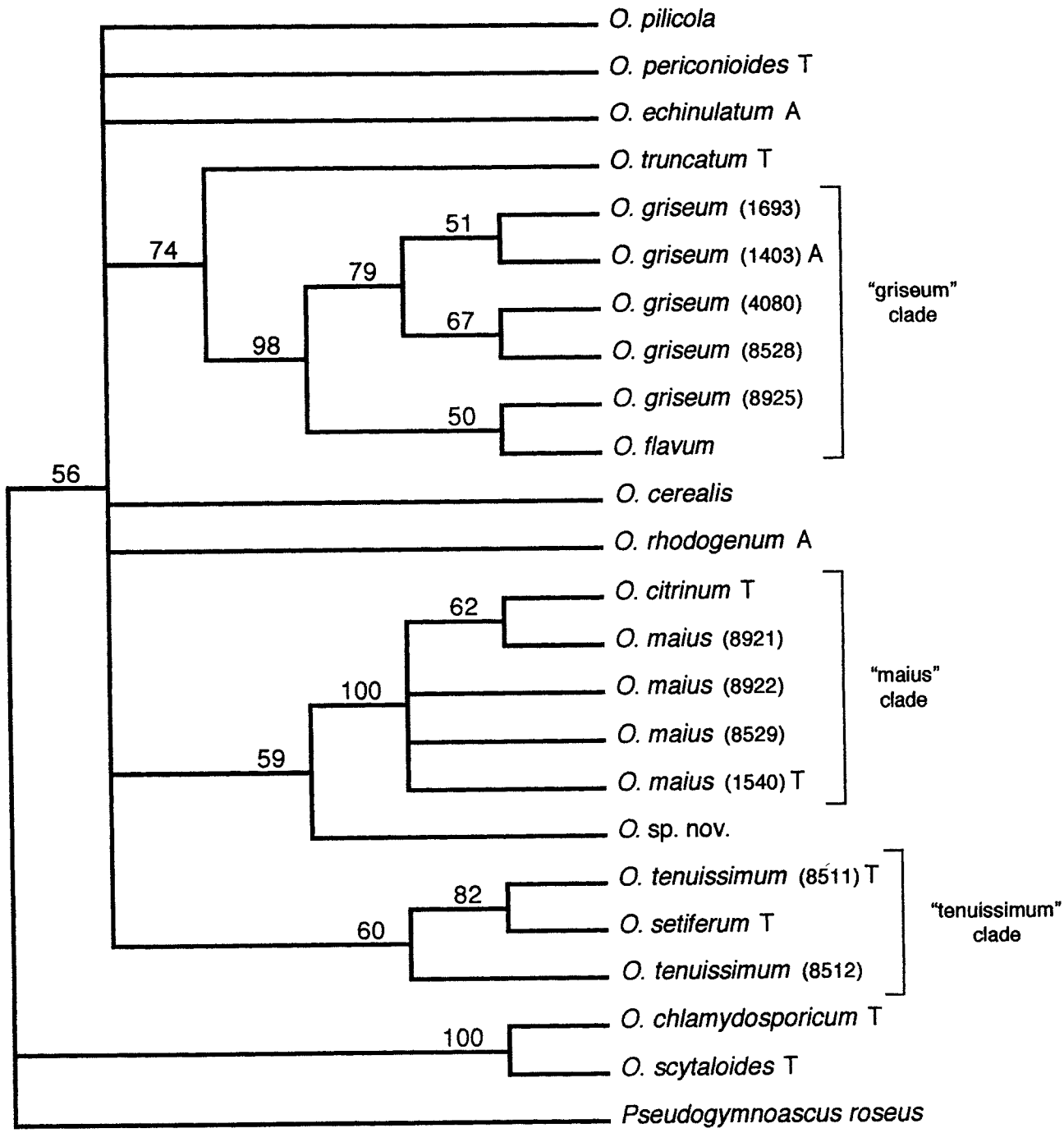

FIG. 1. Majority rule consensus tree (308 steps) resulting from 1000 bootstrap replications of maximum parsimony analysis of the *Oidiodendron* data set using the heuristic search algorithm of PAUP 3.1.1. Bootstrap values above 50% are given adjacent to the corresponding node. UAMH numbers are given to identify multiple strains for a species where needed and ex-type (T) and authentic (A) strains are indicated.

Sequence divergence between species outside these pairings ranged from 6 to 10%, except for *O. echinulatum/O. cerealis* at 4%.

The "griseum" clade (bootstrap 98; FIG. 1) comprised UAMH 1403, an authentic strain; UAMH 4080; two strains that produced a dark amber diffusing pigment, UAMH 1693 and UAMH 8925; and UAMH 8528, which was received as *O. tenuissimum*. Percent sequence dissimilarity between these strains was at most 1%. *O. flavum* clusters with the *O. griseum* isolates and differs from the ex-type strain at 7 nucleotide positions (1.4%; FIG. 2).

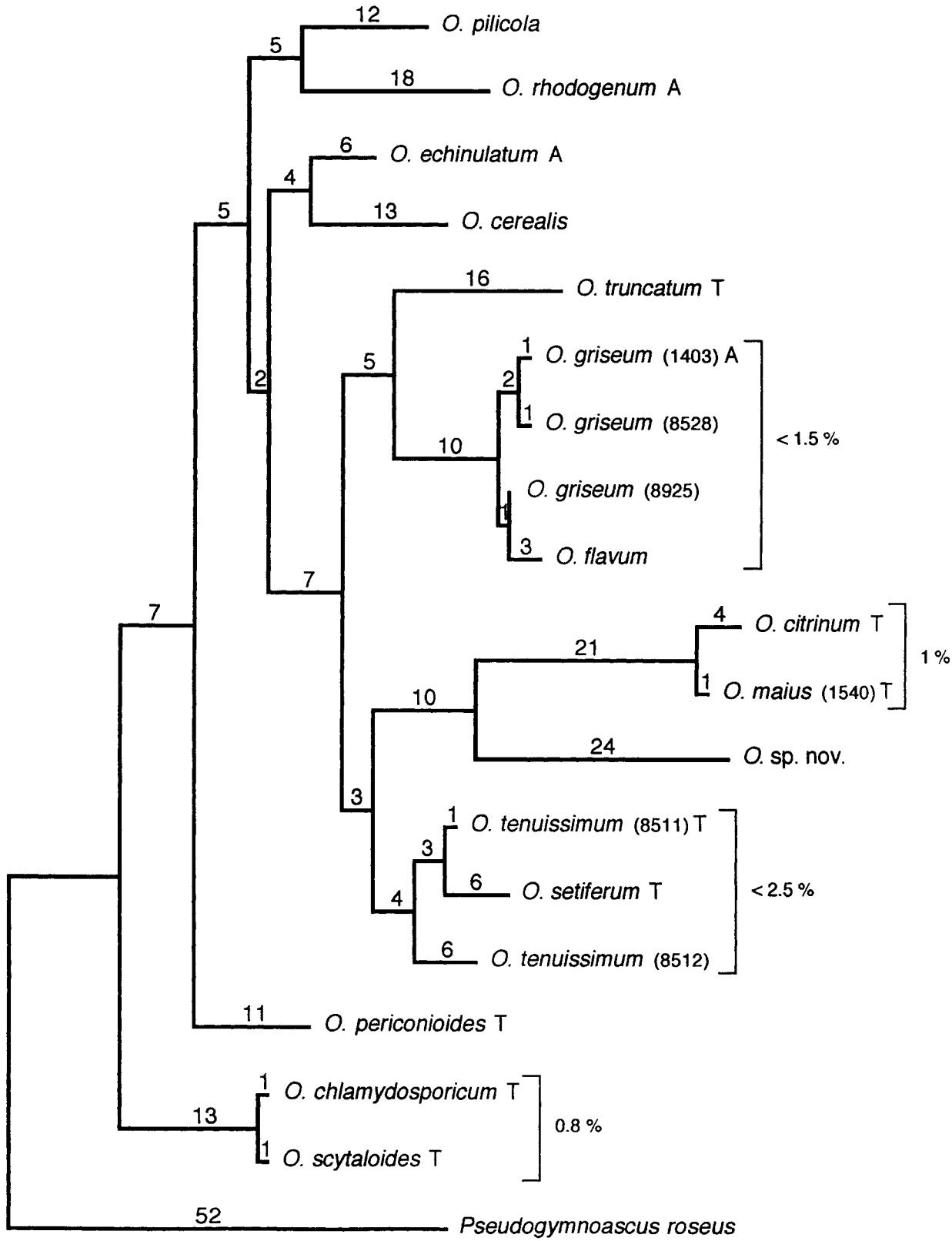

FIG. 2. One of three most parsimonious trees (279 steps, CI = 0.65) resulting from a maximum parsimony analysis of the *Oidiodendron* data set (excluding two strains of *O. griseum* and three of *O. maius*) using the branch and bound algorithm of PAUP 3.1.1. Numbers above the branches indicate the number of nucleotide changes along each branch. UAMH numbers are given to identify multiple strains for a species where needed and ex-type (T) and authentic (A) strains are indicated. Sequence divergence measures for four monophyletic clades are indicated.

The "maius" clade (bootstrap 100; FIG. 1) comprised UAMH 1540, the ex-type strain, as well as three strains isolated from ericoid mycorrhizas, UAMH 8921, UAMH 8922, and UAMH 8529, which was received as *O. griseum*. Percent sequence dissimilarity between these strains was at most 0.8%. *O. citrinum* also clusters with *O. maius* and differs from the ex-type strain at 5 nucleotide positions (1%; FIG. 2), of which one is an insertion/deletion event which is probably due to replication slippage.

The "tenuissimum" clade (bootstrap 60; FIG. 1) comprised UAMH 8511, the ex-type strain; a second strain, UAMH 8512; and the ex-type strain of *O. setiferum*. Percent sequence dissimilarity of the latter two with UAMH 8511 was less than or equal to 2.2% (FIG. 2) with 10 and 7 nucleotide differences respectively.

Aside from these groupings, *O. chlamydosporicum* and *O. scytaloides* formed a closely related species pair (FIGS. 1, 2; bootstrap 100) with only 4 nucleotide differences between them (0.8%), of which three are insertion/deletion events (two were coded out in the data matrix).

Myxotrichaceae analysis.—The aligned DNA sequence data matrix generated using the 25 species listed in TABLE I comprised 547 bases of which 227 were variable and 129 were parsimony informative. The 50% majority rule consensus tree generated by the bootstrap analysis with *Pseudogymnoascus roseus* as outgroup taxon is presented in FIG. 3 (446 steps). *Oidiodendron, Byssoascus,* and *Myxotrichum* formed a monophyletic group within the Myxotrichaceae, while *Gymnostellatospora japonica* clustered with the outgroup taxon *P. roseus,* with high support (bootstrap 100). *M. deflexum* was basal to the rest of the species in the "Myxotrichum" ingroup. Sequence divergence between *P. roseus* and *G. japonica* was 8.2% but much higher at 19% when either species was compared to *M. deflexum.*

Several terminal clusters of species received high support. The species pairs *O. chlamydosporicum/O. scytaloides, O. tenuissimum/O. setiferum, O. griseum/O. flavum,* and *O. maius/O. citrinum,* observed in the *Oidiodendron* analysis, were repeated here. Three species of *Myxotrichum* clustered with anamorphic taxa: *M. arcticum* with *O. griseum/O. flavum* (bootstrap 96), *M. cancellatum* with *O. echinulatum* (bootstrap 97), and *M. setosum* with *O. truncatum* (bootstrap 89). For the first two, the percent sequence divergence from their associated *Oidiodendron* species is very low (FIG. 3). Three other *Myxotrichum* species formed a well supported divergent clade (bootstrap 100) and sequence divergence amongst these species was much higher at 7.0 to 8.5%. Sequence divergence overall between species of *Myxotrichum* ranged from 6 to 12%.

Restriction Fragment Length Polymorphism Analysis.—The RFLP patterns of all five strains in TABLE II, and for both extractions, were identical and matched those published by Hambleton and Currah (1997) for *O. maius.*

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

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

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

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

Colony size for all strains on MEA and PDA was

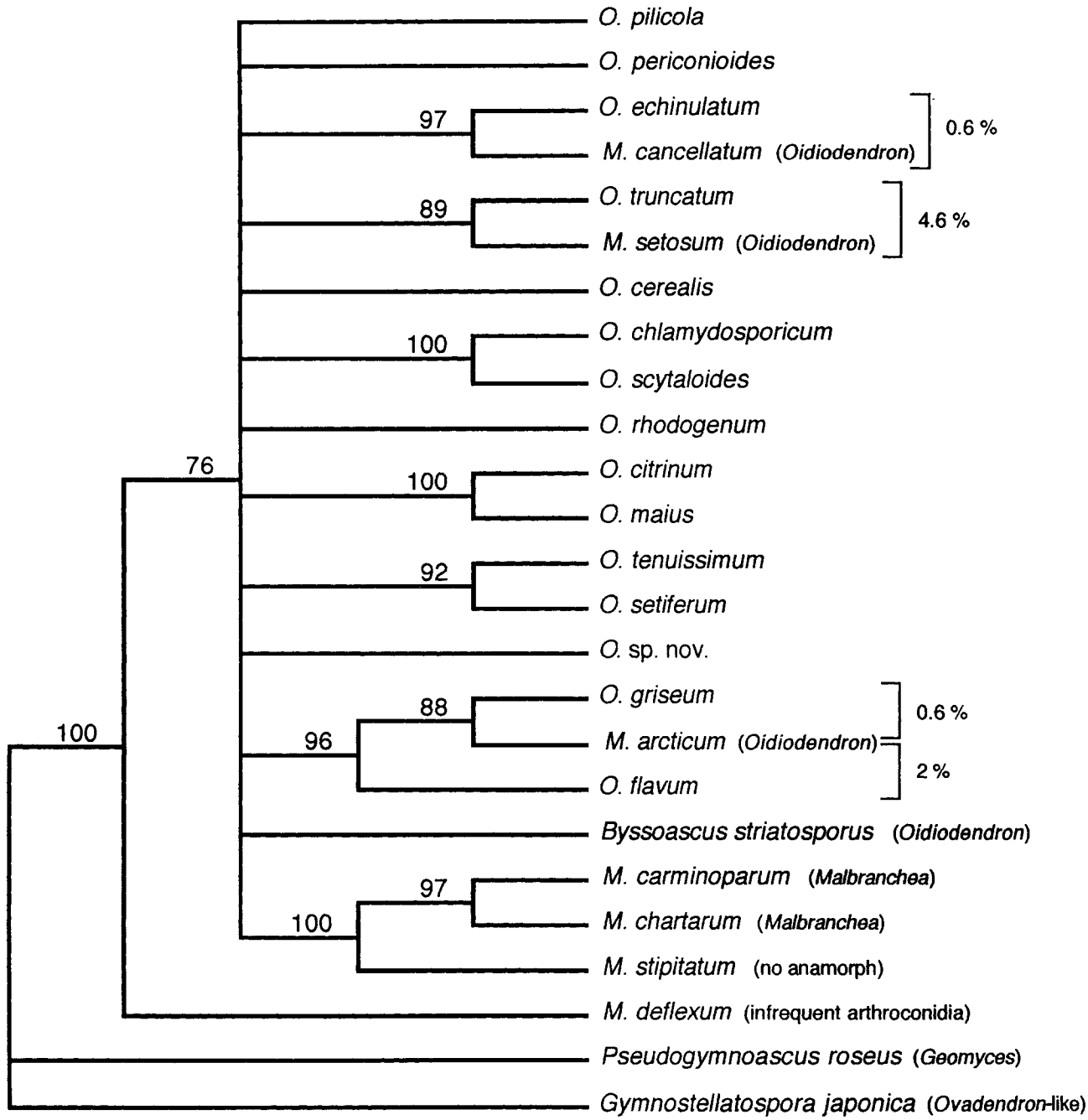

FIG. 3. Majority rule consensus tree (446 steps) resulting from 1000 bootstrap replications of maximum parsimony analysis of the Myxotrichaceae data set using the heuristic search algorithm of PAUP 3.1.1. Bootstrap values above 50% are given adjacent to the corresponding node. Anamorph states for the teleomorphic taxa are mapped on the tree and sequence divergence measures for four meiotic/mitotic species pairs are given.

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

Anamorph states for species in the Myxotrichaceae have been reported as and were observed as follows: *Myxotrichum carminoparum, M. chartarum, M. deflexum, M. stipitatum*: absent to infrequent arthroconidia to chains of arthroconidia assignable to the genus

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

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

TABLE IV. Summary of morphological data for strains of *Oidiodendron griseum, O. tenuissimum* and *O. maius* used in sequence or RFLP analysis, grouped according to their reidentification based on the molecular analyses

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

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

Malbranchea; *M. arcticum, M. cancellatum, M. setosum, Byssoascus striatosporus*: *Oidiodendron*; *Gymnostellatospora japonica*: lacking in the type specimen, *Ovadendron*-like in this strain; *P. roseus*: *Geomyces*.

DISCUSSION

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

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

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

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

All of the major records of ericoid mycorrhizal *Oidiodendron* isolates are now confirmed as strains of *O. maius* (Burgeff, 1961; Couture et al., 1983; Douglas et al., 1989; Stoyke and Currah, 1991; Xiao and Berch, 1992; Hambleton and Currah, 1997) though for two of those studies not all of the deposited strains were examined (Couture et al., 1983; Xiao and Berch, 1992). The importance of this clarification is two-fold. The strains that have been used in experiments aimed at elucidating the role of the fungus as a mycobiont (Dalpé, 1986; Abuzinadah and Read, 1989; Leake and Read, 1990; Haselwandter et al., 1990; Xiao and Berch, 1995) have been those isolated by Couture et al. (1983) (though specific strain identity was not always cited) or by Xiao and Berch (1992), giving the species *O. griseum* an undeserved reputation as a mycobiont. Results may also have been misinterpreted when bonafide strains of *O. griseum* were not observed to form ericoid mycorrhizas in pot cultures (for instance, UAMH 1403 in Douglas et al., 1989).

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

The "griseum" clade contains strains isolated from a wider range of substrates, of which two produce a striking amber diffusing pigment on PDA and MEA. For one of these, UAMH 8925, and for the isolate received as *O. tenuissimum,* UAMH 8528, conidiophore lengths ranged up to 250/225 μm. The molecular analysis, then, shows that conidiophore

length is not a reliable character for these species: *O. maius* may produce very short conidiophores in the range of *O. griseum* and *O. tenuissimum* and vice-versa. Since conidiophore length range for *O. tenuissimum* is reported as intermediate, all three species overlap for this character.

In the original description of *O. tenuissimum* (= *O. fuscum*; Hughes, 1958, p. 790) one important feature noted as absent from *O. griseum* was the production of a dark, diffusing pigment on agar. Unfortunately, surface ornamentation of the conidia was not mentioned, and indeed this is a difficult character to resolve for such small conidia using the light microscope. Barron (1962), after identifying numerous strains isolated from soil as *O. tenuissimum,* noted that the species was highly variable as to pigment production but could be distinguished from *O. griseum* by dark and echinulate conidia. Although UAMH 8513, received as *O. tenuissimum,* demonstrates these conidium characteristics and is also positive for pigment production, it diverges significantly from the "tenuissimum" clade in the phylogenetic analyses (FIGS. 1, 2).

In the ex-type strain UAMH 8511, and UAMH 8512, the conidia were pale brown and only slightly roughened and in these respects similar to *O. griseum.* Based on the divergent sequences and on other distinctive morphological features, UAMH 8513 is considered to represent an undescribed species. The production of a dark diffusing pigment on agar media is not a species specific attribute since it has been observed for several species in addition to *O. tenuissimum* and *O. griseum,* but because production varies depending on agar type, it still may be useful for sorting species groups.

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

Interspecific relationships within Oidiodendron.—Molecular analysis reveals that within the genus *Oidiodendron* there is a high degree of sequence substitution in the ITS region of the rDNA. Sequence divergence varies from 6 to 10% among species at the internal nodes of the phylogenetic trees shown in FIGS. 1, 2 but a lack of resolution of these interspecific relationships is evident from the number of branches which received bootstrap support of less than 50. The relatively low consistency index of the tree in FIG. 2 indicates that data from a more conserved region, such as the 28S subunit, are needed to resolve the phylogeny. In contrast, several species pairs at the terminal nodes are supported by high bootstrap values and also exhibit low sequence dissimilarity measures, similar to those observed at the intraspecific level.

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

In the "tenuissimum" clade, *O. setiferum* is more closely related phylogenetically to the ex-type strain of *O. tenuissimum* than is a second strain, and though the sequence divergence is still under 2.5% for that species, bootstrap support for the clade is low. In many respects the description of *O. setiferum* is similar to that of *O. tenuissimum* but the species is unique in its production of dematiaceous setae at the apex of the conidiophore (Udagawa and Toyazaki, 1987). More sequence data are needed for both species in order to evaluate the cohesiveness of their inferred relationship.

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

A comparison of the divergence measures of other ascomycetous taxa based on both ITS1 and ITS2 with

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

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

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

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

Anamorph-teleomorph relationships.—Within the Myxotrichaceae, species of *Oidiodendron, Myxotrichum* and *Byssoascus* form a well-supported monophyletic group that has diverged significantly from the two other genera in the family. We judged *Pseudogymnoascus roseus* and *Gymnostellatospora japonica* to be equally effective as outgroup taxa because they were unalignable to the rest of the data matrix for the first 58 nt and were less divergent from each other than from *M. deflexum,* the most basal species of the ingroup. In addition, neither produces an *Oidiodendron* anamorph state.

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

Both *M. arcticum* and *M. cancellatum* exhibit less than 1% sequence divergence with a paired anamorphic species (FIG. 3). Based on earlier arguments, this result could indicate conspecific identity for these taxa. *M. arcticum* produces an *Oidiodendron* state that is morphologically typical of *O. griseum* though some conidiophores form a geniculate head with single conidia produced over the surface (Udagawa et al., 1994). The conidial state of *M. cancellatum* was observed to be very similar to the strain of *O. echinulatum* used in this study but neither produced the distinctly roughened conidia reported for the latter species. In both cases, more strains need to be examined before any judgments concerning conspecificity are made.

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

iaceous peridial hyphae, often ornamented with pigmented appendages.

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

Historically, *M. deflexum* was renamed as *Eidamella deflexa* (also a monotypic genus) by Benjamin (1956) with the statement that it did not fit the then-current concept of the genus *Myxotrichum,* but more recent treatments do not concur (Orr et al., 1963; Benny and Kimbrough, 1980; Currah, 1985). Though Benjamin's criteria were not clearly defined, they were apparently based on whether appendages are differentiated from the peridial hyphae. However, appendage characteristics have since been used for interspecific distinctions. Data from more strains and from more conserved regions of DNA need to be evaluated to assess the phylogenetic stability of ascomatal characters for inferring relationships in this group.

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

The molecular analysis confirms the initial hypothesis that the hyphomycete genus *Oidiodendron* is, as a group, phylogenetically a member of the Myxotrichaceae, and is closely related to the genera *Byssoascus* and *Myxotrichum,* especially those species with *Oidiodendron* states. The inferred phylogeny also indicates a marked divergence within the Myxotrichaceae between the genus *Myxotrichum* (and *Byssoascus*), and the other two genera in the family. Sequence analysis of a more conserved portion of the rDNA gene is underway to determine whether *Myxotrichum* shares closer evolutionary ties with taxa outside the current circumscription of the Myxotrichaceae.

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