Utility of a cultural method for identification of the ericoid mycobiont *Oidiodendron maius* confirmed by ITS sequence analysis

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Abstract: A simple cultural method was investigated for its reliability in distinguishing the ericoid mycobiont *Oidiodendron* maius from selected other species of *Oidiodendron*. Forty three isolates were grouped by morphology after 28 d growth on cereal agar overlaid with a cellophane membrane. All isolates of *O. maius* and its close relative *O. citrinum* expressed characteristic colonial morphologies allowing recognition regardless of sporulation. Isolates grouped by colonial features correlated with strongly supported groupings obtained by analysis of nuclear ribosomal internal transcribed spacer (ITS) region sequences, including *O. maius* with *O. citrinum*, *O. griseum* with *O. flavum*, and *O. truncatum* as an independent group. Isolates of *O. tenuissimum*, including the ex-type of the purported synonym *O. fuscum*, demonstrated cultural variation and were dispersed among several different groups in the ITS analysis. *O. fuscum* is here regarded as a distinct taxon.

Key words: ericoid endophyte, ITS region sequences, *Oidiodendron*, *Oidiodendron echinulatum*.

INTRODUCTION

Members of the genus *Oidiodendron* Robak are readily recognizable by their distinctive microscopic morphologies. They have branched hyaline fertile hyphae that divide into arthroconidia borne on erect, basally darkly pigmented conidiophores. Identification to species is often difficult, in part due to variation in both colonial and microscopic features when isolates are grown under differing conditions. These problems have been noted in recent reports with the suggestion that molecular approaches provide the most reliable means of identification (Hambleton *et al*. 1998, Lacourt *et al*. 2001). Correct identification of the mycobiont is essential to gaining a true understanding of the species involved in forming mycorrhizas. Hambleton *et al*. (1998) re-examined available isolates from prior studies and noted that several had been misidentified as *O. griseum* Robak, leading to incorrect assumptions about the mycorrhizal ability of this species. Their results indicated that only *O. maius* Barron had been found to form ericoid mycorrhizas. The close relationship between *O. maius* and *O. citrinum* Barron led Hambleton *et al*. to regard these as conspecific. Lacourt *et al*. suggested that *O. citrinum* could be retained as a subspecies of *O. maius*.

Currently, 23 isolates identified as *O. maius* or *O. citrinum* are on deposit at the University of Alberta Microfungus Collection and Herbarium (UAMH). Among these are eight initially identified as *O. griseum*, including those of Burgeff (1961), Couture *et al*. (1983), Stoyke & Currah (1991) and Xiao & Berch (1992, 1995, 1996). The isolates of Xiao & Berch were misidentified originally by the author of the present paper (LS) prior to acquisition of sufficient experience in the recognition of *O. maius*.

As part of the UAMH depository procedures, all isolates are grown on media overlaid with a cellophane membrane (Carmichael 1961, Currah & Sigler 1986). When the colony is mature, the cellophane membrane is lifted off, transferred to specialized drying racks, and air-dried in a biological safety cabinet. The resulting dried colonies are retained as herbarium specimens. As isolates of *O. maius* were processed, it became apparent that colonies of members of this species demonstrate a highly characteristic appearance when grown on cereal agar plus cellophane. To assess the reliability of this cultural feature as an aid to the identification of *O. maius*, all isolates were regrown and compared with selected isolates of other *Oidiodendron* species. Nuclear ribosomal internal transcribed spacer (ITS) region sequences are available for a number of representative strains and these were compared with some newly derived ones to confirm cultural observations.

MATERIALS AND METHODS

Material examined included all isolates of *O. maius* and *O. citrinum* (Table 1) on deposit at UAMH and selected representatives of *O. echinulatum* Barron, *O. flavum* Szilvinyi emend. Barron, *O. griseum*, *O. tenuissimum* (Peck) Hughes and *O. truncatum* Barron (Table 2). Ex-type or authentic cultures were included for each species. Isolates were acquired from their original

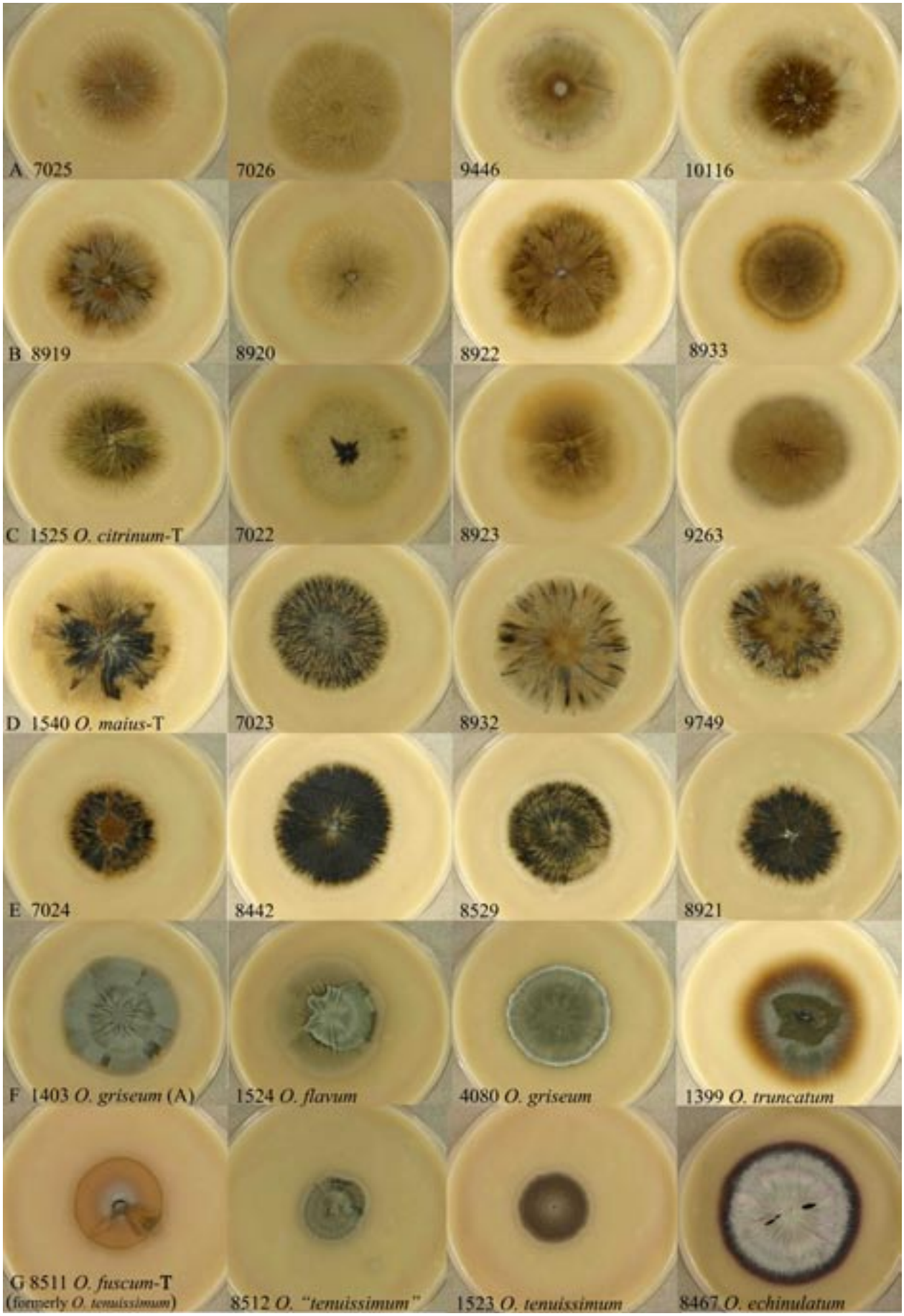

Fig. 1. Colonies of *Oidiodendron* species grown on cereal agar overlaid with cellophane membrane (CERC) for 34 d at 22 ºC. Rows A – E show colonies of *O. maius*, including *O. citrinum*, identified by UAMH number. See Table 1 for information on provenance. Rows F – G show selected isolates of other species, identified by original name and UAMH number. See Table 2 for information on provenance.

isolators or from other culture collections including ARON - Division of Botany and Plant Physiology, Department of Biology, University of Oslo, Oslo, Norway; CBS – Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CDC – Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.; DAOM – National Mycological Herbarium, Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada; IMI - CABI Bioscience Reference Collections, Egham, Surrey, U.K.; MUCL - Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; OAC - Ontario Agricultural College Herbarium, Department of Botany and Genetics, University of Guelph, Guelph, ON, Canada.

Isolates were recovered from freeze-dried material or from water storage vials onto potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI, U.S.A.). All media were dispensed into 100 mm Petri plates. PDA plates were grown for 18 d at 22 ºC in the dark. Ten per cent cereal agar (w:v) (CER) was prepared using mixed cereal baby-food following the protocol outlined in Sigler & Flis (1998) to eliminate aerobic spore-bearing bacteria sometimes present in the baby food. Either Pablum or Heinz (both manufactured by H.J. Heinz Co. of Canada Ltd, North York, ON) is equally acceptable but oatmeal, rice or other cereal formulation should not be substituted for mixed cereal. Each plate of CER was overlaid with a single piece of presterilized cellophane prepared as follows. A stack of Cellophane™ sheets (350 POO, UCB Films, Bath Road Bridgwater Somerset, U.K.) was precut into 6.5 cm squares. The squares were fanned to slightly separate them, then placed into a 10 × 8 cm deep petri dish (Pyrex™ Fisher Scientific, Nepean, ON) half full of distilled water, and autoclaved at 121 ºC for 20 min and allowed to cool. (Unused cellophane pieces in water can be re-autoclaved prior to each use.) In a biological safety cabinet, a single piece of cellophane was removed using a sterile forceps, drained by touching a corner onto a sterilized paper towel, and then laid onto the CER. The curved edge of the forceps was used to smooth out any wrinkles. The plates of CER-cellophane (CERC) so prepared were left at room temperature 24–48 h to absorb residual water. For inoculation, a suspension of conidia was prepared in a small amount of semi-solid detergent agar (SSD; Pitt 1985) dispensed into a 1 mL cryovial. A sterile wooden applicator stick was placed into the SSD, then touched lightly onto the centre of the cellophane membrane. In an attempt to avoid scatter and obtain a single colony, the plate was held upside down during inoculation. Plates were labelled by number only, incubated at 22 ºC in the dark for 28 d, and examined weekly to record colonial features. Colony colours were assessed using Kornerup and Wanscher (1978). After 34 d, the colonies were photographed and dried.

ITS sequences were obtained from seven isolates and compared with those on deposit in GenBank for UAMH strains (Tables 1, 2). DNA extractions from approximately 100 mg of mycelium followed the procedure outlined by Cubero *et al*. (1999) with minor modification. Ground mycelium was incubated with 2 % (w/v) cetyl-trimethyl ammonium bromide for 2–3 h at 65 °C. Crude DNA was purified using the QIAquick PCR Purification kit (QIAGEN Inc., Mississauga, ON, Canada). The nuclear ribosomal ITS region that includes ITS1, 5.8S and the ITS2, was amplified with primer pair BMBC-R (Lane *et al*. 1985) / ITS4 (White *et al*. 1990). PCR reactions were subjected to 30 cycles on a Perkin Elmer GeneAmp Thermal Cycler (Applied Biosystems, Foster, CA, U.S.A.). Both strands were sequenced with primers BMBC-R, ITS2, ITS3 and ITS4 using the BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems) and run on an ABI 377 Automated Sequencer (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, U.S.A.). DNA sequences were edited using the Sequencher™ v. 4.0.2 (Gene Codes Corp., Ann Arbor, MI, U.S.A.) and aligned manually by eye using the sequence alignment programme Se-Al v.1.0a1 Fat (Rambaut 1995). The data matrix was analyzed using PAUP* v.4.0b10 (Swofford 2003). Heuristic searches were conducted on the data matrix under the maximum parsimony criterion. Robustness of the resultant tree was evaluated using 500 bootstrap replicates (Felsenstein 1985).

RESULTS

After 28 d, inoculated CERC plates were grouped visually according to similarities in fungal colonial morphology. Twenty-one cultures were selected as having features typical of *O. maius*. Representative isolates are shown in Fig. 1, rows A–E. All morphologies were in agreement with the original identifications of the isolates. Included among this group of isolates was the ex-type of *O. citrinum*, UAMH 1525 (row C). On CERC, *O. maius* colonies were characteristically thin, glabrous and radially striate, with no or sparse sporulation occurring along the darker striae (e.g. row D, ex-type UAMH 1540). The striations became evident within 21 d. Striae of one isolate (UAMH 7026) developed in a spiral pattern. Colour varied from yellowish white [4A2] to orange-white [5A2/3] or orange-grey [5B2] at the margin to grey [F1], brown [6E8], dark brown [6F8] or greyish-black centrally. In Fig. 1, colonies are arranged according to colour to show the gradation from light to dark. Colony diameters ranged from 3.2–4.5 cm (median 4 cm)

Table 1. Isolates of *Oidiodendron maius* and *O. citrinum* examined.

Name	UAMH number	Original name, if different (reference)	Location	Substrate	Isolator & date	Collector (#)	Other #	Sequence (reference)
O. citrinum	1525 (T)		CANADA: Ontario (ON), Guelph	soil, cedar bog	Barron G.L., 1960	Barron G.L., (OAC 9245)	DAOM 195972	AF062790 (Hambleton *et al*. 1998)
O. maius	1540 (T)		CANADA: ON, Guelph	peat soil, cedar bog	Barron G.L., 1960	Barron G.L., (OAC 9232)	DAOM 195971	AF062798 (Hambleton *et al*. 1998)
	6514	*O. griseum* (Stoyke & Currah 1991)	CANADA: Alberta (AB), Jasper National Park, Arrowhead Lake	roots of *Loiseleuria procumbens* (L.) Descv.	Stoyke G., 1989	Stoyke G., (LPS3)		RFLP analysis (Hambleton *et al*. 1998)
	7022	*O. griseum* (Xiao & Berch 1992, confirmed L. Sigler)	CANADA: British Columbia (BC), Vancouver Island, Port McNeill	roots of *Gaultheria shallon* in *Tsuga heterophylla* reforestation site	Xiao G., 1991	Xiao G. (S4) = UBC S4		AF081424 (ITS2 only) (Monreal *et al*. 1999)
	7023	*O. griseum* (Xiao & Berch 1992, confirmed L. Sigler)	CANADA: BC, Vancouver Island, Port McNeill	roots of *Gaultheria shallon* in *Tsuga heterophylla* reforestation site	Xiao G., 1991	Xiao G. (S18) = UBC S18		AF081421 (ITS2 only) (Monreal *et al*. 1999)
	7024	*O. griseum* (Xiao & Berch 1992, confirmed L. Sigler)	CANADA: BC, Vancouver Island, Port McNeill	roots of *Gaultheria shallon* in *Tsuga heterophylla* reforestation site	Xiao G., 1991	Xiao G. (S61)		
	7025	*O. griseum* (Xiao & Berch 1992, confirmed L. Sigler)	CANADA: BC, Vancouver Island, Port McNeill	roots of *Gaultheria shallon* in *Tsuga heterophylla* reforestation site	Xiao G., 1991	Xiao G. (S91)		
	7026	*O. griseum* (Xiao & Berch 1992, confirmed L. Sigler)	CANADA: BC, Vancouver Island, Port McNeill	roots of *Gaultheria shallon* in *Tsuga heterophylla* reforestation site	Xiao G., 1991	Xiao G. (S92)		
	8442		IRELAND	roots of *Rhododendron* sp. cv. Pink Pearl	Heslin M.C.		ATCC 66504	RFLP analysis (Hambleton *et al*., 1998)
	8507	*O. griseum* (Burgeff 1961)	SWEDEN	ex root of *Ericaceae*	Burgeff H.		CBS 334.52	RFLP analysis (Hambleton *et al*. 1998)
	8529	*O. griseum* (Couture *et al*. 1983)	CANADA: Québec (PQ), Missisquoi, Frelighsburg	roots of *Vaccinium corymbosum* L.	Couture M. (40), 1981	DAOM	DAOM 184107	AF062799 (Hambleton *et al*. 1998)

Table 1. (Continued).

Name	UAMH number	Original name, if different (reference)	Location	Substrate	Isolator & date	Collector (#)	Other #	Sequence (reference)
O. maius	8919		CANADA: AB, Athabasca	roots of *Chamaedaphne calyculata* in acidic peatland	Hambleton S., 1993	Hambleton S. (S-9Da)		
	8920		CANADA: AB, Athabasca	roots of *Oxycoccus quadripetalus* in acidic peatland	Hambleton S., 1993	Hambleton S. (S-10Aa)		
	8921		CANADA: AB, Slave Lake	roots of *Vaccinium myrtilloides* open *Pinus banksiana* forest on stable sand dune	Hambleton S., 1994	Hambleton S. (S-27b)		AF062800 (Hambleton *et al*. 1998)
	8922		CANADA: AB, Slave Lake	roots of *Vaccinium vitis-idaea* L. in open *Pinus banksiana* on stable sand dune	Hambleton S., 1995	Hambleton S. (S-357Ca)		AF062801 (Hambleton *et al*. 1998)
	8923		CANADA: AB, Jasper National Park, Outpost Lake	roots of *Phyllodoce glanduliflora* in alpine heathland	Hambleton S., 1994	Hambleton, S (S-74Aa)		
	8924		CANADA: AB, Jasper National Park, Outpost Lake	roots of *Vaccinium scoparium* in alpine heathland	Hambleton S., 1994	Hambleton S. (S-81Bc)		
	8932		CANADA: AB, Athabasca	roots *Ledum groenlandi-cum* in acidic peatland	Hambleton S., 1994	Hambleton S. (S-38a)		
	8933		CANADA: AB, Jasper National Park, Outpost Lake	roots *Phyllodoce empetriformis* in alpine heathland	Hambleton S., 1994	Hambleton S. (S-77Aa)		
	9263	*O.* sp.	U.S.A.: Pennsylvania, Center Co., Rothrock State Forest, Little Flat mountain	roots of *Vaccinium angustifolium*	Stevens C.	Goulart B. (LFB)		
	9446	unnamed	U.S.A.: Idaho (ID), Priest Lake	roots of *Vaccinium membranaceum*	McCracken B. (PL3B)	McCracken B. (5)		AY624308 this study

Table 1. (Continued).

Name	UAMH number	Original name, if different (reference)	Location	Substrate	Isolator & date	Collector (#)	Other #	Sequence (reference)
O. maius	9749		CANADA: AB, nr Perryvale	decomposing *Sphagnum fuscum* in bog	Thormann M.	Thormann M. (MNT 1)		
	10116	unnamed	NORWAY: Kragero, Telemark	roots of *Populus tremuloides* seedling from boreal *Quercus-Pinus* forest	Vrålstad T., 1998	Vrålstad T. (ARON 2904.S)		AY624314 this study

T – ex-type culture; Acronyms: ARON - Division of Botany and Plant Physiology, Department of Biology, University of Oslo, Oslo, Norway; CBS – Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CDC – Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.; DAOM – National Mycological Herbarium, Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada; MUCL - Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; OAC - Ontario Agricultural College Herbarium, Department of Botany and Genetics, University of Guelph, Guelph, ON, Canada; UAMH – University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, AB, Canada; UBC – University of British Columbia, Vancouver, BC, Canada.

Table 2. Isolates of other *Oidiodendron* species examined.

Name	UAMH number	Original name, if different	Location	Substrate	Isolator (#) & date	Depositor (#)	Other #	Sequence (reference)
O. echinulatum	8467 (A[a])		CANADA: ON	peat soil in cedar bog	Barron G.L. (OAC 10264), 1964	IMI	IMI 110132	AF062791 (Hambleton *et al.* 1998)
	9246	*O. tenuissimum*	CANADA: AB, Elk Island National Park	well decayed log , *Picea glauca*	Lumley T., 1997	Lumley T. (EI-02-S1H, 1-20C)		
O. griseum	1524	*O. flavum* (Determined G.L. Barron)	CANADA: ON, Aberfoyle	soil ex cedar bog	Barron G.L., 1960	Barron G.L. (OAC 9206)	DAOM 95970 CBS 524.69	AF062792 (Hambleton *et al.* 1998)
O. sp.	1370	*O. griseum*	U.S.A.	paper sample ex mill	Wang C.J., 1959	DAOM	DAOM 70385 MUCL 4146	AF307765 (from MUCL 4146) (Lacourt *et al.*. 2001)
O. griseum	1403 (A)		SWEDEN	wood pulp	Melin E., 1960	DAOM	DAOM 75835 CBS 249.33 IMI 149010	AF062793 (Hambleton *et al.* 1998)

Table 2. (Continued).

Name	UAMH number	Original name, if different	Location	Substrate	Isolator (#) & date	Collector (#)	Other #	Sequence (reference)
O. griseum	1693		CANADA: BC, Fort Langley	timber of *Pseudotsuga menziesii*	Carmichael J.W., 1963			AF062794 (Hambleton *et al.* 1998)
	4080		CANADA: AB, Westlock	wood chips and bark ex logging truck	Sigler L., 1978			AF062795 (Hambleton *et al.* 1998)
	5971	*O. flavum*	CANADA	roots *Cypripedium acaule*	St. Arnaud M.	St. Arnaud M. (3.2WS)		AY624310 this study
	5973	*O.* sp.	CANADA: AB	galleries *Dendroctonus ponderosae* in *Pinus contorta*	Yamaoka Y., 1987	Yamaoka Y. (C1225)		
	7719		CANADA: AB, Fairview	indoor air, from *Apis mellifera* overwintering facility	Abbott S.P. (OHS 336), 1994			
	8364		CANADA: AB	airborne plate contaminant	Abbott S.P. (SA-M4), 1993			
	8528	*O. tenuissimum*	CANADA: AB, Sundance Canyon	*Pinus contorta*	Nordin V.S. (VJN 4500-A-11)	Currah R.S.	DAOM 51071	AF062796 (Hambleton *et al.* 1998)
	8925		CANADA: AB, Slave Lake	roots *Vaccinium myrtilloides* in open *Pinus banksiana* forest on stable sand dune	Hambleton S., 1994	Hambleton S. (S-31b)		AF062797 (Hambleton *et al.* 1998)
	9007	*O.* sp.	USA	sternal fluid, female 65 yr	1997	CDC	CDC B-5762	AY624312 this study
O. tenuissimum	1523		CANADA: Guelph, ON	soil in mixed deciduous wood	1960	Barron G.L. (OAC 9201)		AY624309 this study
O. fuscum	8511 (T[a])	(T) *O. fuscum* (previously considered a synonym of *O. tenuissimum*)	NORWAY	wood pulp	Robak H.	CBS	CBS 238.31 MUCL 1057	AF062807 (Hambleton *et al.* 1998)

Table 2. (Continued).

Name	UAMH number	Original name, if different	Location	Substrate	Isolator (#) & date	Depositor (#)	Other #	Sequence (reference)
O."tenuissimum"	8512	*O. tenuissimum*	SWEDEN	forest soil	Söderstrom B.E. (43)	CBS	CBS 920.73	AF062808 (Hambleton *et al*. 1998)
	8943	*O*. sp.	CANADA: AB, Lac La Biche	lung, northern flying squirrel (*Glaucomys sabrina* Shaw)	Csotonyi J. (9), 1997	Currah R.S. (A24/117-1MT/1c)		AY624311 this study
O. truncatum	1399 (T)		CANADA: ON, Guelph	soil, mixed wood	Barron G.L. (OAC 9203), 1960	DAOM	DAOM 75754	AF062809 (Hambleton *et al*. 1998)
	9064	*O*. sp.	CANADA: AB, Elk Island National Park	well decayed wood, *Picea glauca*	Lumley T., 1996	Lumley T. (EI-01-S5E, 3-3A)		AY624313 this study

[a]T – ex-type culture; A – authentic according to Barron (1962). Acronyms; CBS – Centraalbureau voor Schimmelcultures, Utrecht, NE; CDC – Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.; DAOM – National Mycological Herbarium, Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Canada; IMI - CABI Bioscience Reference Collections, Egham, Surrey, U.K.; MUCL - Mycothèque de l'Université catholique de Louvain, Louvain-la-Neuve, Belgium; OAC - Ontario Agricultural College Herbarium, Department of Botany and Genetics, University of Guelph, Guelph, Ontario, Canada; UAMH – University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, AB, Canada.

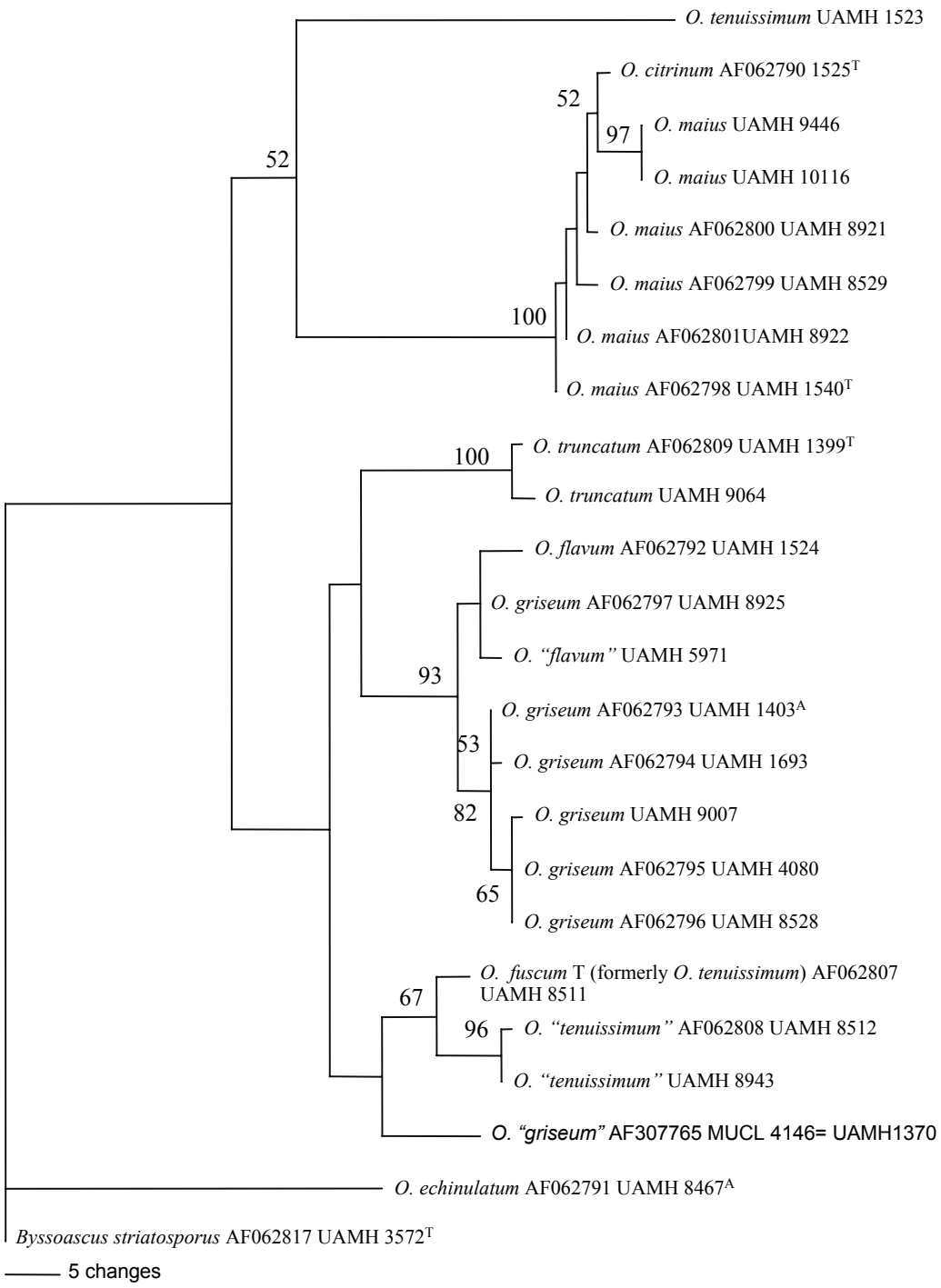

Fig. 2. One of 14 most-parsimonious trees for 24 taxa based on internal transcribed spacer (ITS) region sequences of *Oidiodendron* species. Only bootstrap values above 50 % are given. GenBank numbers for strains newly sequenced are listed in Tables 1 and 2. T = ex-type culture; A= authentic, as determined by Barron (1962).

after 28 d. The medium became discolored greyish to brown in thirteen isolates, but diffusing pigment did not extend beyond the margin of the colony. Two strains (UAMH 9446 and 10116, row A) were initially not selected as *O. maius* in visual examination. Colonies of these isolates were relatively thin, with a glabrous and only weakly striate surface; they were also nonsporulating. The colony of UAMH 10116 also differed in producing droplets of slime centrally. These strains were sequenced and results confirmed their placement in the *O. maius* group (see below).

ITS sequences derived from seven isolates ranged from 496 to 510 bp. The alignment of 24 taxa yielded a total of 489 characters and of these, 392 were constant, 28 were parsimony uninformative and 69 were parsimony informative. Heuristic searches yielded fourteen most-parsimonious trees in 181 steps; one such tree is shown in Fig 2. The trees have a consistency index of 0.669, a homoplasy index of 0.331 and a retention index of 0.837.

In the phylogenetic analysis, isolates of *O. maius* formed a monophyletic group with 100 % support. The ex-types of *O. citrinum* and *O. maius* differed by 1.7 %. The two strains (UAMH 9446 and 10116) that initially were thought to differ from *O. maius* based on culture macromorphology were identical to one another in ITS sequence, but somewhat different from other members of the *O. maius* clade. They differed from other *O. maius* isolates by a 1.4 to 2.3 % difference in ITS bases, with the highest number of differences being found between isolates 10116 and 8922.

Isolates belonging to other species also could be grouped by colonial features but with less reliability due, in part, to the examination of fewer representatives of each species (Fig. 1, rows F and G). No isolate demonstrated thin, glabrous colonies and striae similar to those of *O. maius*. Colony diameters overlapped when *O. maius* was compared to *O. griseum* (2–3.7 cm; median 3 cm) and *O. echinulatum* (3.8–4.2 cm), but *O. maius* colonies were smaller than isolates identified as *O. tenuissimum* (2.3–3 cm; median 2.5 cm) and larger than those classed as *O. truncatum* (4.8–5.5 cm). Colonies of *O. griseum* and *O. flavum* (UAMH 1524) were light to medium grey [1C–E] and velvety (row F). Scant to moderate diffusible pigments were present in seven strains of the *O. griseum* group. Colonies of *O. tenuissimum* showed greater variation. UAMH 8511, ex-type of O. *fuscum* Robak, was thin, glabrous and greyish orange [6B4] with scant diffusible pigment below the colony. Colonies of UAMH 8512 (row G) and 8943 (not shown) were strongly similar to each other and were orange grey to light grey [6B2/1C] and velvety with scant diffusible pigments. Two isolates on deposit as *O. tenuissimum* were dissimilar in colonial features. UAMH 1523 was slow growing (colony diam 2.3 cm) but the colony was dark grey [1F], velvety and slightly zonate (row G) and lacked diffusing pigment. UAMH 9246 matched the authentic isolate of *O. echinulatum* (8467; shown in row G) in having relatively rapidly growing colonies that were grey [1E] centrally and reddish to violet brown [8E5/10F4] at the margin. Both examined isolates of *O. truncatum* demonstrated sectors of thin, greyish white, glabrous mycelium as well as areas that were velvety, brown [6E8] to powdery dark grey [1F]. Diffusible pigment developed below the sporulating areas. The ex-type isolate of *O. truncatum* sporulated copiously and conidia were easily dislodged, leading to the formation of satellite colonies. The isolate was subcultured three times to obtain the single colony shown in row F.

The cultural observations were mostly in agreement with results of the ITS analysis. *Oidiodendron echinulatum* was placed separately. Isolates of *O. griseum* and *O. truncatum* formed monophyletic groups with 93 and 100 % support. The *O. griseum* clade included an authentic isolate of *O. griseum*, UAMH 1403, and two isolates originally determined as *O. flavum*. An isolate from a human source, UAMH 9007, was confirmed as a member of the *O. griseum* group but UAMH 1370 was excluded from this group. The *O. tenuissimum* grouping received a relatively low level of support (67 %). The ex-type isolate of *O. fuscum*, UAMH 8511, was sister to a well supported group (96 %) that included UAMH 8512 and 8943, in agreement with observed cultural differences on CERC (Fig 1, row G). UAMH 1523 was excluded from this group; it was also culturally dissimilar to 8511.

DISCUSSION

Oidiodendron maius can be distinguished from other *Oidiodendron* species by its colonial morphology on CERC after 21 to 28 d. This method was reliable regardless of sporulation. UAMH 8507 was sterile on deposit, but its colonial morphology on CERC was typical for *O. maius*, resembling UAMH 7025 (row A) and 8920 (Row B). Sporulation was relatively strongly inhibited in all isolates of *O. maius* on CERC and some isolates exhibited no or very little sporulation (Fig 1, rows A, B, C). In contrast, sporulation was moderate to profuse in all isolates on plain CER, a medium which has been shown to be valuable for promoting sporulation in many anamorphic fungi and for use in slide culture preparations (Kane *et al*. 1997). UAMH 10116, from roots of *Populus tremuloides* (Vrålstad *et al*. 2000), was sterile when received, but it later developed conidiophores on plain CER, allowing its identification as *O. maius*. Similarly, the ericaceous mycobiont *Rhizoscyphus* (*Hymenoscyphus*) *ericae* (D. J. Read) W.Y. Zhuang & Korf often produces arthroconidia on CER, thus allowing confirmation of the identity of root isolates which are nonsporulating on other media (Hambleton & Sigler 2005–this volume).

Limitations of this cultural method for recognition of *O. maius* are access to the CER medium and cellophane. It is necessary to make the medium in-house since it is not available commercially. However, the baby food product is widely available in grocery stores and in some pharmacies and it is simple to make since no additives other than agar are required. The UAMH technique for preparing dried fungal colonies has been in use for 40 yr and the cellophane has been obtained from a British supplier (now UCB Films as listed above in Materials and Methods).

Three recent studies have examined the use of morphology, physiology and/or molecular techniques to identify *O. maius* and other *Oidiodendron* species (Hambleton *et al*. 1998; Lacourt *et al*. 2001; Rice & Currah 2001). The microscopic features were described in detail but found to be variable and unreliable especially for distinguishing *O. maius* from *O. griseum*

and *O. tenuissimum*. However, *O. maius* was clearly distinguished in molecular data (Hambleton *et al*. 1998, Lacourt *et al*. 2001). The value of physiological data is difficult to assess. Rice & Currah (2001) reported that isolates of *O. maius* demonstrated similar responses in enzymatic tests, but no representatives of other *Oidiodendron* species were analyzed for comparison. All isolates of *O. maius* examined by them, excepting one misidentified strain, demonstrated moderate capacity to break down cellulose in the cellulose azure test after 1 mo of incubation. It is difficult to compare responses using a different method, but in our study, none of the *O. maius* isolates grown on CERC degraded the cellophane membrane within 34 d, nor did they grow and sporulate especially vigorously as do many cellulolytic fungi when grown on a medium with cellophane.

Based on morphological and molecular analysis, Hambleton *et al*. (1998) suggested conspecificity between *O. citrinum* and *O. maius*. Our cultural data support this proposal. The ex-type isolate of *O. citrinum* (Fig 1, row C) was strongly similar to *O. maius* on CERC and the characteristic yellow-green coloration noted by Barron (1962) was not observed. The ITS sequence analysis of Lacourt *et al*. (2001) confirmed *O. citrinum* and *O. maius* as close relatives. These authors found low nucleotide divergence between the two species and no distinguishing morphological features other than colony colour. The grouping of *O. citrinum* isolates together led these authors to take up Hambleton *et al*.'s suggestion that these could be given subspecies status pending further analysis of diversity within this complex. *O. maius* and *O. citrinum* were described simultaneously (Barron 1962) and neither has priority. However, *O. maius* is the better known of the species and the one adopted by Hambleton *et al*.

Since the focus of our study was on *O. maius*, it was not designed to resolve problems within *O. griseum* and *O. tenuissimum*; however, some observations can be made. On CERC, colonies of *O. griseum* are uniformly grey and velvety to powdery due to profuse sporulation. Isolates of *O. flavum* are strongly similar, including UAMH 1524 determined by Barron (1962) as representing this species. Barron emended the species and noted that no original material of *O. flavum* was available for study and that the original description and figures were inadequate for proper identification. The grouping of *O. griseum* with *O. flavum* received 93 % support in the phylogenetic analysis (Fig. 2), in agreement with the results of Hambleton *et al*. (1998), who suggested possible synonymy between these species but reserved judgment pending analysis of additional strains. There appears to be little basis for retaining *O. flavum* as distinct. The results of Lacourt *et al*. (2001) are largely in agreement, except that their strongly supported "*O. griseum* clade" included one strain identified originally as *O. tenuissimum* (CLM 573.96 [Collezione del Laboratorio di Micologia, Department of Plant Biology, University of Turin, Italy]) and excluded another originally determined as *O. griseum* (MUCL 4146 = DAOM 70385 = UAMH 1370). The latter was placed sister to isolates of *O. tenuissimum* and appears to represent a distinct taxon (Fig. 2). Although Barron (1962) regarded *O. tenuissimum* as the most common member of the genus, it appears to have been the species most difficult for subsequent workers to define. Barron placed *O. fuscum* as a synonym of *Periconia tenuissima* Peck (=*Oidiodendron tenuissimum*) based on examination of holotype material for both species, but he described Robak's ex-type culture of *O. fuscum* (UAMH 8511 = CBS 238.31) as atypical and reported that it showed minor differences from its original description. UAMH 8511 had a distinct colony type on CERC (Fig 1, row G). The observed variation suggests that resurrection to species status is warranted for Robak's *O. fuscum* as proposed by Rice & Currah (2005–this volume). These authors determined that Barron's isolate of *O. tenuissimum* (UAMH 1523 = OAC 9201 ; row G) matched the holotype of *P. tenuissima*. As shown in Fig. 2, *O. tenuissimum* UAMH 1523 is distant from *O. fuscum* UAMH 8511 and these clearly represent distinct taxa. Two other isolates identified as *O.* "*tenuissimum*" (UAMH 8512 and 8943) appear to represent a new taxon. These group together but apart from *O. fuscum*, in agreement with observed colonial differences on CERC (row G). The colonial appearance of UAMH 8512 overlaps *O. griseum – O. flavum* (row F). Lacourt *et al*. (2001), who examined most of the same isolates as those examined here, found overlap in morphological features between *O. griseum* and *O. tenuissimum* and isolates identified as these species were interspersed among two groups in their phylogenetic tree, leading them to suggest that *O. griseum – O. tenuissimum* could represent a single genetic and morphologically heterogeneous taxon. We disagree with this conclusion. *O. griseum* is identified by cultural features and strong support in ITS analysis. Confusion over *O. tenuissimum* arose only from misidentified strains.

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