Auxarthron teleomorphs for *Malbranchea filamentosa* and *Malbranchea albolutea* and relationships within *Auxarthron*

L. Sigler,[1*] S. Hambleton,[2] A. L. Flis,[1] and J. A. Paré[3]

[1]*University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, Alberta, Canada T6G 2E1,* [2] *Eastern Cereal and Oilseed Research Centre, Research Branch, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada K1A 0C6,* [3]*Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, USA 53706*

Abstract: *Malbranchea filamentosa* and *M. albolutea* were reexamined and connected to teleomorphs in the genus *Auxarthron*. DNA sequences were obtained from the small subunit (SSU) and internal transcribed spacer (ITS) regions of the nuclear ribosomal rRNA gene to evaluate conspecificity of the *M. filamentosa* isolates and to evaluate relationships among taxa within the *Auxarthron* clade. Five of eight isolates putatively identified as *M. filamentosa* produced fertile ascomata in matings and with F1 progeny. *Auxarthron filamentosum* sp. nov. is described for the teleomorph. Three nonmating isolates appeared conspecific based on morphology, but one was excluded based on sequence divergence. An *Auxarthron* teleomorph was described for *M. albolutea* in 1976, but not named because of uncertainty about its distinction from *A. thaxteri* and *A. umbrinum. Auxarthron alboluteum* sp. nov. is shown to be phylogenetically distinct. Phylogenetic analysis based on newly derived sequences showed that members of the genus *Auxarthron* and *Malbranchea dendritica* formed a strongly supported monophyletic group. In the ITS analysis, most species were placed into two well supported clades that correlated with the shape of the ascospores; the position of the type species *A. californiense*, was not clearly resolved. Differences were found between newly derived SSU sequences and those on deposit in GenBank for the same strains. After re-evaluation of the strains, the following sequences are considered to be incorrect: *M. albolutea* L28063 (UAMH 2846), *A. zuffianum* L28062 (UAMH 4098), *M. dendritica* L28064 (UAMH 2731) and *M. filamentosa* L28065 (UAMH 4097). The sequence for U29389 is correct for *Malbranchea albolutea* not *M. dendritica* as stated in the original GenBank record. The problems with these sequences were not uncovered in prior published analyses because insufficient representatives of *Auxarthron* species were sampled.

Keywords: *Malbranchea filamentosa* , *Malbranchea. albolutea, Malbranchea dendritica, Auzarthron, Onygenaceae*

Introduction

Members of the genus *Auxarthron* Orr & Kuehn produce globose mesh-like gymnothecia composed of branched hyphae with blunt or spiny apices and often bearing elongate appendages. Ascospores are oblate, subglobose or globose and punctate to punctate-reticulate. Anamorphs are common, and separate names in the genus *Malbranchea* Sacc. have been provided for several species with distinctive anamorphs, as was the case for the two species reexamined in this study.

In 1977, Borghi *et al.* reported recovery of seven isolates of *Coccidioides immitis* Stiles ex Rixford & Gilchrist from soil in Argentina. They based their identification on *in vitro* formation of 'spherules'; however, their published illustrations did not show endospores and the microscopic features of the isolates appeared dissimilar to those of *C. immitis*. Two isolates were described as the new species *Malbranchea filamentosa* Sigler & J.W. Carm., characterized by alternate arthroconidia and smooth, branched, yellowish brown setae (Sigler *et al.,* 1982). The presence of the setae suggested the propensity to form a teleomorph, but none was obtained when the two isolates were grown alone under different conditions or when they were mated (Sigler *et al.,* 1982). Two other of Borghi's Argentinian isolates were thought to represent *Malbranchea albolutea* Sigler & J.W. Carm. and *Auxarthron zuffianum* (Morini) G.F. Orr & Kuehn (Sigler *et al.,* 1982). Sigler & Carmichael (1976) described *Malbranchea albolutea* as having an *Auxarthron* teleomorph but did not name it pending further examination of the relationship with the species *A. thaxteri* (Kuehn) G.F. Orr & Kuehn and *A. umbrinum* (Boudier) G.F. Orr & Plunkett (Orr & Kuehn, 1971; Orr *et al*., 1963).

We recently obtained additional isolates of *M. filamentosa* during a survey of the cutaneous fungal biota of healthy captive squamate reptiles (Paré *et al*., in press). The main focus of that survey was to evaluate prevalence of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (Apinis) Currah, which has been identified as an etiologic agent of reptile skin and

* Corresponding author: Lynne Sigler. University of Alberta Microfungus Collection and Herbarium. Devonian Botanic Garden, Edmonton, Alberta, Canada T6G 2E1. email: lynne.sigler@ualberta.ca

deep infection (Paré *et al.*, 1997; Nichols *et al.,* 1999; Thomas *et al*., 2002). All onygenalean fungi recovered were identified to species. Two *M. filamentosa* isolates were mated and yielded fertile ascomata typical of the genus *Auxarthron.* This prompted a broader study of compatibility among all available isolates. We obtained DNA sequences from the small subunit (SSU) and internal transcribed spacer (ITS) regions of the nuclear ribosomal rRNA operon and compared them to published sequences to further evaluate conspecificity, and to assess the relationships of *M. filamentosa* and *M. albolutea* to species of *Auxarthron.*

Methods

Mating and morphology

Eight isolates identified as *M. filamentosa* were on deposit at the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, Alberta (Table 1). Two strains derived from skins of reptiles demonstrated mating compatibility when paired on Takashio agar (a medium used to promote ascosporulation among onygenalean fungi) (TAK, Kane *et al.,* 1997). These were designated as + (UAMH 9986) and – (UAMH 9987) mating type and paired individually with all other strains. All matings were done on TAK at 30 °C in the dark. Self-pairings were not done because all isolates had been grown on different sporulation media (i.e., oatmeal agar, TAK; Kane *et al.,* 1997) and at different temperatures at the time of deposit and examined carefully for development of a teleomorph. The resultant cross of 9986 and 9987 was dried and also retained as a living culture (UAMH 10042). Eight F1 progeny were derived and backcrossed with the parent strains (Table 3). Three F1 progeny were deposited as UAMH 10138 (+), UAMH 10139 (–) and UAMH 10140 (–) and backcrossed with three isolates (7163, 7165, 10036) that failed to mate with the parent strains in the first experiment. The three latter isolates were also mated with each other.

Isolates were grown on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI), cereal agar (CER; Kane *et al.,* 1997) and TAK incubated at 30ºC in the dark. Five isolates were grown on PDA at 35ºC to assess thermotolerance. Colony colour terms are according to Kornerup and Wanscher (1978). Ascomata and ascospores were examined by light and scanning electron microscopy (SEM). Material for SEM was fixed in 2.5% glutaraldehyde in Millonig's buffer (Millonig, 1961), pH 7.3 and postfixed in 2% osmium tetroxide in the same buffer. After drying to the critical point, the samples were sputter coated with gold and examined with a Hitachi S-2500 (Hitachi, Ltd.,Tokyo, Japan).

Table 1. Source of *Malbranchea filamentosa* strains examined and sequenced.

UAMH No.	Source	Mating type	Branched Setae	GenBank No.		Other No.
				SSU	ITS	
4096	soil Argentina A.L. Borghi (5)	+	present	ND	ND	CDC Y-334 (77-104309)
4097^{T}	soil Argentina A.L. Borghi (6)	+	present	AY124501 (also L28065; Pan *et al*., 1994)	AY177298	CDC Y-335 (77-104310) ATCC 48174, CBS 581.82, DAOM 184714 IMI 274926
7163	soil, Benatshimpuma, Kasai, Zaire, Africa, C. de Vroey (RV 24803), Aug 1968, from J. Guarro	did not mate	present	AY124500	AY177299	FMR 3858 CBS 198.92
7164	soil, Benatshimpuma, Kasai, Zaire, Africa, C. de Vroey (RV 24801), Aug 1968, from J. Guarro	+	present	ND	ND	FMR 3859 CBS 199.92
7165	soil, Kuriange, Burundi, Africa, C. de Vroey (RV 24810), Jun 1968, from J. Guarro	did not mate	present	ND	AY177300	FMR 3860 CBS 200.92
9986	shed skin ex frilled lizard (*Chlamydosaurus kingii*), male >7 yr, San Diego Zoo, San Diego, CA	+	present	ND	ND	
9987	shed skin, twin-spotted rattlesnake (*Crotalus pricei*), female 7 yr Audubon Park & Zoological Garden, New Orleans, LA, J. Paré Jan 2001	–	present	ND	AY177301	
10036	shed skin, Eastern indigo snake (*Drymarchon corais couperi*), female 6 yr, Buffalo Zoological Gardens, Buffalo, NY	did not mate	absent	AY124502	AY177302	

T - ex-type culture. ND – not done

Table 2. Other isolates sequenced in this study.

Species	UAMH No.[1]	Source	GenBank No.			Other No.[2]
			New Sequences		Previously published SSU sequences; ref.	
			SSU	ITS		
Malbranchea albolutea Sigler & J.W. Carm.	2846[T]	soil, Utah, from G.F. Orr as O-3508	AY124494	AY177303	L28063; Pan *et al*., 1994	ATCC 34522 CBS 125.77 IMI 211193
	4119	soil, Argentina, A.L. Borghi (9)	AY124495	AY177304	U29389 as *Malbranchea dendritica,* Bowman *et al.,* 1996	CDC 77-104311 = Y-336
Auxarthron thaxteri (Kuehn) G.F. Orr & Kuehn	3912[T]	dung of opossum shrew (*Selenodon*), Haiti, R. Thaxter, from G.F. Orr as O-532 (T *Myxotrichum thaxteri*)	AY124497	AY177305		ATCC 15598 CBS 248.58 NRRL 1717
Auxarthron zuffianum (Morini) G.F. Orr & Kuehn	1875[NT]	lung ex prairie dog, Texas, G.F. Orr as O-514 (Neotype of *A. zuffianum;* T *Gymnoascus brevisetosus*)	AY124493	AY177306	U29395; Bowman *et al*., 1996	ATCC 13484 CBS 219.58 Emmons E5001
	4098	soil, Argentina, A.L. Borghi (13)	AY124492	AY177307	L28062; Pan *et al*., 1994	CDC 77-104312 = Y-337
Auxarthron umbrinum (Boudier) G.F. Orr & Plunkett	1874	laboratory contaminant, Univ. of Calif., Los Angeles, from G.F. Orr as O-1040	AY124499	AY177308		
	3952[T]	soil, U.K., from G.F. Orr as O-1030; T *Gymnoascus umbrinus*	AY124498	AY177309		ATCC 15606 CBS 105.09 NRRL 3657
Malbranchea dendritica	2731[T]	soil, Dugway, Utah, G.F. Orr DPG-141	AY124496	AY177310	L28064; Pan *et al.,* 1994	ATCC 34527 CBS 131.77 IMI 211199

1. T - ex-type culture; NT – neotype culture.
2. Other collections: ATCC – American Type Culture Collection, Manassas, VA; CBS – Centraalbureau voor Schimmelcultures, Utrecht, NE; CDC – Centers for Disease Control, Atlanta, GA; IMI – International Mycological Institute, Egham, UK; NRRL –National Center for Agricultural Utilization Research, ARS-USDA, Peoria, IL

Molecular Analysis

SSU and ITS rDNA sequences were determined for five strains identified as *M. filamentosa*, including UAMH 4097 ex-type, 9987, and three that did not mate (UAMH 7163, 7165 and 10036) (Table 1). The provenance of eight other strains sequenced for this study and included in the analyses is provided in Table 2. Although sequence data for some of the strains sampled were already on deposit in the public nucleotide database GenBank, new sequences were obtained in order to provide data from both DNA regions of interest, and to re-evaluate the results of prior analyses of small subunit sequence data for this group of taxa. UAMH 4119, *Malbranchea albolutea,* was resequenced because of uncertainty about the sequence on deposit as U29389. The GenBank record indicates the provenance as UAMH 4119, but the taxon name as *M. dendritica* rather than *M. albolutea* (Table 2).

Genomic DNA was extracted from mycelium grown on PDA or TAK using a FastDNA® Extraction Kit (BIO 101 INC., Carlsbad CA) and a FastPrep™ Cell Disruptor machine (BIO 101, Carlsbad, CA). DNA amplification and cycle sequencing reactions were performed on a Techne Genius thermocycler (Princeton, NJ). PCR reactions were performed in 25 µL volumes using Ready-To-Go™ PCR Beads (Amersham Pharmacia Biotech Inc., Piscataway NJ) and 2 µL of template DNA. PCR cycling parameters included 30 cycles of denaturation at 95°C for 1.5 min, annealing at 56°C for 1 min, and extension at 72°C for 2 min, with an initial denaturation of 4 min and a final extension step of 10 min. Primers NS1 and ITS4 (White *et al.,* 1990) were used to amplify over 1700 base pairs of the SSU with the complete ITS region, including the ITS1, 5.8S and ITS2. Amplified products were purified using the UltraClean PCR Purification Kit (BIO 101 INC., Carlsbad, CA). DNA concentrations were estimated from fragments stained by ethidium bromide and separated by agarose gel electrophoresis.

Sequencing reactions were performed using the BigDye™ Terminator Cycle Sequencing System (Applied Biosystems, Foster, CA) with the recommended cycling parameters. Reactions were purified by ethanol /sodium acetate precipitation and resuspended as recommended for processing on an ABI PRISM™ 310 DNA sequencer (Applied Biosystems, Foster, CA). Sequencing primers were selected from the range of SSU and ITS primers given in White *et al.* (1990) and Landvik *et al.* (1997) to obtain sequence data for the whole region amplified. Consensus sequences were determined from overlapping sequence data for both DNA strands using

the software Sequencher™ (Gene Codes Corp., Ann Arbor, MI).

To examine phylogenetic relationships among species of *Auxarthron* and related *Malbranchea* spp., DNA sequences were manually aligned in two separate data matrices. Eleven new SSU sequences for six species were aligned with 26 sequences retrieved from GenBank, chosen to represent the phylogenetic diversity of onygenalean fungi. Four species in the *Eurotiales* served as the outgroup. Thirteen new ITS sequences for the same six species were aligned in a second data matrix with eight additional GenBank sequences. *Amauroascus kuehnii* von Arx and *Am. pseudoreticulatus* Currah were selected as outgroup taxa, based on the results of the SSU analysis. Accession numbers for the sequences retrieved from GenBank are given in Figs. 1 and 2, and in Tables 1 and 2 for the sequences used in the analysis shown in Fig. 1A. Both data matrices were subjected to parsimony analysis using the heuristic search option of PAUP* v. 4.0b8 (Swofford, 1999) using the options for 100 replicates of random stepwise addition of taxa, tree bisection-reconnection (TBR) branch swapping, and gaps treated as missing data. Bootstrap percentages used to assess support for the branching topologies were determined from 1000 resamplings of each dataset using the full heuristic search option and simple stepwise addition.

Results

Four strains, including the ex-type strain of *M. filamentosa*, UAMH 4097, mated with UAMH 9987 to produce gymnothecia of the *Auxarthron* type and were categorized as plus or minus mating types (Table 3). Eight F1 progeny derived from UAMH 10042 (cross of 9986 x 9987), when backcrossed with the parents, yielded fully fertile crosses and segregated as two plus and five minus mating types (Table 3). One progeny strain (sai A) failed to produce any ascomata with either parent after 10 weeks. Another progeny strain (sai D) produced a few infertile ascomata. Three isolates (7163, 7165, 10036) failed to mate.

Nearly complete SSU sequences, 1650 to 1750 nucleotides (nt) in length, and complete ITS1/5.8S/ITS2 sequences, 487 to 524 nt, were obtained for eleven (SSU) and thirteen (ITS) strains in six species. GenBank accession numbers are given in Tables 1 and 2. SSU sequences for UAMH 9987 and 7165 of *M. filamentosa* were not determined. Except where noted below, sequences of strains within species were identical. Three of four isolates identified as *M. filamentosa,* two mating (4097, 9987) and one nonmating (7165), had identical ITS sequences; UAMH 7163 (nonmating) differed at one position. Another nonmating isolate putatively identified as *M. filamentosa* (10036) differed at 38 positions (7.5%). The SSU sequence of *Auxarthron thaxteri* (3912) was identical to that of the ex-type of *M. filamentosa* but the ITS sequences differed by 6%. *Malbranchea albolutea* 4119 differed from 2846 (ex-type) at one position in both the SSU and ITS sequences. Our SSU sequence for *M. albolutea* 4119 was identical to U29389, indicating that this sequence is derived from UAMH 4119 as stated in the GenBank record, but that the taxon name was given incorrectly as *M. dendritica* (Table 2) (Bowman *et al.,* 1996). The SSU sequence of 4119 differed at 16 positions from that of the ex-type strain of *M. dendritica* 2731. Two ITS sequences for *A. umbrinum* were identical and matched that of a strain identified as *A. conjugatum* (AJ271573; no SSU data available for this strain). ITS sequences for the two strains of *A. zuffianum* differed at two positions.

The complete SSU data matrix comprised 41 taxa and 1668 aligned characters. Of these 1465 were constant and 117 were parsimony-informative. The *Auxarthron* clade as represented in a preliminary analysis is shown in Fig. 1A. The tree illustrates the anomalous placement of L28063 *M. albolutea* outside of the *Auxarthron* clade and discrepancies between strains newly sequenced and sequences obtained from GenBank based on the same UAMH strains.

Table 3. Mating reactions among isolates of *Auxarthron filamentosum* on TAK incubated at 30ºC.

	Plus mating type					
Minus mating type	4096	4097[T]	7164	9986	10138 (sai B)	sai E
9987	+	+	+	+	+	+
10139 (sai G)	ND	+	ND	+	+	ND
10140 (sai H)	ND	+	ND	+	+	ND
sai C	ND	ND	ND	+	ND	ND
sai D	ND	ND	ND	IF	ND	ND
sai F	ND	ND	ND	+	ND	ND

T – ex-type strain of *Malbranchea filamentosa.* sai – single ascospore isolate derived from cross of UAMH 9986 x 9987. + - ascomata with ascospores present. ND – not done. IF – infertile ascomata

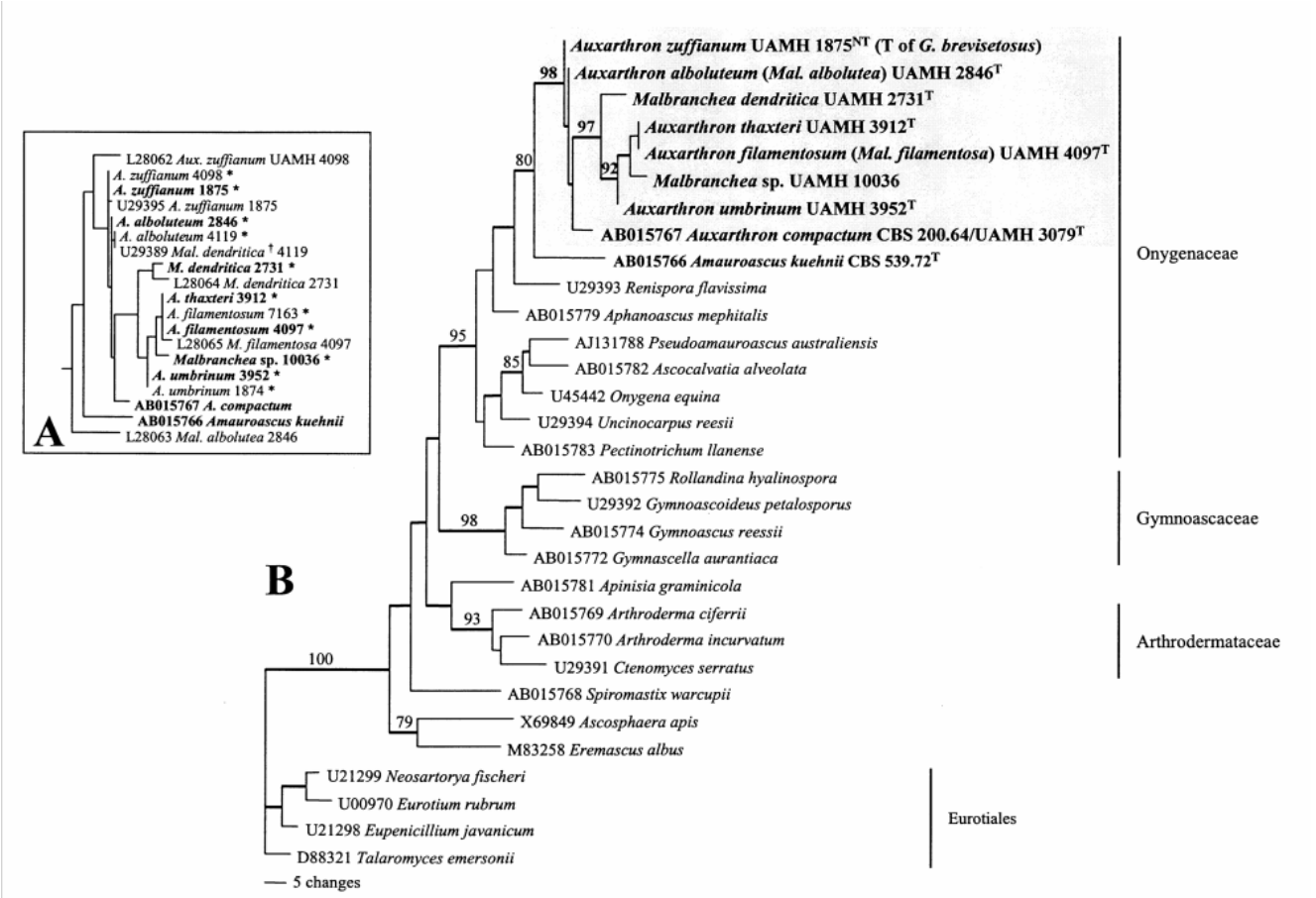


Fig. 1. Results of a maximum parsimony analysis of the small subunit (SSU) data matrix using the heuristic search algorithm of PAUP. **Fig.** 1A. The inset shows the *Auxarthron* clade resulting from a preliminary analysis of all 41 taxa in the alignment. Differences between newly derived SSU sequences (marked with *) and those on deposit in GenBank are shown (see results for detailed explanation). L28063 is shown outside the *Auxarthron* clade. †U29389 is based on *Malbranchea albolutea* not *M. dendritica* as stated in the GenBank record. **Fig.** 1B. The complete phylogram is one of eight equally parsimonious trees (408 steps, CI = 0.556) from an analysis of the reduced taxon set (31 taxa). The *Auxarthron* clade is highlighted and bootstrap values above 70% are given adjacent to the corresponding node. Branches in bold were present in the strict consensus. Bold type indicates the strains included in both analyses.

For U29395 (UAMH 1875 *A. zuffianum*; Bowman *et al*. 1996) the character state at aligned position 12 was 'G' (nt 42 of the deposited sequence) compared with a 'T' for our sequence from 1875, as well as for all of the other sequences included in the SSU data matrix. For other strains, the number of differences at aligned positions was 16 for *A. zuffianum* (L28062, UAMH 4098), 22 for *M. albolutea* (L28063, UAMH 2846), 10 for *M. dendritica* (L28064, UAMH 2731) and 5 for *M. filamentosa* (L28065, UAMH 4097). All our sequences were confirmed by analysis of the reverse strand and by re-sequencing from a second DNA extraction. Cultures were verified as authentic by a match with the original description and with specimens on deposit at UAMH. Therefore these GenBank sequences were considered incorrect and eliminated from further analysis.

Parsimony analysis of the reduced taxon set (31 taxa, 1475 constant and 113 parsimony informative characters) resulted in eight equally parsimonious trees (MPTs) of 408 steps, with a consistency index (CI) of 0.556 and a retention index (RI) of 0.730. Results of bootstrap analysis are shown on one MPT (Fig. 1). Species of *Auxarthron* and *Malbranchea* formed a strongly supported monophyletic group (highlighted clade; 98% bootstrap support) within the *Onygenaceae*. Within this group, *A. filamentosum, A. umbrinum, A. thaxteri*, and *M. dendritica,* for which no teleomorph is known, clustered together in a strongly supported clade (97% support) with *A. zuffianum, A. alboluteum* and *A. compactum* in a basal position. UAMH 10036 differed phylogenetically from *A. filamentosum* and the other species and was redesignated "*Malbranchea* sp." pro tem. Other well-supported clades corresponded to three families in the *Onygenales*, namely, the *Arthrodermataceae* (93%), *Gymnoascaceae* (98%) and *Onygenaceae* (95%). The other MPTs differed only in the position of UAMH 10036 with respect to *A. filamentosum* and *A. thaxteri*, and in the arrangement of taxa within the *Arthrodermataceae* clade.

The ITS data matrix comprised 21 taxa and 582 aligned characters. Of these, 378 were constant and 107 were parsimony-informative; 55 ambiguously

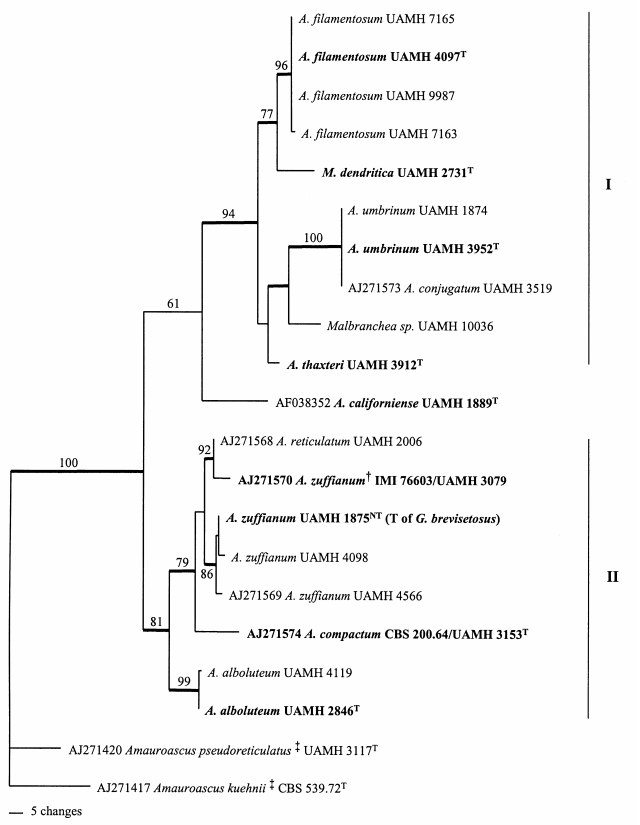


Fig. 2. One of 21 equally parsimonious trees (289 steps, CI = 0.689) resulting from a maximum parsimony analysis of the ribosomal internal transcribed spacer (ITS) data matrix using the heuristic search algorithm of PAUP. Bootstrap values above 60% are given adjacent to the corresponding node. Branches in bold were present in the strict consensus. Bold type indicates ex-type (T) and ex-neotype (NT) strains. †*Auxarthron zuffianum* UAMH 3079 is reidentified as *A. reticulatum.* ‡The GenBank sequences AJ271420 and AJ271417 are derived from ex-type cultures *Amauroascus pseudoreticulatus* (UAMH 3117) and *Am. kuehnii* (CBS 539.72), but the sequences were deposited under the names *Auxarthron pseudoreticulatum* and *Aux. kuehnii* (Solé *et al*. 2002) (see discussion). The corresponding SSU sequence for CBS 539.72 is deposited in GenBank under *Am. kuehnii* (see also Fig. 1).

aligned characters were excluded from the analysis. Parsimony analysis resulted in 21 MPTs of 289 steps, with CI of 0.689 and RI of 0.825. Results of bootstrap analysis are shown on one MPT in Fig. 2.

In the phylogenetic schema shown in Fig. 2, species of *Auxarthron* clustered in two main groups. Clade I, well supported with a bootstrap value of 94%, comprised *A. filamentosum*, *A. umbrinum*, *A. conjugatum*, *A. thaxteri* and *M. dendritica.* The mating and nonmating *A. filamentosum* isolates clustered with strong support (96%) except for the nonmating isolate UAMH 10036, which was excluded. Its distinction from other *Auxarthron* species supported the suggestion from the SSU analysis that it represents an anamorph of another undescribed *Auxarthron* species. The position of *A. californiense,* the type species, within the *Auxarthron* clade was unresolved. Clade II (bootstrap 81%) comprised *A. alboluteum*, *A. compactum, A. reticulatum* and *A. zuffianum*. The *A. alboluteum* strains clustered together with strong support, and the ITS data provide further evidence that the GenBank sequence L28063 is not correct for *M. albolutea.* Strains identified as *A. zuffianum* were distributed in two sister clades.

Taxonomy

Auxarthron filamentosum Sigler, Hambleton & Flis, *sp. nov*. — Figs. 3-7, 12-17

Ascomycota, Onygenales, Onygenaceae

Ascomata gymnothecia, 200 – 350 µm *diam, globosa, discreta, rubro-brunnea, hyphae peridiales septatae, ramosae, reticuloperidium formantes, crassiter tunicatae, asperulatae,* 4.5 - 6 µm *latae; appendices elongatae, orientes ex apicibus libris, rectae, leves, nonseptatae, flavo-brunneae, a septo basali 100-290* µm *longitudine, aliquando ramosae prope basim; asci octospori, subglobosi, deliquescentibes; ascosporae globosae vel subglobosae, 2.8 –3.2* µm*, punctatae-reticulatae, flavo-aurantiacae in massa; heterothallicae.*

Holotypus UAMH 10042, colonia exsiccata e mixtura UAMH 9986 x 9987.

Status anamorphosis: *Malbranchea filamentosa* Sigler & J.W. Carm. 1982. Mycotaxon 15:468.

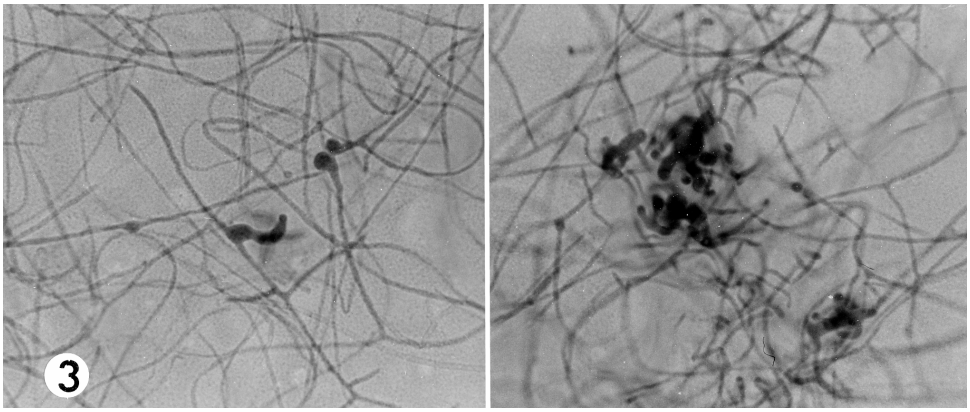
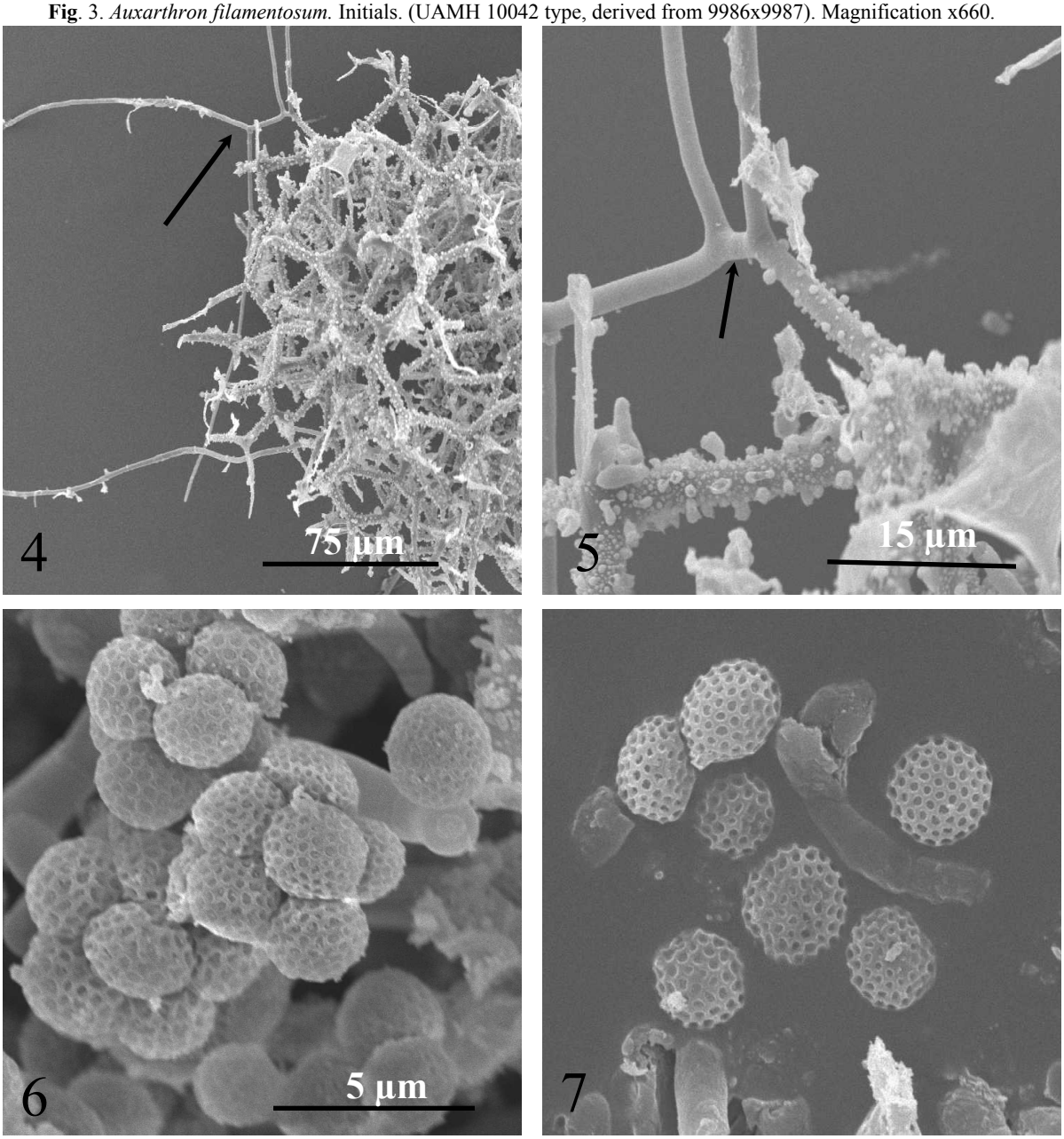


Fig. 3. *Auxarthron filamentosum.* Initials. (UAMH 10042 type, derived from 9986x9987). Magnification x660.



Figures. 4- 7. *Auxarthron filamentosum.* **Fig.** 4. Gymnothecium with elongate appendage showing basal branching (arrow). (UAMH 9986x10139). Bar = 75 µm. **Fig.** 5. Branching at base of appendage (arrow) (UAMH 9986x10139). Bar = 15 µm. **Figs**. 6-7. Punctate ascospores (UAMH 10042, holotype). Bar = 5 µm.

Ascomata (Fig. 4) gymnothecia 200 – 350 µm diam, globose, discrete; peridial hyphae septate, thick-walled, asperulate, reddish brown, 4.5 to 6 µm wide, branched and forming a network of hyphae (reticuloperidium); appendages (Fig. 5) arising from free apices, straight, smooth, yellowish-brown, 100 to 290 µm long from basal septum, often bearing two or three branches near the base, tapering to 2 µm in width at tip, nonseptate; appendages arising also from the vegetative hyphae, branched, smooth, nonseptate, yellowish-brown. Initials (Fig. 3) arising from swollen hyphal cells of adjacent hyphae. Asci 10 x 7 µm, subglobose, 8-spored, evanescent and infrequently observed. Ascospores (Fig. 6-7) punctate-reticulate by SEM, globose to subglobose, 2.8 – 3.2 µm, yellow in mass. Heterothallic. Arthroconidia cylindrical, straight or curved, sometimes rounding up in age and becoming subglobose or globose, hyaline, tan in mass, 2.5 – 4 x 1.5 – 2 µm.

Colonies (Fig. 12-17) are slow growing. On PDA at 30ºC, colonies reach a diam of 3 – 5.5 cm in three weeks (4.5 – 7 cm in 6 weeks), and are pale yellow (4B3) to greyish orange (6B2/3) with reverse light brown (6D5), with central area slightly raised or radially furrowed, velvety to powdery, and peripheral region thinner, flat, or furrowed, sometimes zonate with age. Diffusible tan pigment may be present. Colonies on CER reach 3.2 – 4.5 cm diam after three

weeks (5.8 – 7.5 cm after 6 weeks). They are initially flat, thin, with light orange surface mycelium (6A4/5), becoming pale to brownish orange (5A3 to 5B/C), centrally velvety to powdery, strongly furrowed, and cracking along the folds, sometimes sectoring or zonate. The medium shows a tan discoloration below the colony (e.g. Fig. 13). Colonies on TAK are 3 – 5 diam in three weeks (4 – 6 cm in five weeks), orange white (5A2) becoming greyish orange (6B3), with reverse light brown to brownish orange (7C4) or scarcely coloured, with central area dense, powdery, flat or raised, marginal area thin, sometimes zonate or with sectors of thin mycelium. Good growth occurred at 35ºC with colonies almost equivalent to those at 30ºC after 1 week.

Comments

Auxarthron filamentosum is distinguished by branched brown setae that develop from the vegetative mycelium when isolates are grown on sporulation media such as TAK or oatmeal salts agar (Sigler *et al.*, 1982). Heterothallism in *Mal. filamentosa* was suspected when the species was first described, but only two isolates were then available and these are shown here to be of the same mating type. Of the three isolates that failed to mate with other isolates or with the progeny of crosses, two are regarded as conspecific with *A. filamentosum*. They demonstrated the setae typical of this species (Table 1) and ITS sequences were identical or differed at one (7163) nucleotide position. The failure of these isolates to mate successfully may have been the result of suboptimal experimental conditions or may indicate that a partial fertility barrier exists for some strains. The study was not designed to distinguish these possibilities. The third non-mating strain, UAMH 10036, is excluded from the species because it failed to mate and, more importantly, because the ITS sequence differed at 38 positions from that of the ex-type strain of *A. filamentosum* (Table 1, Fig 2). This strain also produced no setae, and grew more slowly at 35°C than did authentic *A. filamentosum* isolates. Isolates of *A. filamentosum* differed from each other in growth rates and colonial appearance (Figs. 12-17) but these differences were not found to be associated with mating type. All isolates produced diffusible brownish pigments on PDA.

The SSU sequence of *A. filamentosum* was identical to that of *A. thaxteri* but the ITS sequences differed by 6%. These species are similar in their slow growth rates, but differ in their abilities to grow at higher temperatures. Isolates of *A. filamentosum* grew equally well at 30°C and 35°C, whereas the ex-type culture of *A. thaxteri* was strongly inhibited (see below in the comments section on *A. alboluteum*).

Auxarthron alboluteum Sigler & Hambleton *sp. nov.* — Figs. 8-9.

Ascomata gymnothecia, 280 – 400 μm diam, globosa, discreta; hyphae peridiales septatae, ramosae, reticuloperidium formantes, crassiter tunicatae, asperulatae, brunneae; appendices 400 – 800 μm elongatae, leves, non septatae, rectae vel aliquando uncinatae, flavo-brunneae prope basim, fastigatae ad apicem hyalinum qui, si defringit, remanet hebes. Asci octospori, evanescentes, 6.5 – 8 μm diam. Ascosporae globosae, punctatae-reticulatae, flavo-aurantiacae in massa; (2.2) 2.5 – 3.5 μm diam.
Holotypus UAMH 2846, colonia exsiccata.

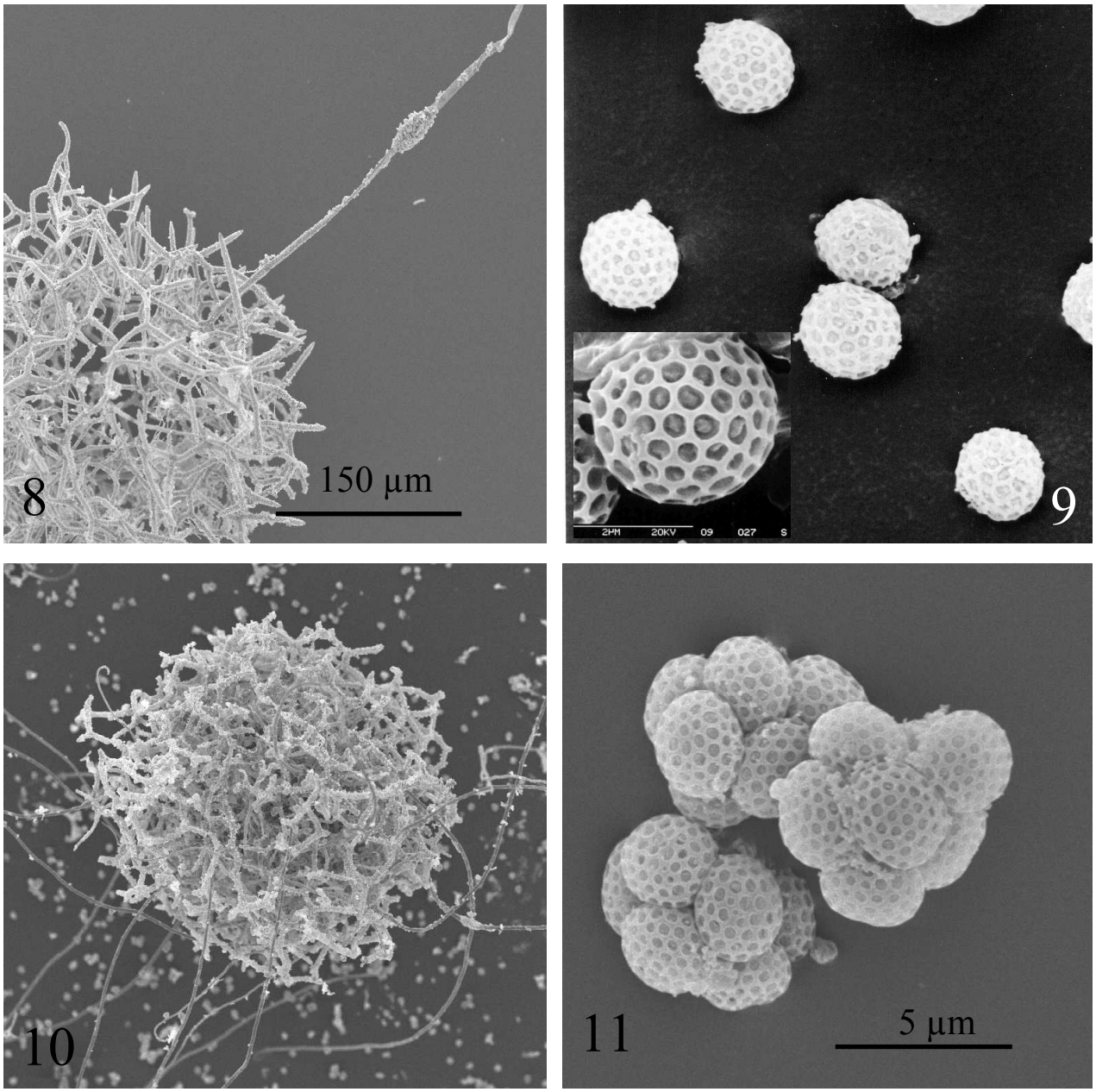
Status anamorphosis: *Malbranchea albolutea* Sigler & J.W. Carm. 1976. Mycotaxon 4:416.

Ascomata (Fig. 8) gymnothecia, brown, globose, discrete, 280 – 400 μm diam (excluding appendages), composed of a branched network (reticuloperidium) of thick-walled, delicately asperulate, septate, hyphae, 3-5 μm wide, with free apices terminating in bluntly pointed spines; appendages arising from a bifurcate base, 400 – 800 μm long, smooth, straight, rarely uncinate, thick-walled and yellowish brown over half the length, tapering to a hyaline apex that may break off leaving the tip blunt. Asci evanescent, hyaline, 8-spored, 6.5 – 8 μm diam. Ascospores (Fig. 9) yellow, appearing finely asperulate by light microscopy, punctate-reticulate by scanning electron microscopy, globose, (2.2) 2.5 – 3.5 μm diam.

Colonies on PDA are 5.5 to 7.8 cm diam after 21 days at 30ºC, yellowish white (4A2/3), powdery, sometimes with sectors of thin mycelium, faintly zonate. Colonies are similar at 25ºC, but growth is slower at 35ºC (2.2 to 3.5 cm diam in 21 days). On OAT, colonies are flat, yellowish-white (3A2), powdery, often cracked, and attain a diam of 5.8 to 7 cm in 21 days.

Comments

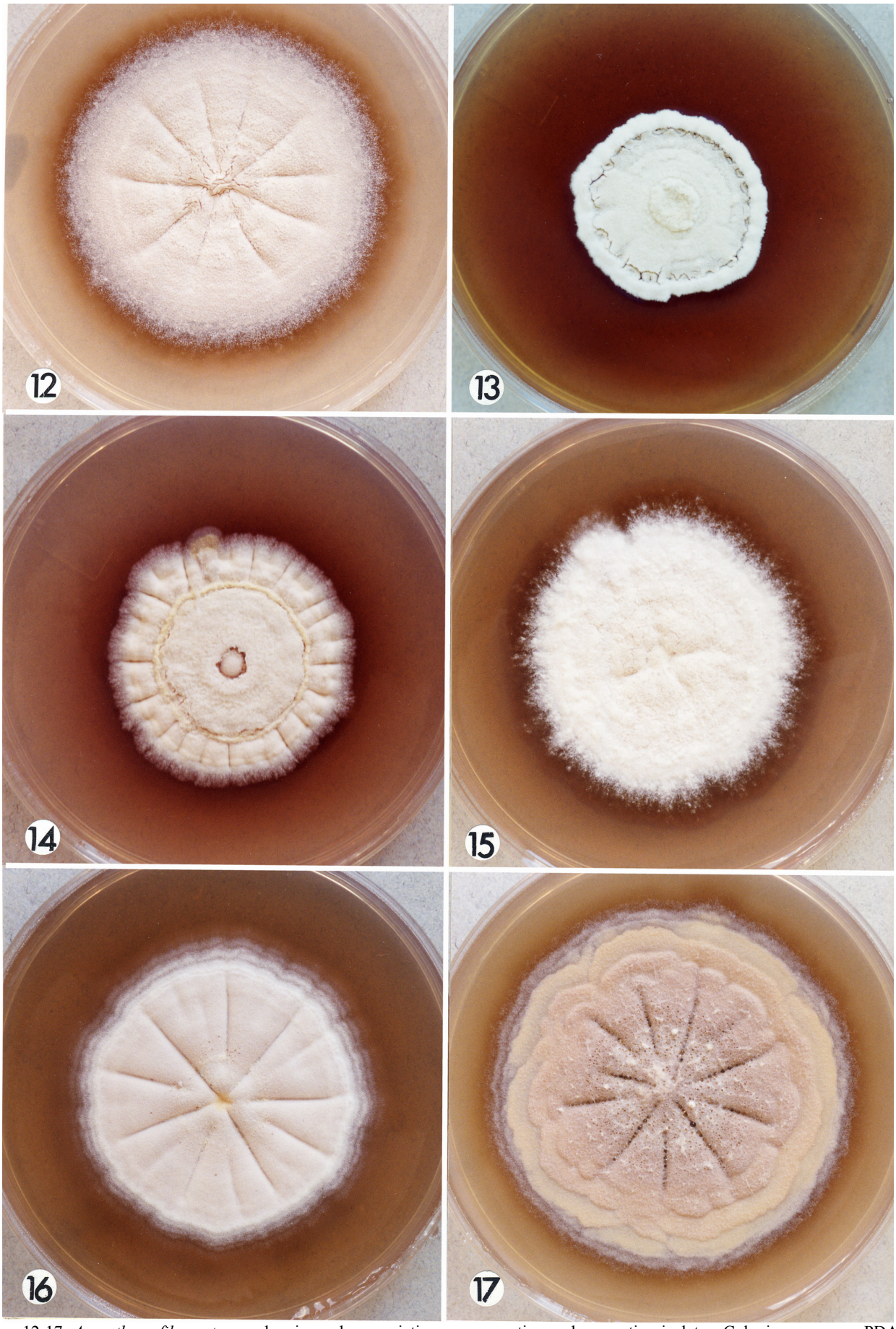
Sigler & Carmichael (1976, p 416-420) described *Mal. albolutea* as having an *Auxarthron* teleomorph similar to that described for *A. thaxteri* (Kuehn) Orr & Kuehn but were hesitant to name it because of uncertainty about the circumscription of *A. thaxteri* and *A. umbrinum*. Both species have had complicated nomenclatural histories. Orr *et al*. (1963) placed *Myxotrichum thaxteri* Kuehn into synonymy with *A. brunneum* (Rostrup) Orr & Kuehn (≡*Myxotrichum brunneum* Rostrup) and based their concept of *A. brunneum* on its type which they selected as neotype. They noted that Rostrup's material and description were inadequate to permit recognition of the species. Apinis (1964) considered *Myx.. brunneum* Rostrup to be a synonym of *Gymnoascus umbrinus* var. *umbrinus* Boudier, based on his examination of type specimens



Figures. 8-9. *Auxarthron alboluteum.* 8. Gymnothecium with elongate appendage (UAMH 2846, ex-type). Bar = 150 µm. 9. Punctate ascospores (inset in higher magnification). Magnification same as Fig. 11. **Figures**. 10-11. *Auxarthron thaxteri.*. 10. Gymnothecium with elongate appendages (UAMH 3912, ex-type). Magnification same as Fig. 8. 11. Punctate ascospores. Bar = 5 µm

for both species, and he redisposed Kuehn's *Myx. thaxteri* as a variety, *G. umbrinus* var. *thaxteri* (Kuehn) Apinis. Orr & Kuehn (1971) reexamined Rostrup's and Boudier's specimens and disagreed with Apinis's decisions. They placed *Myx. brunneum* Rostrup as a synonym of *A. umbrinum* (Boudier) G.F. Orr & Plunkett and made a new combination for *Myx. thaxteri* as *A. thaxteri* (Kuehn) G.F. Orr & Kuehn. They listed *A. brunneum* sensu Orr & Kuehn (Orr *et al*. 1963) as a synonym. Sigler & Carmichael observed some morphological differences between the teleomorph of *Mal. alboluteum* and that of the ex-type of *A. thaxteri;* however, they found another strain of *A. thaxteri* examined by Orr & Kuehn to be identical, thus adding further to the uncertainty concerning this species.

Our SSU and ITS sequence analyses confirm that *A. alboluteum* is genetically distant from *A. thaxteri* and *A. umbrinum* and provide justification for a new name for the teleomorph. The morphological differences are as follows. *A. thaxteri* grows optimally at 25ºC, slower at 30ºC (3.5 cm and 3 cm diam in 21 days respectively) and is strongly inhibited at 35ºC (< 1 cm in 21 days). Ascomatal appendages are shorter, ascospores are ovoid or subglobose (2.8-3 x 2.2-2.5 µm) rather than globose, and the arthroconidial anamorph is less well developed (Figs. 10-11). (Sigler & Carmichael 1976). *A. umbrinum*



Figures. 12-17. *Auxarthron filamentosum* showing colony variation among mating and nonmating isolates. Colonies grown on PDA for 6 weeks at 30^{O} C **Fig.** 12. UAMH 4097, ex-type of *M. filamentosa.* **Fig**. 13. UAMH 9986, mating type strain. **Fig**. 14. UAMH 9987, mating type strain. **Fig.** 15. UAMH 7163. **Figs**. 16-17. UAMH 7165 showing colonial variants derived from different sectors.

colonies (1874, 3952) are in shades of orange and in the ITS analysis, this species grouped with *A. conjugatum* (Kuehn) G.F. Orr & Kuehn, also having orange-buff colonies. Currah (1985) noted only minor differences between *A. umbrinum* and *A. conjugatum,* including size and ornamentation of ascospores and apically branched (*A. conjugatum*) versus unbranched (*A. umbrinum*) elongate appendages. However, absence of ascosporulation in the ex-type culture of *A. umbrinum* precluded assessment of conspecificity based on morphology. Although the ITS sequence data presented here grouped isolates of these species together (Fig. 2), isolates assigned to them need re-evaluation and it would be prudent to sequence the ex-type culture of *A. conjugatum* (UAMH 3156)*,* before reaching any conclusions. The molecular phylogeny study of Solé *et al*. (2002) introduces an error with respect to UAMH 3912 which is correctly listed as *A. thaxteri* among the isolates examined, but is named *A. umbrinum* in their phylogenetic tree.

Discussion

The results of our SSU analysis show that members of the genus *Auxarthron* form a strongly supported monophyletic group within the *Onygenaceae* (*Onygenales*). Based on ITS sequence analysis, the species of *Auxarthron* sampled here, and in particular *A. filamentosum* and *A. alboluteum,* cluster in two distinct clades. The position of the type species, *A. californiense*, is not clearly resolved, although it suggests a closer relationship to clade I than to Clade II. The delineation of the two groups appears to correlate with slight differences in ascospore shape. Ascospores of species in Clade I are oblate (*A. conjugatum, A. umbrinum*), as are those of *A. californiense* (UAMH 1889 ex-type), or globose to subglobose or ovoid (*A. filamentosum, A. thaxteri*). Ascospores of species in Clade II are globose except in *A. zuffianum*, which has globose or subglobose ascospores (Currah, 1985; Orr *et al.,* 1963; Sigler & Carmichael, 1976). In our SSU analysis, *Amauroascus kuehnii* was excluded from the strongly supported *Auxarthron* clade. Transfer of this species and *Am. pseudoreticulatus* to *Auxarthron* was proposed by Solé *et al.* (2002) but it would be prudent to evaluate the placement of these species within a broader analysis including the species described here.

Although heterothallism is common among members of the *Onygenales*, it has not been described previously for a species of *Auxarthron;* however testing of compatibility has rarely been done. Sigler & Carmichael (1976) investigated a possible relationship between *A. conjugatum* and the morphologically similar anamorph, *Malbranchea aurantiaca* Sigler & J.W. Carmich. They found that all single ascospore isolates obtained from five isolates of *A. conjugatum* were self-fertile. Mating studies among ten *M. aurantiaca* strains yielded a single fertile ascoma consistent with the genus *Auxarthron* in just one mated pair. The supposition that *M. aurantiaca* is the anamorph of an *Auxarthron* species is supported by the results of Sugiyama & Mikawa (2001), based on LSU rDNA sequences, which grouped it with *A. compactum,* and those of Herr *et al.,* (2001) based on SSU rDNA sequences which placed it in a strongly supported clade with *A. filamentosum (M. filamentosa), A. zuffianum,* and *M. dendritica*. The results of Herr *et al.* which show *A. alboluteum (M. albolutea*), based on GenBank sequence L28063, as basal to other *Auxarthron* species, confirm our finding as shown in Fig. 1A and as discussed above, that this sequence is incorrect.

The anomalous placement of *M. albolutea* outside of the *Auxarthron* clade*,* even though this species was known to have an *Auxarthron* teleomorph (Sigler & Carmichael 1976), prompted us to re-sequence the ex-type strain and the others included in this study. This work uncovered several discrepancies between our results and sequences on deposit in GenBank based on the same UAMH strains. The source of these differences is difficult to establish as nearly 10 years has passed since the original sequencing was done. One possible explanation is that nucleotides were mis-read during sequence determination. We found the nucleotide differences are scattered along the length of the SSU sequences and include both substitutions and insertions or deletions. The discrepancies are unlikely to be due to contamination occurring at some point in the process because the sequences cluster as expected within the *Onygenales* but have some unique characters not shared by any other sequences in the alignment. Our sequences were derived from cultures that were re-examined morphologically and were confirmed by sequencing two separate DNA extracts for each strain. Thus, we are confident that our data are correct. For *A. alboluteum,* our SSU and ITS sequences for UAMH 2846 and 4119 differed at one position and our SSU sequence for 4119 matched with U29389 which is now corrected in GenBank as *A. alboluteum*, not *M. dendritica*.

Auxarthron species have not been the subject of recent re-evaluation and concepts are still largely based on those of Orr *et al*. (1963). For reliable interpretation of molecular results it will be necessary to re-examine strains studied by them to assess the extent of deviation from the types. For example, Koufopanou *et al.* (2001) identified cryptic species within *A. zuffianum* based on the analysis of gene genealogies for three protein-coding genes, but accumulated evidence suggests that they probably uncovered a misidentified strain. We compared our analysis of ITS sequence data with their results and

those of Solé *et al*. (2002) since some sequences from the latter study were used in our analysis. In our ITS tree (Fig. 2), strains of *A. zuffianum* were distributed in two sister clades. Three strains clustered together with a bootstrap value of 86%. This group included the neotype UAMH 1875 (designated TX, Group I by Koufopanou *et al.* 2001) and an Argentinian strain received from Dr. Borghi (UAMH 4098) (Koufopanou *et al.* examined UAMH 4082 with the same provenance and designated it AG, Group II). However, IMI 76603 (UAMH 3079) (designated UK, Group III) clustered in our analysis (Fig. 2) with *A. reticulatum* (UAMH 2006) (bootstrap 92%), another species lacking elongate appendages. Although Orr *et al*. included IMI 76603 (A207/2) in their circumscription of *A. zuffianum*, these molecular data and our morphological reexamination suggest that this strain represents *A. reticulatum*; however its conspecificity with the neotype (UAMH 1585 = UAMH 3154) should be verified. The identification of UAMH 4566 as *A. zuffianum* is confirmed by our data, but this strain is incorrectly listed as neotype by Solé *et al*.

In our attempt to assess the phylogenetic relationships between *A. filamentosum* and *A. alboluteum* and other *Auxarthron* species, we encountered problems at different levels. In the case of the incorrect sequences, problems were not detected in prior published work because the sequences were shown to have homology with those of onygenalean taxa and only became apparent when more *Auxarthron* taxa were added to the analysis.

Acknowledgments

We gratefully acknowledge financial support for various aspects of this work from the Natural Sciences and Engineering Research Council of Canada (L.S.), the University of Alberta Small Faculties Fund Support for the Advancement of Science (L.S.), the Companion Animal Fund of the University of Wisconsin School of Veterinary Medicine (J.P.), and a Visiting Fellowship in Canadian Government Laboratories (NSERC) to S.H. We thank Michael Hertwig (Latin) and Ming Chen (SEM) for expert assistance.

Literature cited

BORGHI, A.L., ROSSI DE BENETTI, M.S. & CORALLINI DE BRACALENTI, B.J. 1977 — *Coccidioides immitis* su aislamiento de muestras de suelos de las provincias de San Luis y Mendoza — Sabouraudia **15**:51–57.

BOWMAN, B.H., WHITE, T.J. & TAYLOR, J.W. 1996 — Human pathogenic fungi and their close nonpathogenic relatives. — Mol. Phylogenet. Evol. **6**:89–96.

CURRAH, R.S. 1985 — Taxonomy of the *Onygenales: Arthrodermataceae, Gymnoascaceae, Myxotrichaceae* and *Onygenacea*e. — Mycotaxon **24**:1-216.

HERR, R.A., TARCHA, E.J., TABORDA, P.R., TAYLOR, J.W., AJELLO, L. & MENDOZA, L. 2001 — Phylogenetic analysis of *Lacazia loboi* places this previously uncharacterized pathogen with the dimorphic *Onygenales*. — J. Clin. Microbiol. **39**:309–314.

KANE, J., SUMMERBELL, R.C., SIGLER, L., KRAJDEN, S. & LAND, G. 1997 — *Laboratory handbook of dermatophytes. A clinical guide and laboratory manual of dermatophytes and other filamentous fungi from skin, hair and nails.* — Star Publishing Co., Belmont, CA.

KOUFOPANOU, V., BERT, A., SZARO, T. & TAYLOR, J.W., 2001 — Gene genealogies, cryptic species, and molecular evolution in the human pathogen *Coccidioides immitis* and relatives (*Ascomycota*, *Onygenales*). — Mol. Biol. Evol. **18**:1246–1258.

KORNERUP, A. & WANSCHER, J.H. 1978 — *Methuen Handbook of Color*, 3rd ed. Methuen, London, U.K.

LANDVIK S., EGGER K.N. & SCHUMACHER T., 1997 — Towards a subordinal classification of the *Pezizales* (*Ascomycota*): phylogenetic analyses of SSU rDNA sequences. — Nordic J. Bot.**17**: 403–418.

MILLONIG, G., 1961. — Advantages of a phosphate buffer for OsO4 solution in fixation. — J. Appl. Phys. 32: 1637.

NICHOLS, D.K., WEYANT, R.S., LAMIRANDE, E.W., SIGLER, L. & MASON, R.T., 1999 — Fatal mycotic dermatitis in captive brown tree snakes (*Boiga irregularis*). — J. Zoo Wildlife Medicine **30**:111–118.

ORR, G.F. & KUEHN, H.H., 1971 — Notes on *Gymnoascaceae*. I. A review of eight species. —Mycologia **63**:191-203.

ORR, G.F., KUEHN, H.H. & PLUNKETT, O.A., 1963 — New genus of the *Gymnoascaceae* with swollen peridial hyphae. — Canad. J. Bot. **41**:1439–1456.

PAN, S., SIGLER, L. & COLE, G.T., 1994 —Evidence for a phylogenetic connection between *Coccidioides immitis* and *Uncinocarpus reesii* (*Onygenaceae*). —Microbiology **140**:1481–1494.

PARÉ, J.A., SIGLER, L., HUNTER, D.B., SUMMERBELL, R.C., SMITH, D.A. & MACHIN, K.L. 1997 — Cutaneous mycoses in chameleons caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (Apinis) Currah. — J. Zoo Wildlife Med. **28**:443–453.

PARÉ, J.A, SIGLER, L., RYPIEN, K.L. & GIBAS, C.F., 2002 — Survey for the *Chrysosporium* anamorph of *Nannizziopsis vriesii* on the skin of healthy captive squamate reptiles and notes on their cutaneous fungal mycobiota— J. Herpetol. Med. Surg. (in press)

SIGLER, L. & CARMICHAEL, J.W., 1976 — Taxonomy of *Malbranchea* and some other Hyphomycetes with arthroconidia. — Mycotaxon **4**:349–488.

SIGLER, L., LACEY, J. & CARMICHAEL, J.W., 1982 — Two new species of *Malbranchea*. —Mycotaxon **15**:465–475.

SOLÉ, M., J. CANO & GUARRO, J., 2002 — Molecular phylogeny of *Amauroascus, Auxarthron,* and morphologically similar onygenalean fungi. — Mycol. Res. **106**:388-396.

SWOFFORD, D L., 1999 — PAUP*: phylogenetic analysis using parsimony (*and other methods), Version 4. — Sinauer Associates, Sunderland, Massachusetts.

THOMAS, A.D., SIGLER, L., PEUCKER, S., NORTON, J.H. & NIELAN, A., 2002 — *Nannizziopsis vriesii*-like fungus associated with fatal cutaneous mycoses in the salt-water crocodile (*Crocodylus porosus*). — Med. Mycol. **40**:143-151.

WHITE, T.J., BRUNS, T., LEE, S. & TAYLOR J., 1990 — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. — Pp. 315–322. In: INNIS, M.A., GELFAND, D.H., SNINSKY, J.J., & WHITE T.J., (eds.): PCR Protocols: a guide to methods and applications. — Academic Press, New York.