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The Ajellomycetaceae, a new family of vertebrate-associated Onygenales

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Abstract: Phylogenies inferred from the analysis of DNA sequence data have shown that the Onygenales contains clades that do not correspond with previously described families. One lineage identified in recent molecular phylogenetic studies includes the dimorphic pathogens belonging to the genera *Ajellomyces, Emmonsia* and *Paracoccidioides*. To evaluate the degree of support for this lineage and determine whether it includes additional taxa, we examined relationships among the members of this clade and selected saprobic onygenalean taxa based on maximum-parsimony analyses of partial nuclear large RNA subunit (LSU) and internal transcribed spacer (ITS) sequences. A clade distinct from the Onygenaceae was found to encompass *Ajellomyces* (including the anamorph genera *Blastomyces, Emmonsia* and *Histoplasma*) and *Paracoccidioides brasiliensis*. The members of this lineage are saprobic and pathogenic vertebrate-associated taxa distinguished by their globose ascomata with coiled appendages, muricate globose or oblate ascospores, and lack of keratinolytic activity. Anamorphs are solitary aleurioconidia or irregular alternate arthroconidia. Based on molecular data and on morphological and physiological similarities among these taxa, we propose the new family, Ajellomycetaceae.

Key words: *Ajellomyces, Blastomyces dermatitidis, Histoplasma capsulatum,* molecular systematics, rDNA sequences, taxonomy

INTRODUCTION

The Onygenales is a monophyletic lineage within the Ascomycota encompassing species with gymnothecial or cleistothecial ascomata, evanescent asci, unicellular ascospores and aleurio- or arthroconidial anamorphs. As circumscribed by Currah (1985, 1994), the order includes four families separated on the basis of anamorph connections, ascospore ornamentation and the ability to enzymatically degrade cellulose or keratin. Keratinolytic activity, as demonstrated through hair degradation tests or inferred from the occurrence of taxa on keratin-rich substrates, defines the Arthrodermataceae and Onygenaceae, whereas the remaining nonkeratinolytic and cellulolytic members of the order have been assigned to the Gymnoascaceae and Myxotrichaceae, respectively.

With the exception of the Myxotrichaceae, a group now recognized to be more closely allied to the Leotiales (Currah 1997, Sugiyama et al 1999), Currah's concept of the Onygenales has been supported by the results of studies of the ecology, molecular systematics and morphology of members of this order. The Gymnoascaceae, a family once thought to represent a heterogeneous assemblage of taxa with affinities to the Arthrodermataceae and Eurotiales (Currah 1985, 1994), forms a monophyletic group in phylogenies based on the analysis of nuclear ribosomal RNA (rRNA) gene sequences (Sugiyama and Mikawa 2001, Sugiyama et al 1999, Untereiner et al 2002). The Arthrodermataceae, which encompasses taxa with smooth ascospores and anamorphs assigned to *Chrysosporium* Corda, *Epidermophyton* Sabour., *Microsporum* Gruby and *Trichophyton* Malmsten, also is represented as a well-supported lineage in analyses of nuclear rRNA and chitin synthase gene sequences (Herr et al 2001, Leclerc et al 1994, Sugiyama et al 1999). The phylogenetic structure of the Onygenaceae, a family that includes species with pitted ascospores and anamorphs placed in *Blastomyces* Gilchrist & Stokes (= *Chrysosporium* fide Carmichael 1962),

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Coccidioides G.W. Stiles, *Chrysosporium, Emmonsia* Ciferri & Montemartini, *Histoplasma* Darling, *Malbranchea* Sacc. and *Paracoccidioides* Almeida, is resolved less clearly. Recent sequence-based phylogenies indicate that the family is polyphyletic (Gibas et al 2002, Herr et al 2001, Sugiyama and Mikawa 2001, Sugiyama et al 1999, Untereiner et al 2002).

One clade recognized consistently in molecular phylogenetic studies of the Onygenaceae includes a group of medically important taxa encompassing the dimorphic systemic pathogens. Taxa identified as members of this clade in phylogenies inferred from nuclear small subunit (SSU) rRNA, nuclear large subunit (LSU) rRNA and internal transcribed spacer (ITS) sequences include *Ajellomyces capsulatus* (anamorph *Histoplasma capsulatum* Darling), *A. crescens* (anamorph *Emmonsia crescens*), *A. dermatitidis* (anamorph *Blastomyces dermatitidis* Gilchrist & Stokes) and species of the anamorph genera *Emmonsia* and *Paracoccidioides* (Herr et al 2001, Peterson and Sigler 1998, Sugiyama et al 1999, Vidal et al 2000). *Spiromastix* Kuehn & Orr, a nonpathogenic member of the Onygenaceae, recently was positioned within this clade based on the comparison of nuclear LSU sequences (Sugiyama and Mikawa 2001). This finding was corroborated by Untereiner et al (2002) in an investigation that examined phylogenetic relationships of species of *Ajellomyces* McDonough & Lewis, *Polytolypa* Scott & Malloch and *Spiromastix* inferred from the analysis of nonmolecular characters and sequences from the nuclear LSU and mitochondrial SSU rRNA genes. Based on the results of their study, Untereiner et al (2002) transferred *Spiromastix grisea* Currah & Locquin-Linard to *Ajellomyces* and restricted *Spiromastix* (typified by *S. warcupii*) to species isolated from soil that possess oblate ascospores and peridial appendages that are wavy to helical but with only 1–2 turns per helix. *Polytolypa hystricis,* a species described from porcupine dung (Scott et al 1993), also was shown to be closely related to *Ajellomyces* and *Spiromastix,* but its phylogenetic position was not sufficiently resolved to propose its transfer to either genus (Untereiner et al 2002).

In the present investigation, we examined the phylogenetic structure of the Onygenaceae *sensu lato* based on the analysis of nuclear LSU and ITS rDNA sequences for an expanded set of taxa. Our results provide further evidence for the recognition of the clade encompassing *Ajellomyces* (including the anamorph genera *Blastomyces, Emmonsia* and *Histoplasma*) and *Paracoccidioides* that we describe formally as a new family.

MATERIALS AND METHODS

Fungal isolates.—Isolates and sequences employed in this study are listed in TABLE I. Cultures sequenced during this investigation were maintained at room temperature on modified Leonian's agar (MLA) (Malloch 1981).

DNA extraction, amplification and sequencing.—Cultures of *Ajellomyces, Polytolypa* and *Spiromastix* used for DNA isolations were grown in modified Leonian's broth, harvested, and lyophilized as described previously (Untereiner et al 1995). Total nucleic acids were extracted from ground, lyophilized cultures as described by Untereiner et al (2002). A DNA fragment that extended from the 3′ end of the nuclear SSU rRNA gene to approximately 1000 base pair (bp) positions downstream from the 5′ end of the nuclear LSU gene was amplified for these taxa using the primers WNS9 (Untereiner and Naveau 1999) and LR5 (Vilgalys and Hester 1990) following the parameters described by Untereiner and Naveau (1999). Residual primers, salts and unincorporated dNTP were removed using a QIAquick PCR purification kit (Qiagen Ltd., Mississauga, Ontario) following the manufacturer's instructions. Sequencing reactions were performed using a Prism dye terminator cycle sequencing ready reaction kit (Applied Biosystems Inc., Foster City, California) and primers 5.8SR, LR1 (Vilgalys and Hester 1990), WITS3 (Untereiner et al 1995) and WNS9. Excess dye terminators were removed by centrifugation using Centri-sep columns (Princeton Separations Inc., Adelphia, New Jersey) before analysis employing an Applied Biosystems 373A or 377 DNA sequencer.

Data analysis.—Sequences were edited and assembled into larger consensus sequences using Sequencher 3.0 software (Gene Codes Corp., Ann Arbor, Michigan). Multiple alignments were produced using Clustal X version 1.7 (Thompson et al 1994). The final multiple alignments were adjusted manually after visual inspection and areas of sequence ambiguity were eliminated. The first alignment (TreeBase SN1748-5533), which included partial LSU rDNA sequences (924 bp) for 61 taxa, was analyzed to determine the phylogenetic positions of species assigned presently to the Arthrodermataceae, Gymnoascaceae and Onygenaceae *sensu lato.* The second alignment (TreeBase SN1748-5534) consisted of the combined ITS-LSU rDNA sequences (1149 bp) of 21 taxa. Outgroup taxa were *Auxarthron californiense* (21-taxon phylogeny) and *Byssochlamys nivea, Eurotium herbariorum* and *Petromyces alliaceus* (61-taxon phylogeny).

Phylogenetic relationships were inferred from aligned sequences using the maximum parsimony (MP) method found in PAUP* (beta version 4.0b10) (Swofford 2002). Gaps were treated as missing in all analyses. Heuristic searches of the 21- and 61-taxon datasets were performed employing tree bisection-reconstruction (TBR) branch swapping with the MulTrees and steepest descent options activated. Heuristic searches of the ITS-LSU alignment for new optimal trees were conducted using 1000 random-addition-sequence replicates. Constraint trees for the 21-taxon alignment were constructed using MacClade 3.05 (Maddison and Maddison 1992), imported into PAUP* and compared to the most-parsimonious tree (MPT) inferred from MP analysis using the Kishino-Hasegawa test. Phylogenies inferred from a pruned 12-taxon alignment also were generated from exhaustive searches of the ITS and combined ITS-LSU datasets.

TABLE I. Sources and accession numbers of the isolates used in this study

Taxon	Source[a]	GenBank accession numbers
Ajellomycetaceae		
Ajellomyces capsulatus (Kwon-Chung) McGinnis & Katz	CBS 137.72	AF071950
A. capsulatus	UAMH 7141	AF038353
A. capsulatus	UAMH 3536 mt[b]−	AF038354
A. crescens Sigler	CBS 177.60 (T[c] of *Emmonsia crescens* Emmons & Jellison)	AF071864
A. crescens	UAMH 137	AF038342
A. crescens	UAMH 349 T mt+	AF038336
A. crescens	UAMH 394	AF038340
A. crescens	UAMH 4077	AF038349
A. dermatitidis McDonough & Lewis	ATCC 18187 T mtA	AF038355, AY176704
A. dermatitidis	UAMH 5438	AF038358
A. grisea (Currah & Locquin-Linard) Untereiner & Scott	CBS 128.88 T	AB040677
A. grisea	UAMH 6836	AY176721, AY527404[d]
E. parva (Emmons & Asburn) Ciferri & Montemartini	UAMH 130	AF038333
E. parva	UAMH 6312	AF038330
Emmonsia sp.[e]	UAMH 141	AF038321
Emmonsia sp.[e]	UAMH 2304	AF038320
Emmonsia sp.[e]	UAMH 7425	AF038323
Paracoccidioides brasiliensis (Splendore) Almeida	IMT 556	U81263
P. brasiliensis	UAMH 8037	AF038360
Arthrodermataceae		
Arthroderma benhamiae Ajello & Cheng	UAMH 10389	AY176742
Ar. ciferrii Varsavsky & Ajello	CBS 272.66 T	AB040681
Ar. curreyi Berkeley	CBS 138.26	AY176726
Ar. incurvatum (Stockdale) Weitzman et al.	CBS 174.64 T	AY176738
Ar. otae (Hasegawa & Usui) McGinnis et al.	ATCC 28328 T mt−	AY176739
Ar. quadrifidum Dawson & Gentles	ATCC 22954 T mt+	AY176728
Ar. simii Stockdale et al.	UAMH 10390	AY176745
Chrysosporium vallenarense Oorschot & Piontelli	UAMH 6914	AY176732
Ctenomyces serratus Eidam	CBS 187.61 NT[f]	AY176733
Epidermophyton floccosum (Harz) Langeron & Milochevitch	CBS 553.84	AY176734
Microsporum canis Bodin	UAMH 2338	AY176735
M. cookei Ajello	OMH H1-10	AY176736
M. persicolor (Sabouraud) Guiart & Grigorakis	OMH, strain unnumbered	AY176737
Shanorella spirotricha Benjamin	ATCC 12594 T	AY176720
Trichophyton krajdenii Kane et al.	UAMH 3244 T	AY176740
T. mentagrophytes (Robin) Blanchard	OMH 607678	AY176741
T. raubitschekii Kane et al.	OMH 6-1286	AY176743
T. rubrum (Castellani) Sabouraud	UAMH 2129	AY176744
Gymnoascaceae		
Arachniotus ruber (van Tieghem) Schroeter	CBS 352.90 NT	AY176746
Gymnascella aurantiaca Peck	ATCC 22394 T	AY176747
G. citrina (Massee & Salmon) Orr et al.	NRRL 5973	U17915
Gymnoascoideus petalosporus Orr et al.	ATCC 34351 T	AY176748
Gymnoascus reessii Baranetsky	CBS 410.72	AY176749
Rollandina hyalinospora (Kuehn et al.) Roy et al.	CBS 548.72	AB040687

TABLE I. Continued

Taxon	Source[a]	GenBank accession numbers
Onygenaceae *sensu lato*		
Amauroascus albicans (Apinis) Arx	NRRL 5141 T	U17914
Am. aurues (Eidam) Arx	ATCC 18654 NT (= CBS 593.71)	AJ271431, AY176705
Am. kuehnii Arx	CBS 539.72 T	AB040691
Am. niger Schroeter	ATCC 22339 NT	AY176706
Am. purpureus Ito & Nakagiri	IFO 32622 T	AY176707
Aphanoascus fulvescens (Cooke) Apinis	CBS 111.58	AY176708
Aph. fulvescens	UAMH 5117	AF038357
Aph. mephitalis (Malloch & Cain) Cano & Guarro	ATCC 22144 T	AY176725
Aph. terreus (Randhawa & Sandhu) Apinis	ATCC 16413 T	AY176714
Apinisia graminicola La Touche	CBS 721.68 T	AY176709
Ap. racovitzae (Lagarde) Guarro et al.	CBS 156.77	AB040696
Ascocalvatia alveolata Malloch & Cain	ATCC 22147 T	AY176710
Auxarthron californiense Orr & Kuehn	ATCC 15600 T (= UAMH 1889)	AF038352, AY176711
Aux. zuffianum (Morini) Orr & Kuehn	CBS 219.58 NT	AY176712
Chrysosporium keratinophilum D. Frey ex Carmichael	CBS 392.67 T	AY176730
Coccidioides immitis Rixford & Gilchrist	ATCC 7366	AY176713
Malbranchea aurantiaca Sigler & Carmichael	CBS 127.77 T	AB040704
Malbranchea sp.[g]	JCM 11275	AB040705
Nannizziopsis vriesii (Apinis) Currah	ATCC 22444 T	AY176715
Neogymnomyces demonbreunii (Ajello & Cheng) Orr	ATCC 18394 NT	AY176716
Onygena equina (Wildenow) Persoon	ATCC 22731	AY176717
Pectinotrichum llanense Varsavsky & Orr	CBS 882.71 T	AB040698
Polytolypa hystricis Scott & Malloch[g]	UAMH 7299 T	AY176718, AY527405[d]
Renispora flavissima Sigler et al.	ATCC 38503 T mt+	AY176719
Spiromastix tentaculatum Guarro et al.[g]	CBS 184.92 T	AY176722, AY527406[d]
S. warcupii Kuehn & Orr[g]	CBS 576.63 T	AB040679
S. warcupii[g]	UAMH 7099	AY176723, AY527407[d]
Spiromastix sp.[g]	JCM 11276	AB040680
Uncinocarpus reesii Sigler & Orr	ATCC 34533 T mt−	AY176724
Trichocomaceae		
Byssochlamys nivea Westling	CBS 100.11 T	AY176750
Eurotium herbariorum (Wiggers : Fr.) Link	ATCC 16469 NT	AY176751
Petromyces alliaceus Malloch & Cain	ATCC 16891 T	AY176752

[a] Cultures were obtained from the following collections: ATCC, American Type Culture Collection, Manassas, VA, U.S.A.; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; IMT, Instituto de Medicina Tropical de São Paulo, São Paulo, Brazil; JCM, Japanese Collection of Microorganisms, Saitama, Japan; MUCL, Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NRRL, Agricultural Research Service Collection, Peoria, IL, U.S.A.; OMH, Ontario Ministry of Health, Toronto, ON, Canada; UAMH, University of Alberta Microfungus Collection and Herbarium, Edmonton, AB, Canada.

[b] Mating type.

[c] Strain derived from the type specimen.

[d] Sequences generated in this study.

[e] Identified originally as *E. parva* in Sigler (1996).

[f] Strain derived from the neotype specimen.

[g] Disposition uncertain.

Bremer support (Bremer 1994) was determined heuristically by searching for trees up to five steps (61-taxon phylogeny) or 10 steps (21-taxon phylogeny) longer than the MPT and is given as the number of additional steps necessary for the collapse of a particular clade. Bootstrap support (Felsenstein 1985) for internal branches was evaluated from 100 (LSU dataset) or 1000 (ITS-LSU dataset) heuristic searches, and groups with a frequency of greater than 50% were retained in the bootstrap consensus trees. Congruence between the ITS and LSU datasets for the 21-taxon dataset was measured based on 1000 searches using the partition-homogeneity test (PHT) (Farris et al 1995) included in PAUP*.

RESULTS

Sequences employed in the molecular datasets ranged from 1146 to 1212 bp (ITS-LSU) and 552 to 953 bp (LSU) in length before deletion of ambiguous or unalignable bp (data not shown). The larger LSU dataset (61 taxa, 924 bp) contained sequences of 58 members of the Onygenales and consisted of 192 phylogenetically informative characters. MP analysis of this dataset produced three MPT 969 steps in length (L) with a consistency index (CI) of 0.362 and a retention index (RI) of 0.698. The strict consensus of these trees (FIG. 1) contained a large, well-supported clade (bootstrap support 100%) corresponding to the Onygenales. Three major lineages within the Onygenales receiving bootstrap support (≥ 70%) included the *Ajellomyces-Paracoccidioides* clade (73%), the *Spiromastix-Malbranchea* sp. clade (86%), and a large, well-supported group (94%) encompassing these subclades: *Amauroascus kuehnii-Auxarthron-Malbranchea aurantiaca* (79%), *Amauroascus niger-Coccidioides* (74%), *Aphanoascus-Chrysosporium keratinophilum* (98%), *Ascocalvatia-Onygena* (100%), *Am. purpureus-Neogymnomyces-Renispora* (75%) and the Arthrodermataceae (85%). Less robustly supported groups (<70%) within the largest lineage were the *Apinisia-Am. albicans* subclade (69%) and the Gymnoascaceae (64%). The position of *Polytolypa hystricis* was unresolved in the strict consensus.

A single MPT (L = 803 steps, CI = 0.654, RI = 0.634) was obtained in an heuristic search of the ITS-LSU dataset (1149 bp, 258 phylogenetically informative characters) for 21 taxa (FIG. 2). Data from these two rRNA gene regions were combined based on congruence demonstrated by the partition homogeneity test ($P = 0.163$). Shorter trees were not found in a search based on 1000 random-addition-sequence replicates. In this phylogeny, species of *Ajellomyces, Emmonsia, Paracoccidioides, Polytolypa* and *Spiromastix* formed a strongly supported group (bootstrap support 100%, Bremer support >10) that contained two well-supported subclades. The first of these (bootstrap support 97%, Bremer support >10) included species of *Ajellomyces, Emmonsia* and *Paracoccidioides* and also was recovered from the LSU sequences. Within this subclade, the clinically important taxa formed a moderately well-supported group (bootstrap support 62%, Bremer support 7) that did not encompass *A. grisea*. *Polytolypa hystricis* again was shown to be sister of the *Ajellomyces-Emmonsia-Paracoccidioides* clade, but its position was not supported strongly. Comparison of the MPT with constraint trees that grouped *A. grisea* with *Polytolypa* and *Spiromastix* or *A. grisea* with *Spiromastix* supported this result, but we were unable to reject the hypothesis of the monophyly of *Polytolypa* and *Spiromastix* (TABLE II). The second subclade included *Spiromastix tentaculatum* and *S. warcupii* (bootstrap support 72%, Bremer support 8).

The topology of the 21-taxon phylogeny was identical to the single MPT (L = 586 steps, CI = 0.706, RI = 0.405) inferred from an exhaustive search of the combined ITS-LSU dataset for a 12-taxon alignment that included *Ajellomyces capsulatus* UAMH 3536, *A. crescens* UAMH 349, *A. dermatitidis* ATCC 18187, *A. grisea* UAMH 6836, *Auxarthron californiense* (outgroup), *Emmonsia* sp. UAMH 2304 and UAMH 7425, *E. parva* UAMH 6312, *Polytolypa hystricis, Paracoccidioides brasiliensis* UAMH 8037, *Spiromastix tentaculatum* and *S. warcupii* UAMH 7099 (data not shown). An exhaustive search of ITS rDNA sequences for the same 12 taxa produced three MPTs (L = 352 steps, CI = 0.772, RI = 0.437) and the strict consensus of these trees differed from the phylogenies based on analyses of the combined ITS-LSU datasets for 12 and 21 taxa only in the positions of the members of the *Ajellomyces-Emmonsia-Paracoccidioides* clade (data not shown).

DISCUSSION

As circumscribed currently, the Onygenaceae *sensu lato* includes keratinolytic and keratinophilic taxa with pitted or punctate ascospores and a variety of types of peridial hyphae (Currah 1985, 1994). The family has been considered to be relatively homogeneous, but this study and other recent molecular phylogenetic studies indicate that the Onygenaceae is polyphyletic and confirm that ascomatal and ascospore morphology are of limited value as predictors of phylogenetic relationship (Sugiyama and Mikawa 2001, Sugiyama et al 1999, 2002). Analyses of rDNA sequence data divide the Onygenaceae into a number of clades. One of these clades, represented by species of *Ajellomyces* (encompassing the anamorphic genera *Blastomyces, Emmonsia* and *Histoplasma*), *Lacazia* and *Paracoccidioides* is resolved in phylogenies inferred from nuclear SSU (Herr et al 2001, Sugiyama et al 1999), LSU (Sugiyama and Mikawa 2001) and combined LSU-SSU rDNA sequences (Sugiyama et al 2002). The *Ajellomyces* clade was shown in a recent nuclear SSU rDNA phylogeny to be sister of the Arachnomycetales, a lineage encompassing species of *Arachnomyces* Massee & Salmon, and of the Eurotiales (Gibas et al 2002), but its position relative to these taxa and to other members of the Onygenales was not resolved. A second clade identified in phylogenies inferred from rDNA sequences encompasses *Polytolypa hystricis,* species of *Malbranchea* and members of the genus *Spiromastix* (Sugiyama and Mikawa

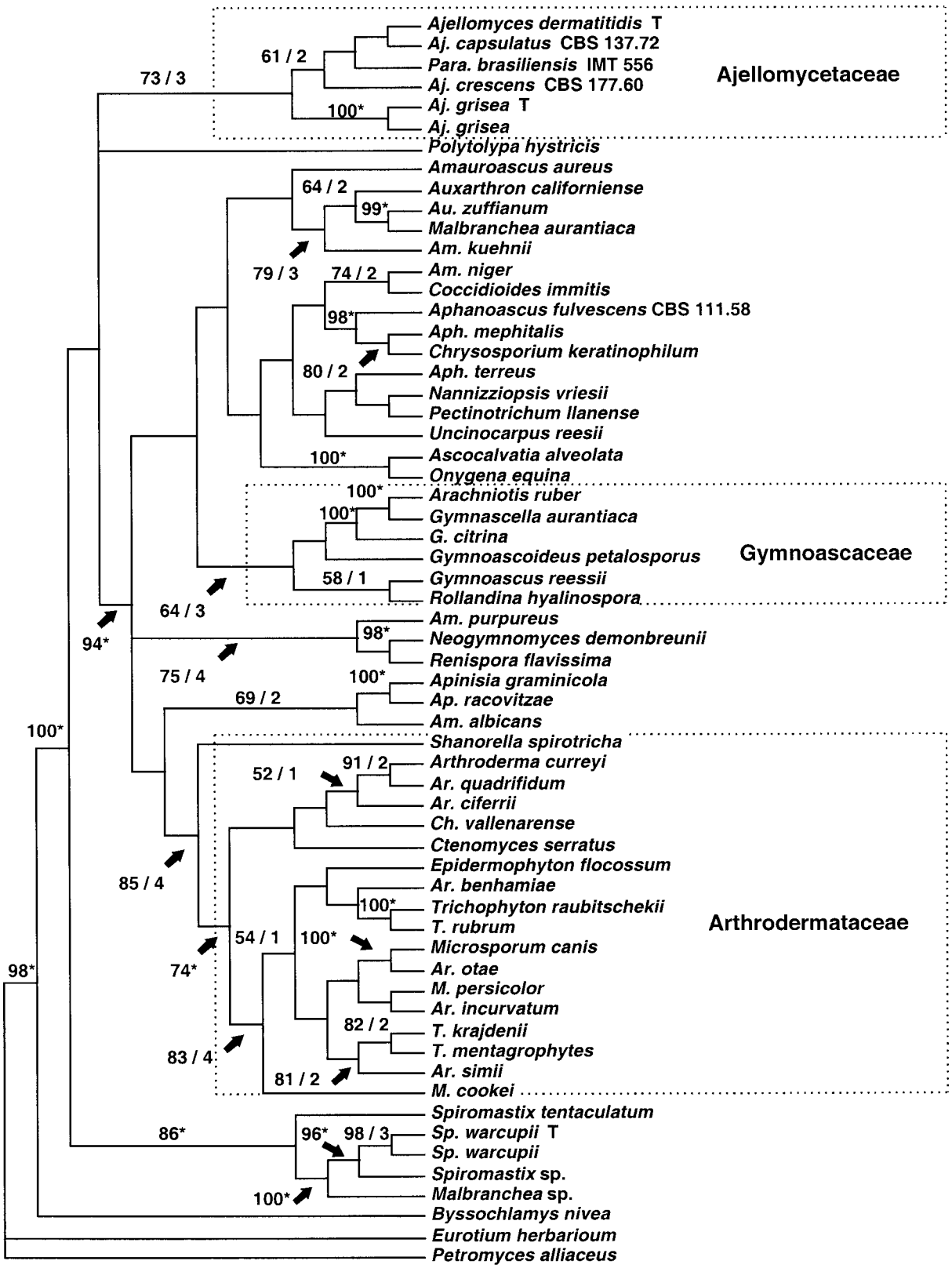

FIG. 1. Phylogenetic relationships of the Onygenales inferred from partial LSU rDNA sequence data. This is the strict consensus of 3 MPT (L = 969 steps) generated from an heuristic analysis of 924 bp for 61 taxa (CI = 0.362, RI = 0.698). Bootstrap values greater than 50% calculated from 100 replicates are given above either the branches or the diagonal lines adjacent to branches. Bremer support is shown either below the branches or the diagonal lines adjacent to branches. An asterisk indicates clades retained in trees five steps longer than the MPT. A "T" designates strains derived from the type specimen. Outgroup taxa are *Byssochlamys nivea, Eurotium herbariorum* and *Petromyces alliaceus.*

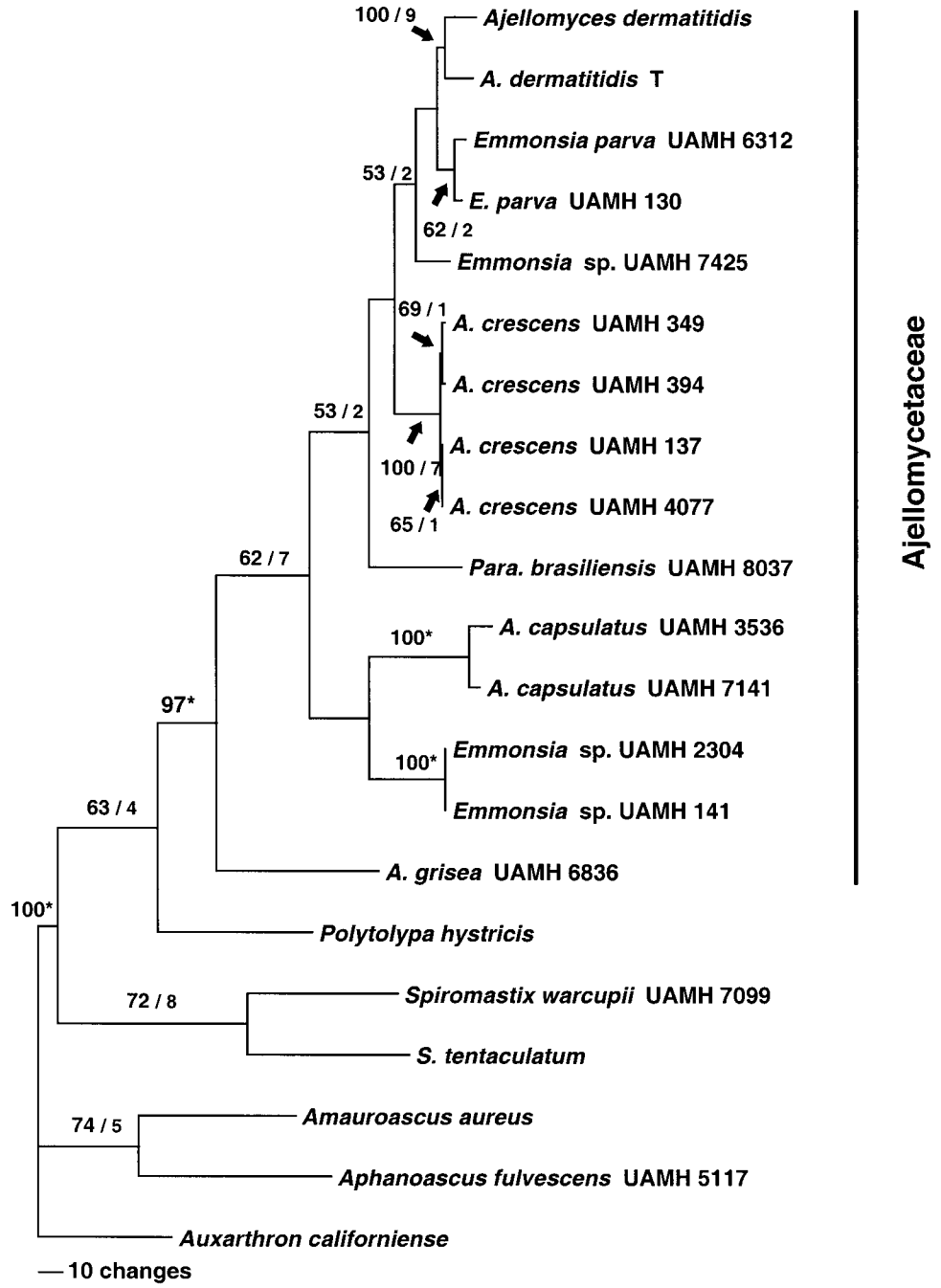

FIG. 2. Phylogenetic relationships within the Ajellomycetaceae inferred from the combined dataset (ITS and partial LSU rDNA sequences). This is the single MPT (L = 803) generated from an heuristic analysis of 1149 bp for 21 taxa (CI = 0.654, RI = 0.632). Bootstrap values greater than 50% calculated from 1000 replicates are given either above branches or to left of the diagonal lines adjacent to branches. Bremer support is shown either below the branches or the diagonal lines adjacent to branches. An asterisk indicates clades retained in trees 10 steps longer than the MPT. A "T" designates strains derived from the type specimen. Outgroup taxon is *Auxarthron californiense*.

2001, Sugiyama et al 2002, Untereiner et al 2002). The largest clade representing the Onygenaceae contains the dimorphic pathogen *Coccidioides immitis* and the remaining members of the family (FIG. 1, this study; Sugiyama and Mikawa 2001, Sugiyama et al 2002).

Within the *Ajellomyces* clade, the vertebrate pathogenic members of the genus form a moderately well-supported group. The teleomorphic taxa (*A. capsulatus, A. crescens* and *A. dermatitidis*) are the closest relatives of anamorphic taxa from both clinical and environmental sources (*Emmonsia* sp., *E. parva,*

TABLE II. Results of the Kishino-Hasegawa tests inferred from alignments of ITS-LSU sequences of 21 taxa

Tree	Parsimony tree length	−ln L	Difference in −ln L	*P* value	Significantly different at $P < 0.05$
MPT with the lowest ln L (FIG. 2)	803	−5628.73	—	—	—
Polytolypa with *Spiromastix*	810	−5635.42	6.69	0.345	no
Ajellomyces grisea with *Polytolypa*	819	−5674.22	45.49	0.001	yes
A. grisea with *Spiromastix*	823	−5684.04	55.31	0.000	yes
A. grisea with *Polytolypa* and *Spiromastix*	824	−5672.04	43.31	0.005	yes

Paracoccidioides brasiliensis) (FIG. 2). *Ajellomyces dermatitidis* (anamorph *Blastomyces dermatitidis*) is the closest relative of *E. parva,* and these taxa form a group that is sister of a well-supported clade that includes mating and nonmating isolates of *A. crescens* (FIG. 2, this study; Peterson and Sigler 1998). As shown by Peterson and Sigler (1998) and confirmed in the present study, *P. brasiliensis* is closely related to species of *Ajellomyces* but its position is not clearly resolved. The phylogenetic position of *A. capsulatus* (anamorph *Histoplasma capsulatum*) also requires further study. *Ajellomyces grisea,* a species transferred by Untereiner et al (2002) from the genus *Spiromastix,* is confirmed as a member of the strongly supported *Ajellomyces* clade (81–97% bootstrap support) (FIG. 2, this study; Sugiyama et al 2002, Untereiner et al 2002).

Species of *Ajellomyces* form globose ascomata with coiled or appendages and small, finely ornamented ascospores that appear smooth by light microscopy (Currah 1985, Kwon-Chung 1973, McDonough and Lewis 1986, Sigler 1996, 2002). Ascospores are hyaline, globose and muricate or oblate and finely punctate, <2.5 μm diam (Currah and Locquin-Linard 1987, Sigler 1996, 2002). Anamorphs are prominent and have been the primary means of recognition and identification of these taxa in the clinical setting. Conidia are smooth to slightly echinulate or tuberculate solitary aleurioconidia borne on stalks that often are slightly swollen at the end nearest to the conidium (Carmichael 1962, Sigler 1996, 2002). Intercalary arthroconidia are formed irregularly in *Paracoccidioides brasiliensis* (Sigler 2002).

Not every member of this lineage is pathogenic, but all are vertebrate-associated and they share similar substrates and physiological characteristics. Species of *Ajellomyces* and *Paracoccidioides* are isolated from animal hosts, dung, or more rarely soils associated with animals and animal dung (Kwon-Chung and Bennett 1992, Peterson and Sigler 1998, Sigler 2002). All exhibit growth at 35 C or higher, but growth may be strongly inhibited (Sigler 1996, 2002, Untereiner et al 2002). *Ajellomyces capsulatus, A. dermatitidis* and *P. brasiliensis* exhibit thermal dimorphism and grow in a yeast phase in vivo and in vitro at 35–37 C (Kwon-Chung and Bennett 1992, Sigler 2002). *Ajellomyces* and *Emmonsia* show varying degrees of cycloheximide resistance (Scott et al 1993, Sigler 1996, 2002). None of the members of this clade demonstrate keratinolytic activity as measured by hair degradation or by the keratin azure test (Carmichael 1962, Scott et al 1993, Scott and Untereiner 2004, Sigler unpubl data, Untereiner et al 2002).

Polytolypa hystricis and species of *Spiromastix* (*S. tentaculatum, S. warcupii* and *Spiromastix* sp. JCM 11276) are sister of the *Ajellomyces* clade, lack keratinolytic activity and share some morphological features (this study, Sugiyama and Mikawa 2001, Sugiyama et al 2002, Untereiner et al 2002). *Polytolypa* is similar to *Ajellomyces* in having tightly coiled peridial appendages that possess two to many turns per helix and ascospores which are muricate. This taxon differs in having yellow to yellow-orange ascospores that are ellipsoidal and larger (3–4 μm diam) and in producing alternate arthroconidia (Scott et al 1993). Conidia are absent in species of *Spiromastix,* and peridial appendages are wavy to slightly curved or helical (Currah 1985, 1988, Currah and Locquin-Linard 1988). Although we hypothesize that these taxa are closely related phylogenetically, the monophylly of *Ajellomyces, Polytolypa* and *Spiromastix* depicted in our ITS-LSU phylogeny (FIG. 2) and in the phylogenies of Untereiner et al (2002) likely reflects the choice of outgroup taxa. Resolving the phylogenetic position of *P. hystricis* and clarifying the relationship of *Polytolypa* and *Spiromastix* to *Ajellomyces* will require analyses of sequences of a greater number of coprophilous and geophilic onygenalean fungi. There is little question that a number of these ''missing taxa'' await discovery and formal description.

TAXONOMY

Ajellomycetaceae Untereiner, Scott & Sigler, fam. nov.

Type genus: *Ajellomyces* McDonough & Lewis, Mycologia 60:77. 1968

Ascomata gymnothecia, globosa vel irregulariter stellata,

discreta vel aggregata, parva, pallide brunnea; appendices centraliter orientes ex ascogonio, contortae cum helicibus paucis ad compluribus, cum parietibus crassis, flavo-brunneae, leves; hyphae peridiales cum parietibus crassis; hyphae uniformes diametro, sinuosae vel forma inaequales et apud septum constrictae; asci solitarii, irregulariter dispositi, globosi vel subglobosi vel pyriformes, octospori, hyalini, evanescentes; ascosporae globosae vel oblatae, muricatae, hyalinae, foramina germinalia absunt; anamorphoses de aleurioconidiis vel arthroconidiis cum dehiscentia lytica.

Ascomata gymnothecia, discrete or aggregated, globose to stellate, small, tan; appendages arising centrally from ascogonium, thick-walled, coiled with few to several helices, yellowish brown, smooth; peridium composed of branched anatomizing hyphae; hyphae uniform in diameter and sinuous, or constricted at the septa and inflated centrally; asci solitary, irregularly disposed, globose, subglobose to pyriform, eight spored, hyaline, evanescent; ascospores hyaline, globose to oblate, muriculate, lacking germ pores; anamorphs aleurioconidia or irregular alternate arthroconidia with rhexolytic dehiscence.

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