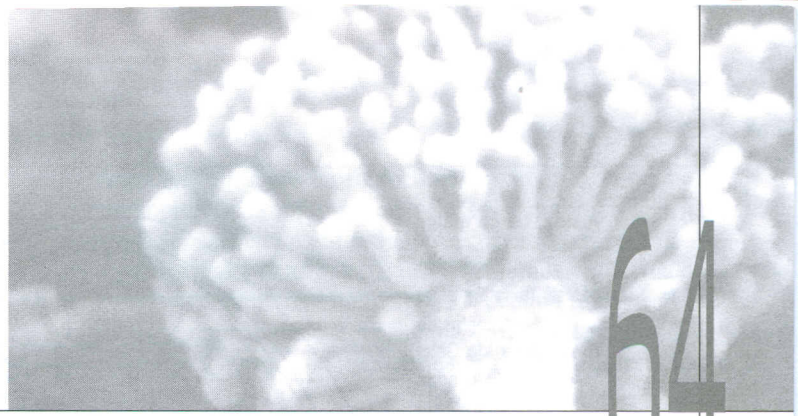


64

Biologia dos Fungos

Olga Fischman Gompertz
Walderez Gambale
Claudete Rodrigues Paula
Benedito Corrêa

22/11/2005

INTRODUÇÃO

Durante muito tempo, os fungos foram considerados como vegetais e, somente a partir de 1969, passaram a ser classificados em um reino à parte denominado *Fungi*.

Os fungos apresentam um conjunto de características que permitem sua diferenciação das plantas: não sintetizam clorofila nem qualquer pigmento fotossintético; não têm celulose na parede celular, exceto alguns fungos aquáticos, e não armazenam amido como substância de reserva. A presença de substâncias quitinosas na parede da maior parte das espécies fúngicas e a capacidade de armazenar glicogênio os assemelham às células animais.

Os fungos são ubíquos, encontrando-se em vegetais, em animais, no homem, em detritos e em abundância no solo, participando ativamente do ciclo dos elementos na natureza.

A dispersão dos fungos na natureza é feita por várias vias: animais, homem, insetos, água e, principalmente, pelo ar atmosférico, através dos ventos.

Os fungos são seres vivos eucarióticos com um só núcleo, como as leveduras, ou multinucleados, como os fungos filamentosos ou bolores e os cogumelos (fungos macroscópicos).

ESTRUTURA DA CÉLULA FÚNGICA

Todas as células fúngicas são eucarióticas, isto é, possuem núcleo com membrana nuclear.

Os fungos originam-se de uma única célula ou de um fragmento da hifa e estas unidades apresentam estruturas variadas, e algumas delas, mais especificamente a parede celular, são de grande auxílio na taxonomia destes microrganismos. Na Fig. 64.1, estão esquematizadas as principais estruturas da célula fúngica.

Parede. É uma estrutura rígida que protege a célula de choques osmóticos (possui até oito camadas e mede de 200 a 350nm). É composta, de modo geral, por glucanas, mananas e, em menor quantidade, por quitina, proteínas e lipídios. As glucanas e as mananas estão combinadas com proteínas, formando as glicoproteínas, manoproteínas e glicomanoproteínas. Estudos citoquímicos demonstraram que cada camada possui um polissacarídeo dominante: as camadas mais internas (8ª e 5ª) contêm beta-1-3, beta-1-3-glucanas e mananas, enquanto as mais externas contêm mananas e beta-1-6-glucanas (Fig. 64.2). A primeira e a terceira camadas são as mais ricas em mananas.

As glucanas nas células fúngicas são normalmente polímeros de D-glicose, ligados por pontes betaglicosídicas.

As mananas, polímeros de manose, representam o material amorfo da parede, e são diferenciados em dois tipos: uma manoproteína não-enzimática, envolvida na arquitetura da parede, e uma manoproteína com características enzimáticas, relacionada com a degradação de macromoléculas.

A quitina, um polímero (1,4) de 2-acetamida-2-deoxi-beta-D-glicose, é o principal componente estrutural do exoesqueleto de invertebrados e da parede celular fúngica. Nas leveduras, a quitina encontra-se em menor quantidade do que nos bolores (na proporção de 1:3) e está restrita à área de blastoconidiação. A quitina é geralmente encontrada como microfibrilas cristalinas, dentro de uma matriz protéica.

Os lipídios representam somente de 1% a 2% do peso seco celular, e estão presentes como compostos polares e apolares. Os principais lipídios apolares são os triacilgliceróis e os esteróis, e os polares são os diacilglicerofosfocolinas e diacilgliceroctanolaminas.

Membrana citoplasmática. Atua como uma barreira semipermeável, no transporte ativo e passivo dos materiais,

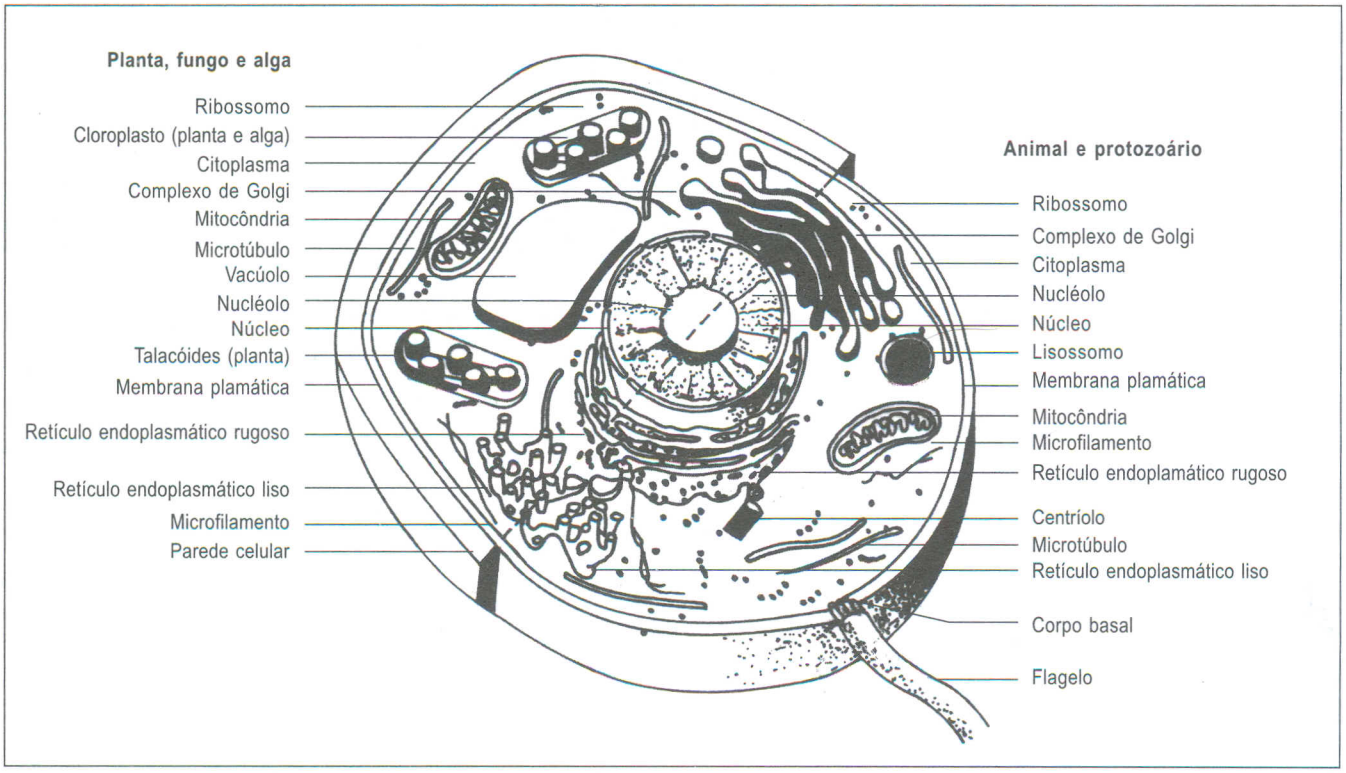


Fig. 64.1 — *Desenho esquemático de uma célula eucariótica.*

para dentro e para fora da célula, sendo constituída de uma porção hidrofóbica e de uma porção hidrofílica. As membranas das células dos fungos têm em sua composição química esteróis, que não são encontrados nas células bacterianas.

Basicamente esta estrutura consiste em lipídios e proteínas. As proteínas servem como enzimas, que fornecem à membrana diferentes propriedades funcionais, enquanto os lipídios dão à membrana sua verdadeira propriedade estrutu-

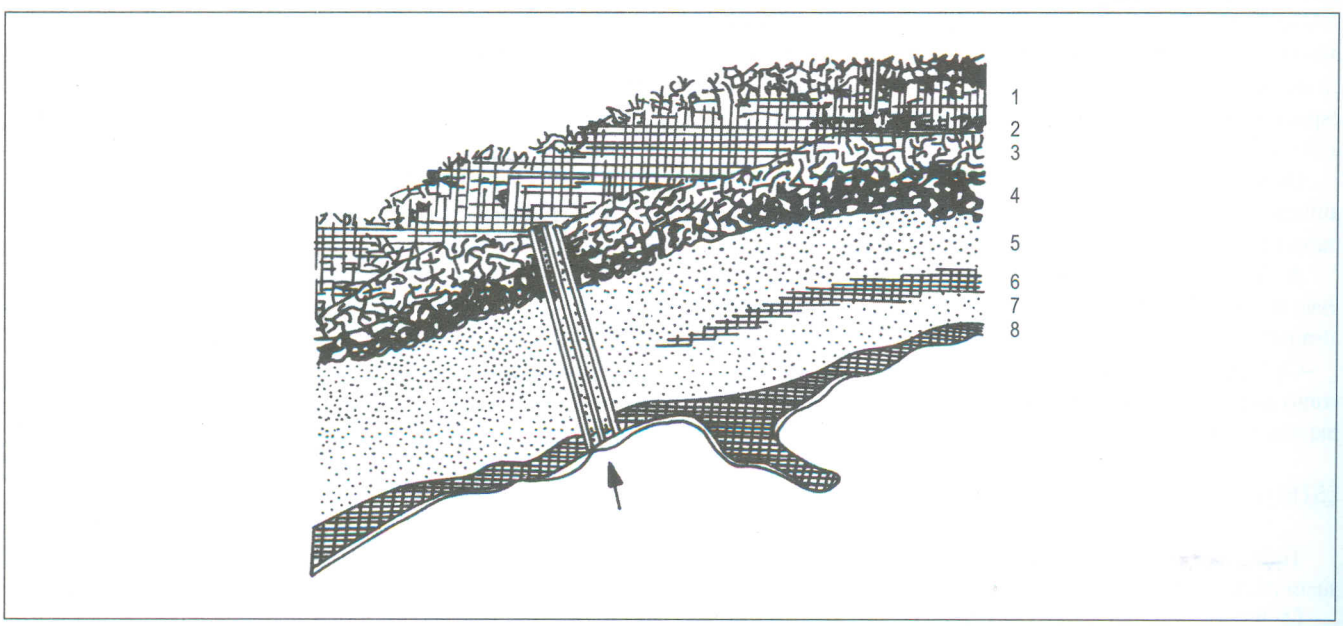


Fig. 64.2 — *Esquema da parede de uma célula fúngica e composição química. Camadas 1, 2, 3 e 4: Mananas, glucanas e proteínas (Camada 1 = composta por finos filamentos; Camada 2 = contínua; Camada 3 = com baixa eletrodensidade; e Camada 4 = com alta eletrodensidade). Camada 5: Glucanas e quitina (não muito bem delineada). Camada 6: Mananas e proteínas (distribuída de modo não-homogêneo). Camada 7: Quitina e glucanas (muito espessa; não é claramente contrastada). Camada 8: Quitina, proteínas e polissacarídeos (de espessura irregular).*

ral. Na Fig. 64.3, pode-se observar um modelo de membrana da célula fúngica, que consiste em uma camada bimolecular de lipídios intermediada por proteínas. As proteínas extrínsecas (externas) estão inseridas perifericamente na superfície polar lipídica, enquanto as proteínas intrínsecas (internas) podem estar em qualquer parte da camada lipídica. Externamente, encontramos cadeias de glicoproteínas inseridas tanto nas proteínas intrínsecas como nas extrínsecas.

Como as proteínas, os lipídios podem estar ligados às moléculas de açúcares formando os glicolipídios, que estão relacionados com importantes fenômenos, como o da aderência das células fúngicas às células do hospedeiro.

Núcleo. Contém o genoma fúngico e está agrupado em cromossomos lineares, compostos de dupla fita de DNA arrumados em hélice. Contém também as histonas que são proteínas básicas, associadas ao DNA cromossomal. A membrana nuclear é de natureza lipídica e possui numerosos poros. Dentro do núcleo, encontra-se o nucléolo, um corpúsculo esférico contendo DNA, RNA e proteínas. Este corpúsculo é o sítio de produção do RNA ribossomal.

Durante a divisão nuclear, observa-se que a membrana desaparece, sendo substituída por um aparato em forma de agulhas (processo mitótico) com numerosos microtúbulos. Após a mitose, a membrana nuclear é novamente sintetizada.

Ribossomos. São os sítios da síntese protéica, compostos por RNA e proteína e ocorrem dentro do citoplasma da célula. São formados por duas subunidades, 60S e 40S, e a partícula ribossomal completa tem 80S.

Mitocôndria. Sítio da fosforilação oxidativa, composta por membranas de fosfolipídios. Possui membrana interna achatada (crista) e contém seu DNA e ribossomos próprios.

Retículo endoplasmático. É uma membrana em forma de rede que se encontra distribuída por toda célula fúngica. Está ligada à membrana nuclear, mas não à membrana citoplasmática. Os ribossomos (80S) podem estar aderidos ao retículo endoplasmático.

Aparelho de Golgi. Esta estrutura (dictiossoma) é uma agregação interna de membranas, que está envolvida no armazenamento de substâncias que serão desprezadas pela célula fúngica. Os vacúolos estão relacionados com o armazenamento de substâncias de reserva para a célula, tais como glicogênio e lipídios.

Lomassomos. São corpúsculos que ocorrem dentro do periplasma (espaço entre a parede celular e a membrana citoplasmática) da célula fúngica, com função ainda não conhecida.

MORFOLOGIA E REPRODUÇÃO

Os fungos se desenvolvem em meios especiais de cultivo formando colônias de dois tipos: leveduriformes e filamentosas.

As colônias leveduriformes, em geral, são pastosas ou cremosas e caracterizam o grupo das leveduras. Esses microorganismos são unicelulares, em que a própria célula cumpre as funções vegetativas e reprodutivas. As estruturas microscópicas mais comuns são os blastoconídios, também denominados gêmulas, que possuem forma em geral arredonda-

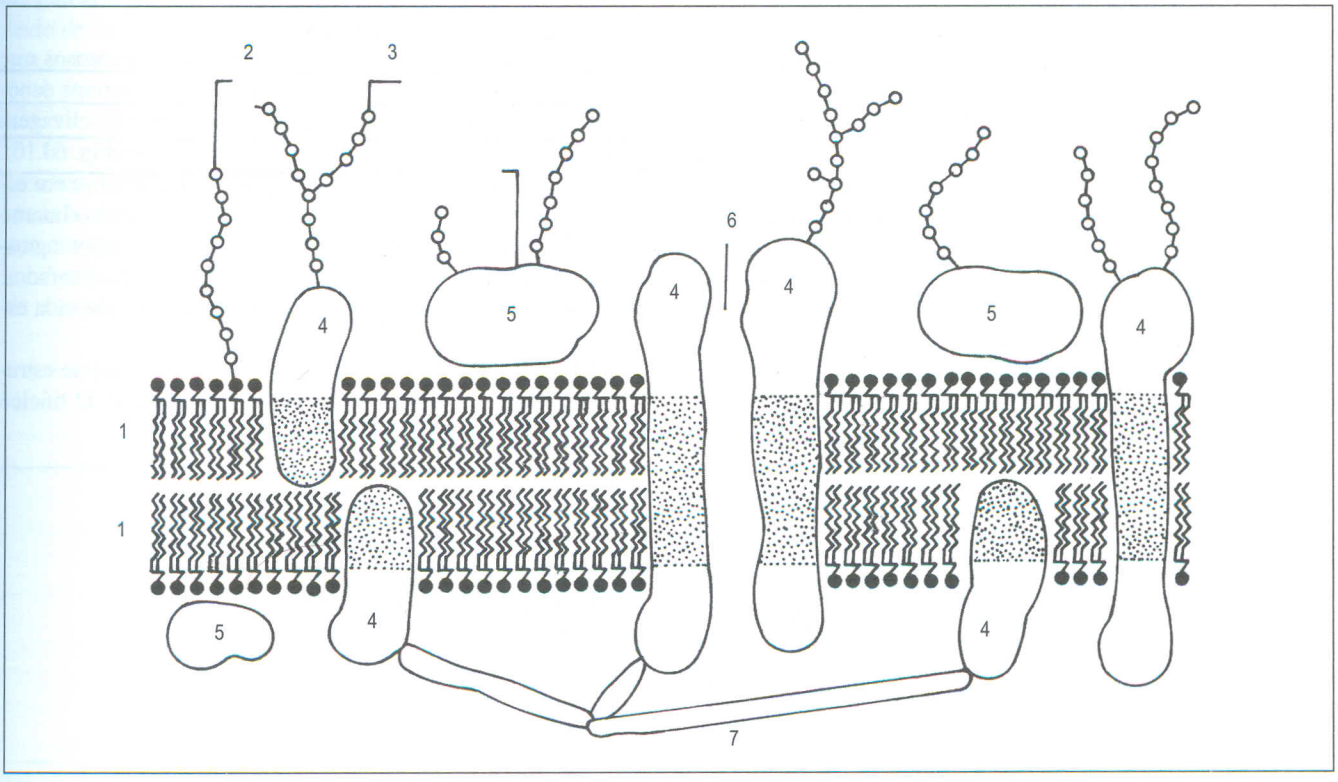


Fig. 64.3 — *Modelo esquemático de membrana de célula fúngica: 1 = camadas lipídicas; 2 = glicolipídeos; 3 = glicoproteínas; 4 = proteína intrínseca; 5 = proteína extrínseca; 6 = poro formado por proteínas intrínsecas; 7 = rede de proteínas.*

da ou ovalada. Por brotamento da célula-mãe, formam-se os brotos ou as células-filha, que podem desprender-se da célula-mãe, ou permanecer ligados à mesma, em cadeia, formando uma estrutura denominada pseudo-hifa, cujo conjunto é o pseudomicélio (Fig. 64.4).

As colônias filamentosas que identificam os bolores podem ser algodonosas, aveludadas, pulverulentas, com os mais variados tipos de pigmentação. Esses organismos são constituídos fundamentalmente por elementos multicelulares, em forma de tubos — as hifas — que podem ser contínuas, não-septadas ou cenocíticas e septadas (Fig. 64.5).

Ao conjunto de hifas dá-se o nome de micélio. O micélio que se desenvolve no interior do substrato, funcionando também como elemento de sustentação e de absorção dos nutrientes, é chamado micélio vegetativo. O micélio que se projeta na superfície e cresce acima do meio de cultivo é o micélio aéreo.

O micélio aéreo dos fungos filamentosos pode diferenciar-se ou formar estruturas de reprodução dos fungos — micélio reprodutivo. Essas estruturas têm origem sexuada ou assexuada e são de importância fundamental na identificação morfológica da maioria das espécies fúngicas.

Os propágulos formados no micélio reprodutivo estão representados na Tabela 64.1.

Os conídios representam o modo mais comum de reprodução assexuada e cumprem importante papel na dispersão dos fungos na natureza. As células que dão origem aos conídios são denominadas células conidiogênicas. Os conídios podem ser hialinos ou pigmentados, geralmente demácios, e apresentam formas diferentes — esféricos, fusiformes, cilíndricos, piriformes etc., com parede lisa ou rugosa, formados por uma única célula ou ter septos em um ou dois planos, apresentando-se isolados ou agrupados.

As hifas especializadas que originam os conídios são chamadas de conidióforos, que podem ou não ser ramificados. Normalmente, os conídios são formados na extremidade do conidióforo (Figs. 64.6 e 64.7). Outras vezes, nascem em qualquer parte do micélio, e são denominados conídios sésseis.

Algumas estruturas são comuns às leveduras e a fungos filamentosos. Os artroconídios são formados por fragmentação de hifas em elementos retangulares (Fig. 64.8).

Os clamidoconídios, estruturas de resistência, são células geralmente arredondadas de volume aumentado com paredes duplas e espessas, nas quais se concentra o citoplasma e formam-se em condições ambientais adversas, como escassez de nutrientes, de água e temperaturas não-favoráveis ao desenvolvimento fúngico. Sua localização pode ser apical ou intercalar (Fig. 64.9).

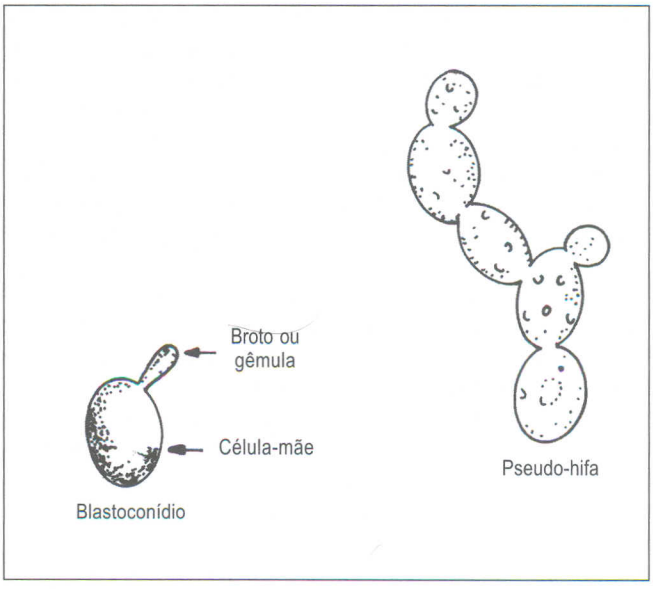


Fig. 64.4 — *Blastoconídio e pseudo-hifa encontrados nas leveduras.*

Entre outras estruturas de resistência devem ser mencionados os esclerotos ou esclerócios que são corpúsculos duros e parenquimatosos, formados por conjuntos de hifas e que permanecem em estado de dormência até que condições adequadas permitam a sua germinação.

Os propágulos assexuados de fungos filamentosos que possuem hifas não-septadas originam-se em estruturas denominadas esporângios por um processo interno de clivagem do citoplasma e são chamados esporangiosporos (Fig. 64.10).

Os propágulos assexuados inferiores originam-se em estruturas denominadas esporângios, por um processo interno de clivagem de seu citoplasma, e são chamados esporangiosporos. Pela ruptura do esporângio, os esporos são liberados. A hifa especial que sustenta o esporângio é denominada esporangioforo (Fig. 64.10).

Os propágulos sexuados originam-se da fusão de estruturas diferenciadas com caráter de sexualidade. O núcleo

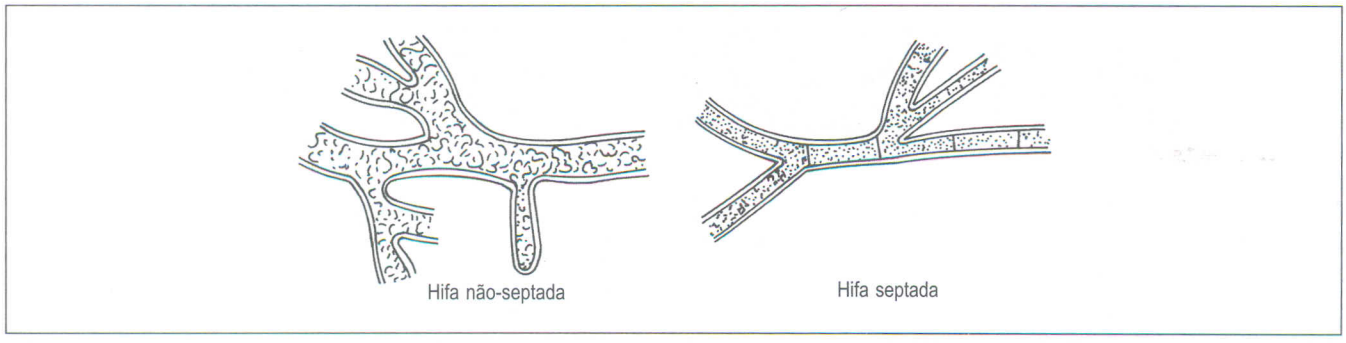


Fig. 64.5 — *Diferentes tipos de hifas.*

Tabela 64.1
Principais Estruturas de Reprodução de Fungos Leveduriformes e Filamentosos

Estruturas de reprodução	Típicas de fungos	Blastoconídios leveduriformes	
	Mais comuns em fungos filamentosos	Propágulos assexuados	Externos - conídios
			Internos - esporangiosporos
		Propágulos sexuados	Externos - basidiosporos
			Internos - ascosporos
	Encontradas em fungos filamentosos e leveduriformes	Artroconídios Clamidoconídios	

haplóide de uma célula doadora funde-se com o núcleo haplóide de uma célula receptora, formando um zigoto. Posteriormente, por divisão meiótica, originam-se quatro ou oito núcleos haplóides, alguns dos quais se recombinarão geneticamente.

Os propágulos sexuados internos são chamados ascósporos e formam-se no interior de estruturas denominadas ascos. Os ascos podem ser simples, como em algumas leveduras, ou distribuir-se em lóculos ou cavidades do micélio — o ascostroma — ou ainda estar contidos em corpos de frutificação, os ascocarpos. Três tipos de ascocarpos são bem conhecidos: cleistotécio, peritécio e apotécio.

O cleistotécio é uma estrutura globosa, fechada, de parede formada por hifas unidas, contendo um número indeterminado de ascos, cada um geralmente com oito ascósporos em seu interior. O peritécio é uma estrutura piriforme com um poro por onde são eliminados os ascos. O apotécio é um ascocarpo aberto em forma de cálice (Fig. 64.11).

Os propágulos sexuados externos são denominados basidiósporos e originam-se no ápice de uma célula fértil chamada basídio (Fig. 64.12). Esses propágulos são característicos dos denominados cogumelos (fungos macroscópicos).

A reprodução sexuada entre os fungos contribui, através da recombinação genética, para a variabilidade necessária ao aperfeiçoamento da espécie. Em geral, esses fungos com reprodução sexuada produzem, em determinadas fases de seu ciclo, estruturas assexuadas, os conídios que asseguram a sua disseminação. Essa característica de mudança de tipo de reprodução reflete-se em características morfológicas diferentes e o mesmo fungo recebe denominações diferentes. Por exemplo, o fungo leveduriforme *Cryptococcus neoformans* em sua fase sexuada é denominado *Filobasidiella neoformans*.

A fase sexuada dos fungos é denominada teleomórfica ou perfeita e a fase assexuada, anamórfica ou imperfeita.

A maior parte das leveduras reproduz-se assexuadamente por brotamento ou gemulação e por fissão binária. No processo de brotamento, a célula-mãe origina uma gêmula, o blastoconídio, que cresce e recebe um núcleo após a divisão do núcleo da célula-mãe. Na fissão binária, a célula-mãe divide-se em duas células de tamanhos iguais. No seu ciclo evolutivo, algumas leveduras podem originar esporos sexuados, ascósporos, após duas células sofrerem fusão celular e nuclear, seguida de meiose.

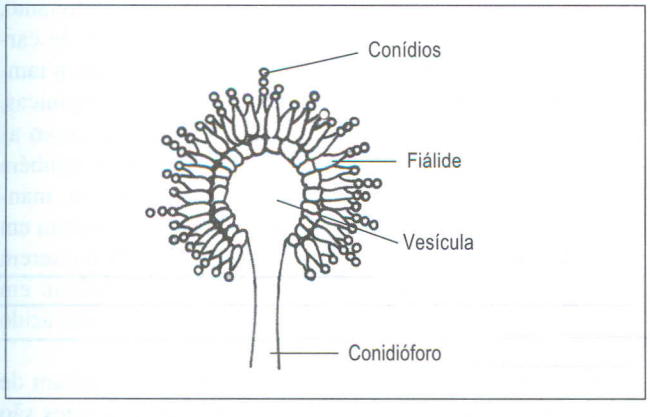


Fig. 64.6 — *Conídios de* Aspergillus *agrupados em forma de cabeça, ao redor de uma vesícula.*

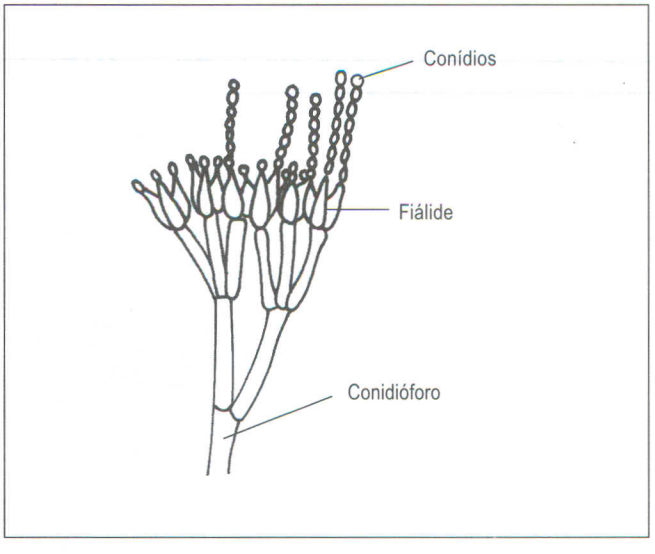


Fig. 64.7 — *Conídios de* Penicillium *agrupados em forma de pincel.*

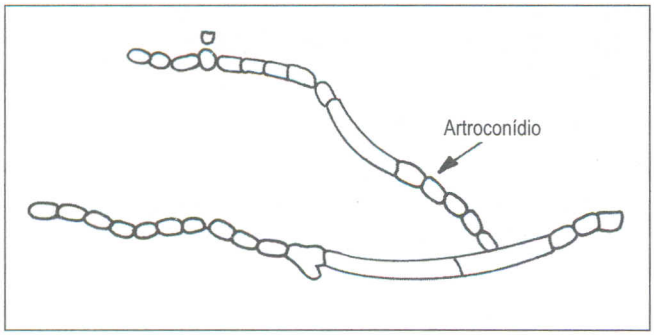


Fig. 64.8 — *Artroconídio.*

O fenômeno da parassexualidade, demonstrado em *Aspergillus*, consiste em fusão de hifas e formação de heterocário que contém núcleos haplóides. Às vezes, esses núcleos fundem-se e originam núcleos diplóides, heterozigóticos, cujos cromossomos homólogos sofrem recombinação durante a mitose. Apesar de estes recombinantes serem raros, o ciclo parassexual é importante na evolução de alguns fungos.

NUTRIÇÃO, CRESCIMENTO E METABOLISMO

Os fungos são microorganismos eucarióticos que se encontram amplamente distribuídos no solo, na água, em alimentos, nos vegetais, em detritos em geral, em animais e no homem, e em sua maioria são aeróbios obrigatórios, com exceção de certas leveduras fermentadoras anaeróbias facultativas, que podem desenvolver-se em ambiente com oxigênio reduzido ou mesmo na ausência deste elemento. Não possuem mecanismos químicos fotossintéticos ou autotróficos para produção de energia ou síntese de constituintes celulares.

Os fungos absorvem oxigênio e desprendem anidrido carbônico durante seu metabolismo oxidativo. Alguns fungos podem germinar muito lentamente em meio com pouco oxigênio. O crescimento vegetativo e a reprodução assexuada ocorrem nessas condições, enquanto a reprodução sexuada se efetua apenas em atmosfera rica em oxigênio.

Na respiração, ocorre a oxidação da glicose, processo essencial para a obtenção de energia.

Em condições aeróbicas, a via de hexose monofosfato é a responsável por 30% da glicólise. Sob condições anaeróbicas, a via clássica usada pela maioria das leveduras é a de Embden-Meyerhof, que resulta na formação do piruvato. Algumas leveduras, como *Saccharomyces cerevisiae*, fazem o processo de fermentação alcoólica de grande importância industrial na fabricação de bebidas e na panificação.

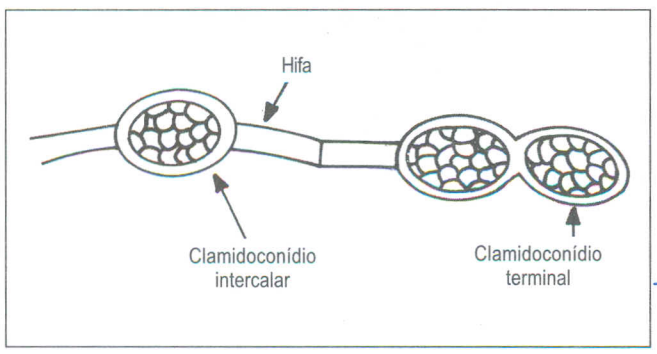


Fig. 64.9 — *Clamidoconídios.*

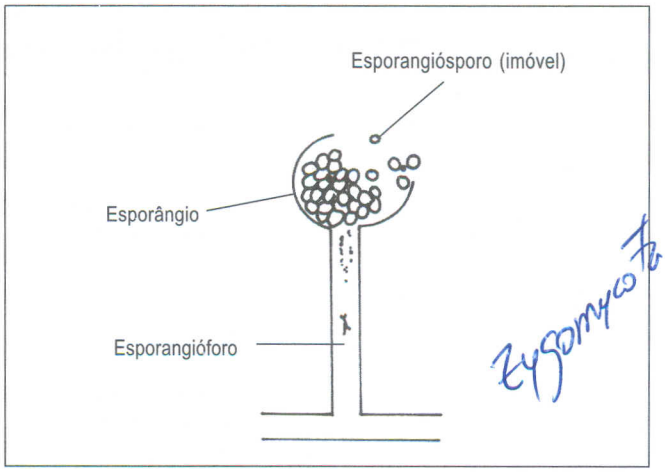


Fig. 64.10 — *Reprodução assexuada interna.*

Devido à ausência de clorofila, os fungos, para se nutrirem, necessitam de substâncias orgânicas que eles próprios são incapazes de elaborar. Assim, são obrigados a viver em estado de saprofitismo, parasitismo ou simbiose.

Os saprófitas utilizam substâncias orgânicas inertes, muitas delas em decomposição. Os parasitas desenvolvem-se em outros organismos vivos, os hospedeiros, e nutrem-se de substâncias existentes em suas células vivas. Os simbiontes associam-se com outros organismos, prestando mútua ajuda em suas funções.

A nutrição da maioria dos fungos dá-se por absorção, processo no qual enzimas adequadas hidrolisam macromoléculas, tornando-as assimiláveis através de mecanismos de transporte. As principais enzimas encontradas nos fungos são: lipases, invertases, lactases, amilases, proteinases etc. Há fungos que têm a capacidade de hidrolisar substâncias orgânicas complexas como quitina, osso, couro, inclusive materiais plásticos.

Para o seu desenvolvimento, os fungos exigem, de preferência, carboidratos simples como a D-glicose. Entretanto, outros açúcares como sacarose, maltose e fontes de carbono mais complexas como amido e celulose podem também ser utilizadas. Substâncias nitrogenadas inorgânicas, como sais de amônia ou nitratos, ou orgânicas, como as peptonas e sais minerais como sulfatos e fosfatos, também são necessárias. Oligoelementos como ferro, zinco, manganês, cobre, molibdênio e cálcio são exigidos, porém em pequenas quantidades. Alguns fungos também requerem fatores de crescimento que não conseguem sintetizar, em especial vitaminas como tiamina, biotina, riboflavina, ácido pantotênico etc.

Os fungos, como todos os seres vivos, necessitam de água para o seu desenvolvimento. Algumas espécies são halofílicas e desenvolvem-se em ambiente com elevada concentração de sal.

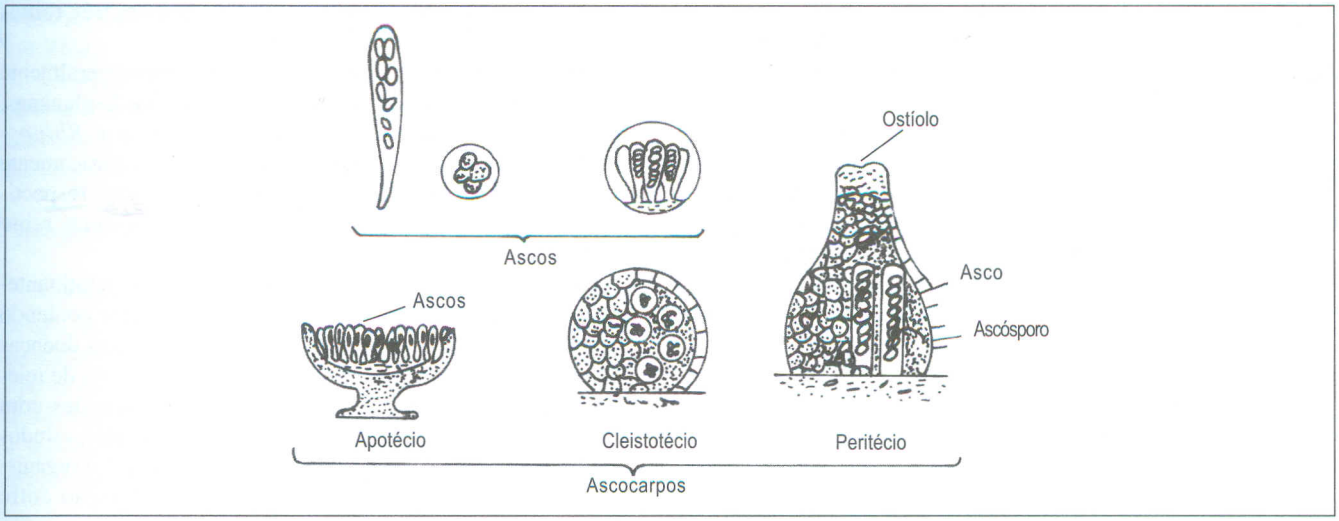


Fig. 64.11 — *Diferentes tipos de ascos e ascocarpos.*

A temperatura de crescimento abrange uma larga faixa, havendo espécies psicrófilas, mesófilas e termófilas. Os fungos de importância médica, em geral, são mesófilos, apresentando temperatura ótima entre 20 e 30°C.

Os fungos podem ter morfologia diferente, segundo as condições nutricionais e a temperatura de seu desenvolvimento. O fenômeno de variação morfológica mais importante em micologia médica é o dimorfismo fúngico, que se expressa por um crescimento micelial entre 22 e 28°C e leveduriforme entre 33 e 37°C. Em geral, essas formas são reversíveis.

A forma micelial (M, *mould*) ou saprofítica é a forma infectante e está presente no solo, nas plantas etc. A forma leveduriforme (Y, *yeast*) ou parasitária é encontrada nos tecidos e *in vitro* em meios enriquecidos a 37°C. Este fenômeno é conhecido como dimorfismo e se observa entre os fungos agentes de micoses sistêmicas e subcutâneas, como *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Blastomyces dermatitidis*. Na *Candida albicans*, a forma saprofítica infectante é a leveduriforme e a forma parasitária, isolada dos tecidos, é a micelial.

Em laboratório, é possível reproduzir o dimorfismo mediante variações de temperatura de incubação, de tensão de O_2 e meios de cultura específicos.

O pleomorfismo nos dermatófitos expressa-se pela perda das estruturas de reprodução ou conídios, com variações morfológicas das colônias. Essas estruturas podem ser recuperadas nos retrocultivos, após inoculações em animais de laboratório ou em meios enriquecidos com terra.

A maioria dos fungos tolera uma ampla variação na concentração de íons de hidrogênio e, de modo geral, um pH em torno de 5,6 é ótimo para o desenvolvimento dos mesmos. Os fungos filamentosos podem crescer em ampla faixa de pH variando de 1,5 a 11. As leveduras não toleram pH alcalino. A pigmentação dos cultivos, muitas vezes, está relacionada com o pH do substrato.

No desenvolvimento vegetativo, os fungos preferem a obscuridade ou luz difusa e, no desenvolvimento da parte reprodutiva, procuram a luz para a sua formação. A luz solar direta, geralmente, é um fator fungicida, devido às radiações ultravioletas.

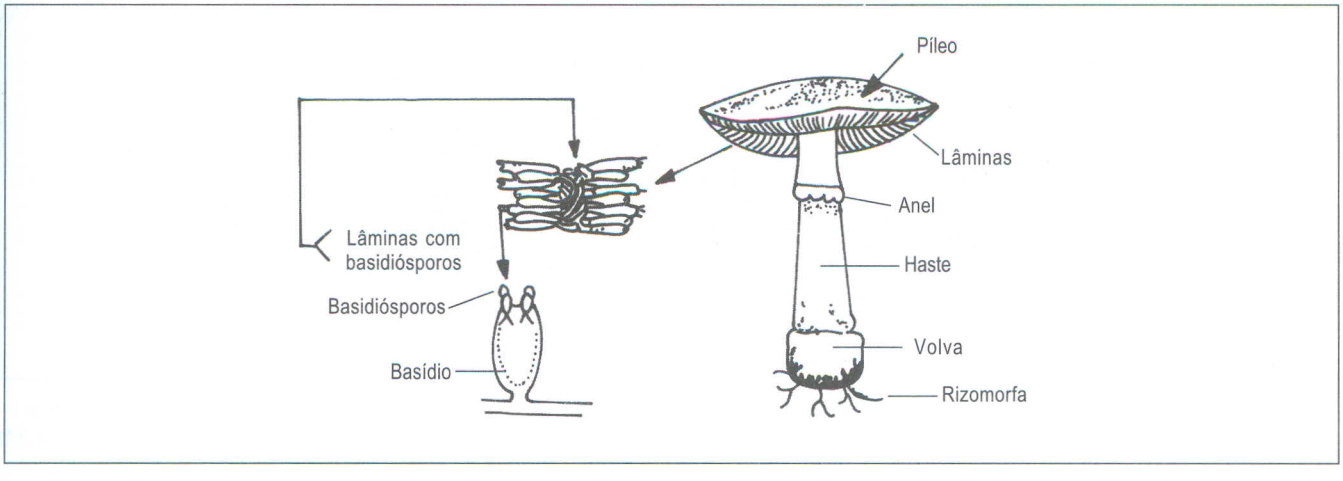


Fig. 64.12 — *Principais estruturas de* Basidiomycota.

Alguns microorganismos podem influenciar o crescimento fúngico, devido à competição que se estabelece no substrato de cultivo. Este antagonismo, muitas vezes, é conseqüência da elaboração de substâncias tóxicas.

O crescimento dos fungos é mais lento que o das bactérias, e suas culturas precisam, em média, de sete a 15 dias ou mais de incubação. Com a finalidade de impedir o crescimento bacteriano, que pode inibir ou se sobrepor ao do fungo, é necessário incorporar aos meios de cultura antibacterianos de largo espectro, como o cloranfenicol. Também se pode acrescentar ciclo-heximida para diminuir o crescimento de fungos saprófitas contaminantes dos cultivos.

TAXONOMIA DOS FUNGOS

Níveis Taxonômicos dos Fungos

Phylum ou filo	sufixo	Mycota
Subfilo	sufixo	Mycotina
Classe	sufixo	Mycetes
Ordem	sufixo	ales
Família	sufixo	aceae
Gênero	sem radical específico	
Espécie	sem radical específico	

A taxonomia dos fungos tem apresentado progressos expressivos baseados em técnicas moleculares, principalmente a prova de PCR e seleção de oligonucleotídeos com sondas específicas.

Os fungos são atualmente enquadrados em três reinos distintos: *Chromista*, *Fungi* e *Protozoa*.

O reino *Chromista* abrange microorganismos geralmente unicelulares com parede celular sem quitina e β-glucanas, mas contendo celulose. *Phytium insidiosum* e *Rhinosporidium seeberi, organismos* hidrofílicos, que classicamente eram estudados no reino *Fungi*, são classificados respectivamente no filo *Oomycota* e *Hyphochytriomycota,* reino *Cromista.*

Os representantes do reino *Protozoa* são predominantemente unicelulares sem verdadeira parede celular contendo cloroplastos. A maior parte das espécies não causa doenças no homem. *Pneumocystis carini*, agente oportunista de relevada importância, principalmente entre os pacientes com AIDS, foi considerado como protozoário. Entretanto, estudos com base na biologia molecular estabeleceram que o organismo pertencia ao reino *Fungi*, onde ocupa posição entre *Ascomycota e Basidiomycota*.

Os fungos patogênicos e oportunistas mais importantes estão distribuídos em três filos do reino *Fungi*: *Zygomycota*, *Basidiomycota*, *Ascomycota* e no grupo dos *Deuteromycetes* (Fig. 64.13).

Filo *Ascomycota*

Agrupa fungos de hifas septadas. A sua principal característica é o asco, estrutura em forma de bolsa ou saco, no interior do qual são produzidos os ascosporos, esporos sexuados, com forma, número e cor variáveis para cada

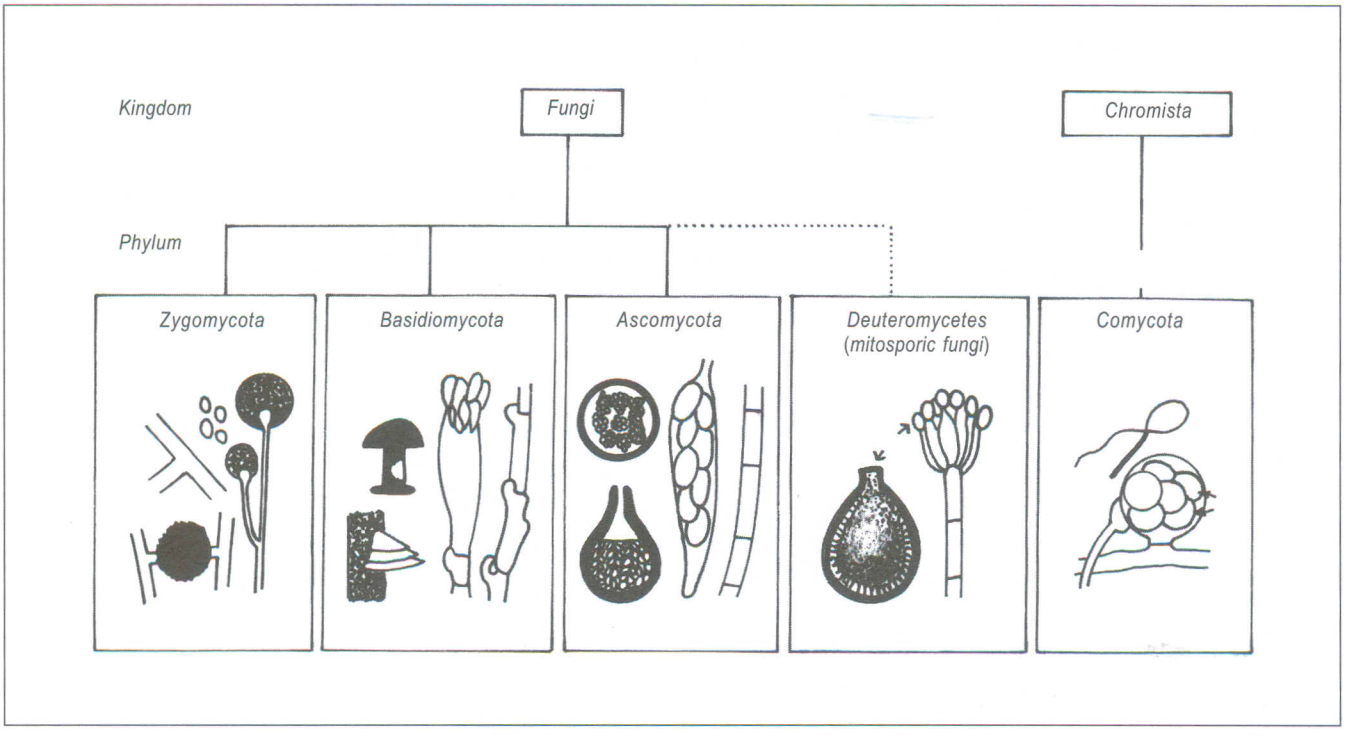


Fig. 64.13 — *Posição taxonômica dos fungos de importância médica (Guarro e col., 1999).* Zygomycota*: a — hifa cenocítica; b — zigosporo; c — esporangiosporo; d — esporangiosporos.* Basidiomycota*: e —esporocarpo; f — basídio; g — basidiosporos; h — hifa com ganchos.* Ascomycota*: i — ascotroma; j — ascos; k — ascoporos; 1 — hifa septada.* Deuteromycetes*: m — picnídio; n — conidóforos; o — célula conidiogênica; p — conídios.* Oomycota*: q — zoosporo; r — gametângio; s — oosporos.*

espécie. Conídios, propágulos assexuados são também encontrados.

Compreende 80% das espécies fúngicas patogênicas e oportunísticas. Três classes no Filo *Ascomycota* possuem espécies patogênicas para o homem: *Hemiascomycetes*, *Loculoascomycetes* e *Plectomycetes*.

Filo *Basidiomycota*

Compreende os fungos superiores ou cogumelos comestíveis. Apresentam hifas septadas e são caracterizados pela produção de esporos sexuados externos, os basidiosporos, típicos para cada espécie. Conídios ou propágulos assexuados podem ser encontrados. A classe *Teliomycetes* contém a espécie patogênica mais importante, *Filobasidiella neoformans*.

Filo *Zygomycota*

Inclui os fungos de micélio cenocítico, ainda que septos possam separar estruturas como esporângios. A reprodução pode ser sexuada pela formação de zigosporos e assexuada com a produção de esporos, os esporangiosporos, no interior de esporângios.

A classe dos *Zygomycetes* contém fungos de interesse médico, encontrados nas ordens *Mucolares e Entomophthorales*.

Deuteromycetes (Fungos Mitospóricos)

Todos os fungos que não têm conexão com *Ascomycetes* e *Basidiomycetes* são incluídos no grupo artificial dos *Deuteromycetes*. Outros termos como fungos imperfeitos, fungos assexuados e fungos conidiais têm sido usados para designar esses organismos. Ainda que outros fungos possuam estruturas assexuadas, como *Omycota e Zygomycota* estes organismos nunca foram tratados como *Deuteromycetes*.

A grande maioria dos fungos desse grupo tem hábitat no solo e são os principais componentes da microbiota atmosférica.

Fungos patogênicos e oportunistas em sua maioria estão no grupo dos *Deuteromycetes* entre as classes *Blastomycetes*, *Coelomycetes* e *Hyphomycetes*.

Filo *Oomycota*

O filo *Oomycota* compreende aproximadamente 700 espécies que possuem características de parede celular com celulose e hábitat próprio, geralmente a água.

Nos filos *Oomycota* e *Hyphochytriomycota*, que pertencem ao reino *Chromista*, são reconhecidos dois agentes fúngicos, *Rhinosporidium seeberi* e *Pythium insidiosum*, de relativa importância em micologia médica.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Ainsworth GC, Sparrow AS. The fungi: an advance treatise. Academic Press, N. York, 1973
2. Madigan MT, Martinko JM, Parker J. Brock Biology of Microorganisms, 8ª Ed. Prentice Hall, 1997.
3. Carlile MJ, Watkinson SC. The fungi. 2ª ed. Academic Press, 1995.
4. Guarro J et al. Developments in fungal taxonomy. Clin. Microbiol. Rev. 12:454-500, ASM Press, Washington, 1999.
5. Silveira VD. Micologia, 4ª ed. Interamericana, R. Janeiro, 1981.

525
528
529
530
531
531
538
538

543
551
561
565

Foreword

Fungi: The threads that keep ecosystems together

When people ask what I do for a living, and I tell them I'm a mycologist, they usually react with surprise. Often they don't know what a mycologist is, but when I tell them, the next question is "why?" Why study fungi?

When someone mentions "fungi" you may think immediately of mushrooms on pizza or maybe moldy food in your refrigerator or the fungus growing on your toes – But in fact fungi are everywhere and affect our lives every day, from mushrooms to industrially important products to plant helpers to plant pathogens to human diseases.

Fungi affect human lives in many and varied ways, so it is important to know something about fungal biology in order to be able to control or exploit them for our own purposes. The study of fungi has increased exponentially in the past 100 years, but they are still being ignored or neglected in many fields of study. For example, more than 90% of fungal species have never been screened for antibiotics or other useful compounds. Many ecologists do not even think about fungi when doing their experiments or observations. However fungi play very important roles in the ecosystem. They are a vital part of the links in the food web as decomposers and pathogens and are important in grassland and forest ecosystems alike. Fungi have many different kinds of associations with other organisms, both living and dead. Since all fungi are heterotrophic, they rely on organic material, either living or dead, as a source of energy. Thus, many are excellent scavengers in nature, breaking down dead animal and vegetable material into simpler compounds that become available to other members of the ecosystem. Fungi are also important mutualists; over 90% of plants in nature have mycorrhizae, associations of their roots with fungi, which help to scavenge essential minerals from nutrient poor soils. Fungi also form mutualistic associations with algae and cyanobacteria in the dual organisms known as lichens.

On the other hand, many fungi are detrimental, inciting a large number of plant diseases, resulting in the loss of billions of dollars worth of economic crops each year, and an increasing number of animal diseases, including many human maladies. Fungi can cause human disease, either directly or through their toxins, including mycotoxins and mushroom poisons. They often cause rot and contamination of foods – you probably have something green and moldy in the

back of your refrigerator right now. They can destroy almost every kind of manufactured good – with the exception of some plastics and some pesticides. In this age of immunosuppression, previously innocuous fungi are causing more and more human disease.

There are many ways in which people have learned to exploit fungi. Of course, there are many edible mushrooms, both cultivated and collected from the wild. Yeasts have been used for baking and brewing for many millennia. Antibiotics such as penicillin and cephalosporin are produced by fungi. The immunosuppressive anti-rejection transplant drug cyclosporin is produced by the mitosporic fungus *Tolypocladium inflatum*. Steroids and hormones – and even birth control pills – are commercially produced by various fungi. Many organic acids are commercially produced with fungi – e.g. citric acid in cola and other soda pop products is produced by an *Aspergillus* species. Some gourmet cheeses such as Roquefort and other blue cheeses, brie and camembert are fermented with certain *Penicillium* species. Stone washed jeans are softened by *Trichoderma* species. There are likely many potential uses that have not yet been explored.

Fungi are also important experimental organisms. They are easily cultured, occupy little space, multiply rapidly, and have a short life cycle. Since they are eukaryotes and more closely related to animals, their study is more applicable to human problems than is the study of bacteria. Fungi are used to study metabolite pathways, for studying growth, development, and differentiation, for determining mechanisms of cell division and development, and for microbial assays of vitamins and amino acids. Fungi are also important genetic tools, e.g. the "one gene one enzyme" theory in *Neurospora* won Beadle and Tatum the Nobel prize for Physiology or Medicine in 1958. The first eukaryote to have its entire DNA genome sequenced was the bakers' and brewers' yeast *Saccharomyces cerevisiae.*

Mycologists study many aspects of the biology of fungi, usually starting with their systematics, taxonomy, and classification (you have to know "what it is" before you can work effectively with it), and continuing on to their physiology, ecology, pathology, evolution, genetics, and molecular biology. There are quite a few disciplines of applied mycology, such as plant pathology, human pathology, fermentation technology, mushroom cultivation and many other fields.

Fungi never fail to fascinate me. They have interesting life cycles and occupy many strange, even bizarre, niches in the environment. Take for example *Entomophthora muscae,* a fungus that infects houseflies. The spores of the fungus land on the unfortunate fly and germinate, then penetrate the exoskeleton of the fly. The first thing the fungus does, according to reports, is grow into the brain of the fly, in order to control its activities. The mycelium of the fungus grows into the particular area of the brain that controls the crawling behavior of the fly, forcing the fly to land on a nearby surface and crawl up as high as possible. Eventually the hyphae of the fungus grow throughout the body of the fly, digesting its guts, and the fly dies. Small cracks open in the body of the fly and the *Entomophthora* produces sporangia, each with a single spore, which are then released in hopes of landing on another fly.

Other fungi, such as the dung fungus *Pilobolus*, produce spore "capsules" that are shot off with great force, up to 3 meters away from their 1 cm sporulating structure. Some fungi are "farmed" by Attine ants and by termites. Some fungi can actually trap and eat small worms called nematodes. Known for their diverse

and amazing physiology, fungi can grow through solid wood, and in lichen associations can even break down rocks. Fungi have intriguing and captivating sex lives, some species with thousands of different sexes. Tetrad analysis in the Ascomycetes has helped to solve some fundamental mysteries about genetics in eukaryotic organisms.

I am pleased to introduce you to THE book for teaching and for learning fungal biology. Michael Carlile, Sarah Watkinson, and Graham Gooday have produced an eminently readable book to introduce students to all aspects of the biology of fungi, including physiology and growth of hyphae and spores, fungal genetics, fungal ecology and how these aspects of the fungi can be exploited in biotechnology. The authors cover many of the topics I have alluded to above in great depth, as well as thoroughly explaining the mostly hidden lives of fungi.

For new students of the fungi, I know you will enjoy learning about these amazing organisms. For those of you who are already mycophiles, this book will serve as a handy reference to fungi and their activities.

Thomas J. Volk
Department of Biology
University of Wisconsin – La Crosse
http://www.wisc.edu/botany/fungi/volkmyco.html

Molecular Phylogenetics and Evolution 65 (2012) 682–694

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular phylogeny of the *Entomophthoromycota*

Andrii P. Gryganskyi [a,f,*], Richard A. Humber [b], Matthew E. Smith [c], Jolanta Miadlikovska [a], Steven Wu [a], Kerstin Voigt [d], Grit Walther [d,e], Iryna M. Anishchenko [f], Rytas Vilgalys [a]

[a] Duke University, Department of Biology, Durham, NC 27708-90338, USA
[b] USDA-ARS BioIPM Research, RW Holley Center for Agriculture & Health 538 Tower Road, Ithaca, NY 14853, USA
[c] Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA
[d] Friedrich-Schiller University of Jena, Institute of Microbiology, 25 Neugasse, Jena 07743, Germany
[e] CBS-KNAW Fungal Biodiversity Centre, 8 Uppsalalaan, Utrecht 3584 CT, The Netherlands
[f] M. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, 2 Tereshchenkivska St., Kyiv 01601, Ukraine

ARTICLE INFO

Article history:
Received 28 February 2012
Revised 3 July 2012
Accepted 23 July 2012
Available online 2 August 2012

Keywords:
Fungi
Monophyly
Divergence time
Ancestral state
Substitution rate

ABSTRACT

The *Entomophthoromycota* is a ubiquitous group of fungi best known as pathogens of a wide variety of economically important insect pests, and other soil invertebrates. This group of fungi also includes a small number of parasites of reptiles, vertebrates (including humans), macromycetes, fern gametophytes, and desmid algae, as well as some saprobic species. Here we report on recent studies to resolve the phylogenetic relationships within the *Entomophthoromycota* and to reliably place this group among other basal fungal lineages. Bayesian Interference (BI) and Maximum Likelihood (ML) analyses of three genes (nuclear 18S and 28S rDNA, mitochondrial 16S, and the protein-coding *RPB2*) as well as non-molecular data consistently and unambiguously identify 31 taxa of *Entomophthoromycota* as a monophyletic group distinct from other *Zygomycota* and flagellated fungi. Using the constraints of our multi-gene dataset we constructed the most comprehensive rDNA phylogeny yet available for *Entomophthoromycota*. The taxa studied here belong to five distinct, well-supported lineages. The *Basidiobolus* clade is the earliest diverging lineage, comprised of saprobe species of *Basidiobolus* and the undescribed snake parasite *Schizangiella serpentis* nom. prov. The *Conidiobolus* lineage is represented by a paraphyletic grade of trophically diverse species that include saprobes, insect pathogens, and facultative human pathogens. Three well supported and exclusively entomopathogenic lineages in the *Entomophthoraceae* center around the genera *Batkoa*, *Entomophthora* and *Zoophthora*, although several genera within this crown clade are resolved as non-monophyletic. Ancestral state reconstruction suggests that the ancestor of all *Entomophthoromycota* was morphologically similar to species of *Conidiobolus*. Analyses using strict, relaxed, and local molecular clock models documented highly variable DNA substitution rates among lineages of *Entomophthoromycota*. Despite the complications caused by different rates of molecular evolution among lineages, our dating analysis indicates that the *Entomophthoromycota* originated 405 ± 90 million years ago. We suggest that entomopathogenic lineages in *Entomophthoraceae* probably evolved from saprobic or facultatively pathogenic ancestors during or shortly after the evolutionary radiation of the arthropods.

1. Introduction

The phylum *Entomophthoromycota* (Humber, in press) is an ecologically important fungal lineage consisting of more than 180 species that are primarily obligate arthropod parasites that are found in diverse habitats worldwide. This group also includes some saprotrophic taxa as well as a handful of species that are parasites of desmid algae, fern gametophytes, tardigrades, and nematodes as well as several facultative parasites of vertebrates including humans (Pfitzer, 1872; Humber, 1981, 1984; Sharma et al., 2003; Hibbett et al., 2007; Koval, 2007).

The *Entomophthoromycota* display a variety of different growth forms. These fungi can grow as a well-developed, multinucleate or septate mycelium that is either walled or protoplastic, depending on the species and the stage of growth. Most of the entomopathogenic species develop inside their arthropod hosts as multinucleate hyphal bodies. Asexual reproduction in species of *Entomophthoromycota* is characterized by the production of forcibly discharged conidia on distinctive conidiophores, and the routine formation of actively or passively dispersed secondary conidia. This repetitive production of forcibly discharged conidia is unique among basal fungal lineages and appears to be a synapomorphy. The thick-walled resting spores form either as zygospores after the fusion

* Corresponding author at: Duke University, Department of Biology, Durham, NC 27708-90338, USA.
E-mail address: Andrii.Gryganskyi@gmail.com (A.P. Gryganskyi).

1055-7903/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2012.07.026

of compatible hyphae (gametangia) or, most commonly in *Entomophthoraceae*, as azygospores formed without a prior gametangial conjugation. It is difficult to determine whether any of these resting spores is the result of sexual reproduction since it appears that the morphological events of conjugation and genetic events of karyogamy and meiosis may not be obligatorily linked in these fungi (Humber, 1981, in press; McCabe et al., 1984). It has been suggested that all *Entomophthoromycota* species are homothallic but the mating biology of this group has not been studied genetically (Humber, in press). Intracellular characteristics such as nuclear size and number, size and position of the nucleolus, and the details of mitotic divisions have long been used to differentiate the taxa, particularly at the familial rank (Humber, 1981, 1984, 1989).

The molecular systematics of the *Entomophthoromycota* was first addressed in the 1990s when a few taxa were included in analyses to elucidate broad phylogenetic patterns in the evolution of basal fungi. The early phylogenetic studies repeatedly revealed a striking lack of concordance between traditional morphological taxonomy and the newer molecular data, both within the *Entomophthoromycota* and across all early diverging fungi (Nagahama et al., 1995; Jensen et al., 1998). Traditional taxonomy had recognized the large, diverse, and heterogeneous phylum *Zygomycota* for fungi with non-septate mycelium and sexual spores formed after hyphal fusion (Moreau, 1954). Molecular phylogenetic studies have verified the heterogeneity of this phylum by recognizing at least five apparently monophyletic groups within the fungi of *Zygomycota* s.l. Based on the results of a six-gene phylogenetic analysis of all major fungal groups (James et al., 2006), the phylum *Zygomycota* was rejected as non-monophyletic and these five molecularly distinct lineages were recognized as the phylum *Glomeromycota* (arbuscular mycorrhizal fungi) and four subphyla of uncertain position (*incertae sedis*): *Mucoromycotina*, *Entomophthoromycotina*, *Kickxellomycotina*, and *Zoopagomycotina* (Hibbett et al., 2007). Fifth subphyllum *Mortierellomycotina* was separated from *Mucoromycotina* recently (Hoffmann et al., 2011). Nomenclature for the major groups of former *Zygomycota* has not yet been standardized (e.g., Kirk et al., 2001; O'Donnell et al., 2001; Hibbett et al., 2007). While evolutionary relationships within the *Entomophthoromycotina* have not been fully resolved, the morphology

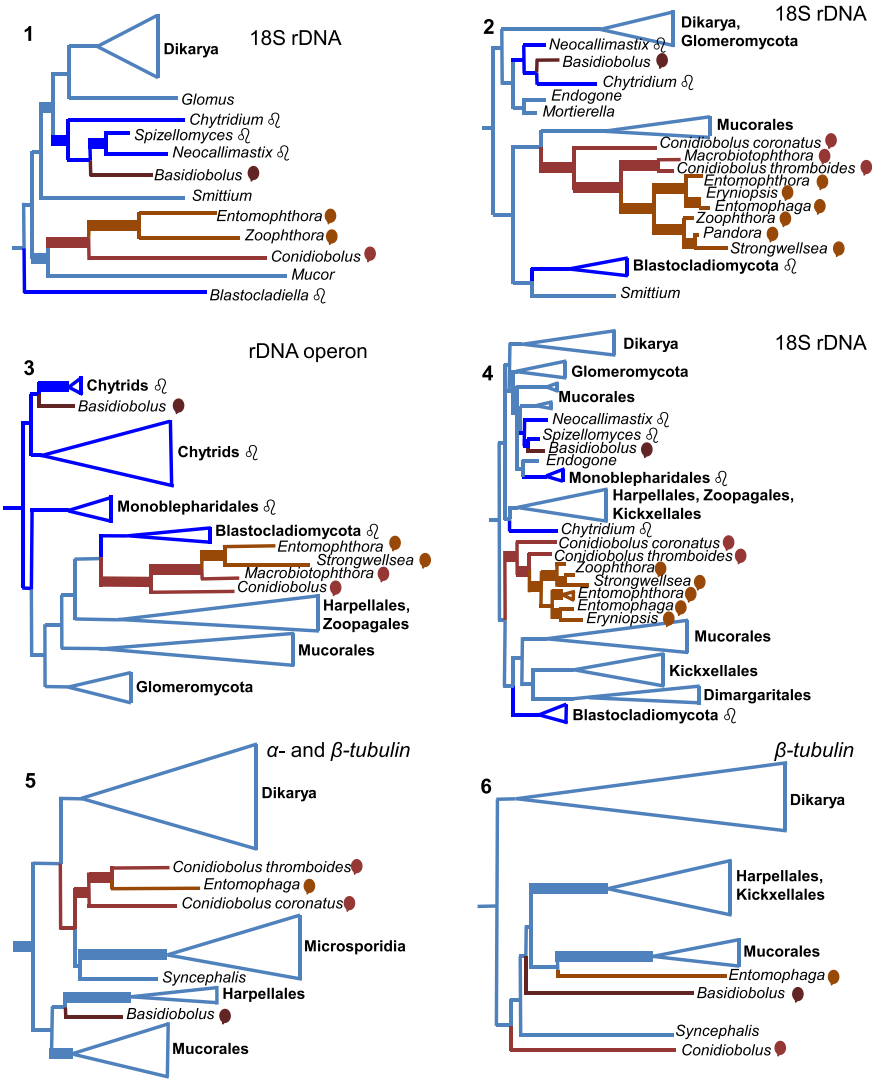


Fig. 1. Placement of *Entomophthoromycota* among the basal fungal lineages in 12 previously published studies. Thickened branches indicate lineages with statistically significant support as reported in the original publication. Filled ovals indicate *Entomophthoromycota* and small open circles with tails indicate flagellated fungi (*Chytridiomycota* and *Blastocladiomycota*). Phylogenies are modified for presentation here based on the following publications: 1 – Nagahama et al. (1995); 2 – Jensen et al. (1998); 3 – James et al. (2000); 4 – Tanabe et al. (2000); 5 – Keeling (2003); 6 – Einax and Voigt (2003); 7 – Tanabe et al. (2004); 8 – James et al. (2006); 9 – White et al. (2006); 10 – Liu and Voigt (2010); 11 – Ebersberger et al. (2011); 12 – Sekimoto et al. (2011).

of this group and the results of James et al. (2006) suggst that it may be a unique monophyletic lineage. Based on these criteria, Humber (in press) reasoned that this lineage was sufficiently distinct to be recognized at the phylum rank as *Entomophthoromycota*.

Previous molecular studies suggest that the *Entomophthoromycota* is a polyphyletic group with uncertain placement of its main lineages (Nagahama et al., 1995; Jensen et al., 1998; James et al., 2006; White et al., 2006; Liu and Voigt, 2010; Voigt and Kirk, 2011). Molecular studies have also failed to identify the closest relatives of *Entomophthoromycota* due to poor phylogenetic resolution, which is probably due to the use of only a few molecular markers with limited ability to discriminate evolutionary patterns within this group of fungi. To date, molecular phylogenetic studies of *Entomophthoromycota* have used three groups of molecular markers: (1) nuclear rDNA loci (18S, 28S or the entire ribosomal operon); (2) protein-coding genes (actin and β-tubulin, core orthologous genes from completely sequenced fungal genomes); or (3) multiple genes (rDNA, *RPB1*, *RPB2*, and *EF-1α*). The majority of these studies included only 2–12 representatives from *Entomophthoromycota* (only 1–4% of the known species in the group – White et al., 2006; Table S1). Earlier phylogenetic analyses based on a single gene suggested a polyphyletic *Entomophthoromycota*, with *Basidiobolus* split from the rest of the group (Nagahama et al., 1995; Jensen et al., 1998) (Fig. 1.1–7 and 1.10). In contrast, multi-locus phylogenetic analyses have delimited a monophyletic *Entomophthoromycota* (Fig. 1.8: James et al., 2006; Fig. 1.9: White et al., 2006). Another characteristic feature of most phylogenetic studies is that most have reconstructed flagellated fungi as a "sister group" to the *Basidiobolus* clade (Fig. 1.1: Nagahama et al., 1995; Fig. 1.2: Jensen et al., 1998; Fig. 1.8: James et al., 2006; Fig. 1.9: White et al., 2006; Fig. 1.12: Sekimoto et al., 2011). Studies in which protein-coding genes were used to evaluate all basal eukaryotic lineages suggested a close relationship of *Entomophthoromycota* with *Microsporidia* (Fig. 1.5: Keeling, 2003) or even with non-fungal groups (Fig. 1.10: Liu and Voigt, 2010; Voigt and Kirk, 2011), or place it as an intermediate group between aquatic and terrestrial fungi (Fig. 1.11: Ebersberger et al., 2011).

Although *Entomophthoromycota* have not received comprehensive phylogenetic study, some molecular data are now available for ca. 30–40% of the described species, with the most commonly sequenced regions being rDNA (18S and 28S), *RPB2*, mitochondrial 16S, *RPB1*, actin, β-tubulin, and *TEF-1* (in decreasing order of

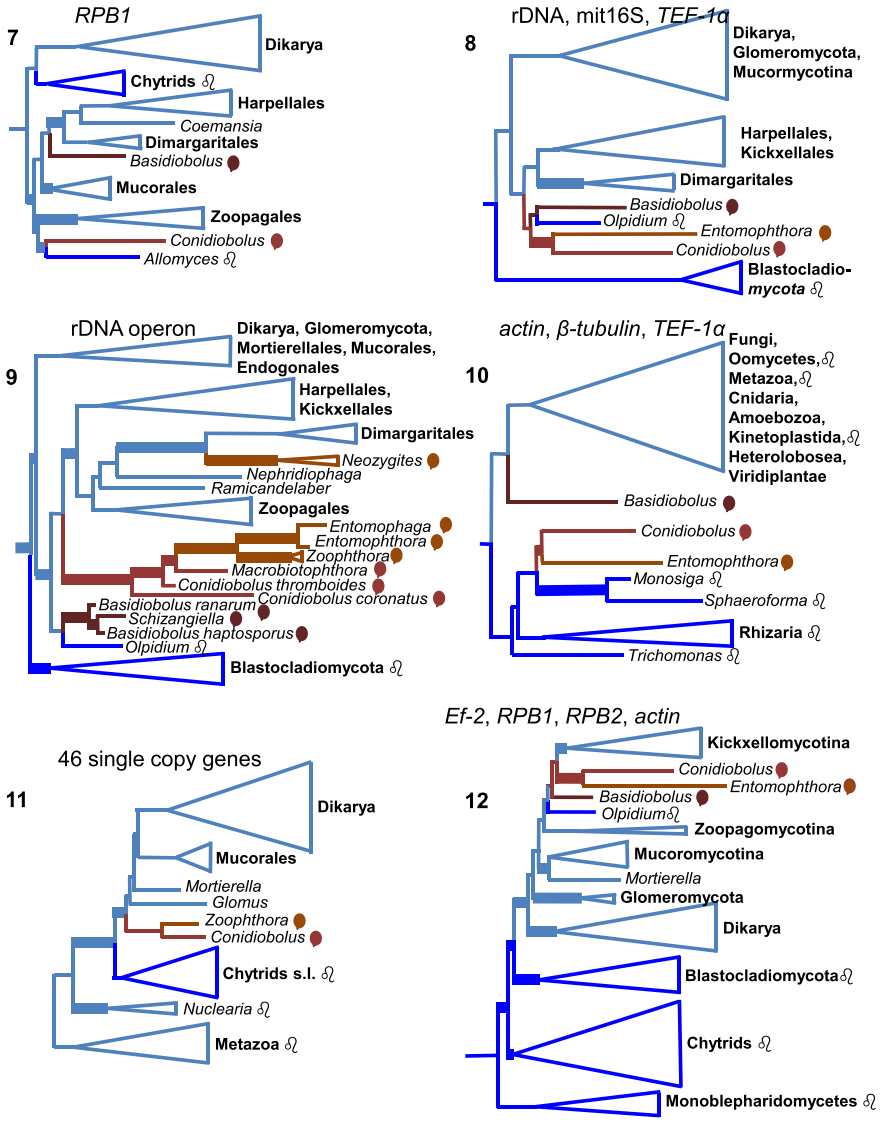


Fig. 1 (*continued*)

available sequences, Table S2). The available data are sufficient to place the *Entomophthoromycota* on the fungal tree of life and to study relationships among the major lineages in this group.

The main aims of this study were to: (1) determine if *Entomophthoromycota* is a monophyletic group, (2) build a taxon-rich phylogeny of this group with all available molecular data to investigate the relationships between different lineages, (3) compare the molecular phylogenetic results with the morphological features and the traditional taxonomy of the group, (4) estimate the divergence time of this group, (5) reconstruct the ancestral phenotype, and (6) determine the closest relatives of the *Entomophthoromycota*. To accomplish these goals we used both Maximum Likelihood (ML) analysis and Bayesian inference (BI) to construct a multi-gene phylogeny of 31 *Entomophthoromycota* taxa representing most major lineages. Our analysis is the first to include a large number of *Entomophthoromycota* species and employ the simultaneous analysis of nuclear, mitochondrial and protein-coding loci.

2. Materials and methods

2.1. Fungal material and molecular protocols

Twenty-two cultures were obtained from the Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF, Ithaca, NY, USA), CBS-KNAW Fungal Biodiversity Centre (CBS-KNAW, Utrecht, the Netherlands), and Jena Microbial Resource Collection (Friedrich-Schiller-University of Jena, Germany). We selected representative fungal cultures from as many genera as possible based on their availability in the culture collections.

Cultures were grown in 10% malt extract broth (VWR International, West Chester, PA, USA) for 3 days on an incubator shaker 25 °C (Lab-Line Instruments Inc., Melrose Park, IL, USA). Mycelium was then filtered using Whatman filter paper (Whatman Int. Ltd., Maidstone, England, UK), lyophilized overnight in a *Freeze Mobile 12SL lyophilizer* (*Virtis Sentry*, Gardiner, USA), and ground in liquid nitrogen using a mortar and pestle or sterile glass beads. DNA was extracted with a CTAB extraction technique (Gardes and Bruns, 1993). Polymerase chain reaction (PCR) was performed with the following primers: (18S rDNA) NSSU1088R (Kauff and Lutzoni, 2002) and NS24 (Gargas and Taylor, 1992); (28S rDNA) LROR (Rehner and Samuels, 1994) and LR5 (Vilgalys and Hester, 1990); (mitochondrial 16S) mtSSU1 and mtSSU2r (Zoller et al., 1999); and (*RPB2*) fRPB2-5F and fRPB2-7cR (Liu et al., 1999). All PCR reactions were performed using Apex Taq DNA polymerase (Genesee Scientific, San Diego, CA, USA) using previously published protocols (Gryganskyi et al., 2010). Amplicons were purified with ExoAP (NEB, Ipswich, MA, USA) according to the manufacturer's recommendations. Purified PCR products were then sequenced using amplification primers and BigDye version 3.1 (Applied Biosystems Inc., Foster City, CA, USA). Sequences were determined with an ABI3700 DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA). Raw sequence data were analyzed and edited using Sequencher v. 4.1.4 software (Gene Codes Corporation, Ann Arbor, MI, USA).

Data and alignments have been submitted to GenBank (Table S3) and TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S12923; last accessed 28 June 2012).

2.2. Multi-gene phylogeny of Entomophthoromycota

The first goal of this study was to construct a well-resolved, multi-gene phylogeny for the *Entomophthoromycota* to test whether the group is monophyletic, to resolve the major lineages within, to estimate the divergence time for the group as a whole, and to calculate the rates of substitution for each of the studied genetic regions. In order to construct the multi-gene phylogeny, we used a complete molecular dataset from 64 fungal taxa, including 31 species of *Entomophthoromycota* (ingroup) and 33 species from other fungal lineages (outgroups). The outgroup taxa include members of all major subphyla within the *Zygomycota* as well as 22 flagellated fungi classified in the *Chytridiomycota* and *Blastocladiomycota*.

We used the sequences from four loci: rDNA (18S and 28S); mitochondrial SSU (mtSSU); *RPB2* (regions 5–7). This analysis included 40 new sequences as well as 209 sequences from GenBank and AFToL (http://aftol.org/) (Table S3).

To assess conflicting phylogenetic signals from the four loci, we searched for strong incongruence of the nodes by 1000 ML bootstrap replicates (>70%) and by posterior probabilities (>0.95) of credible Bayesian trees. Because no supported nodes were in conflict (Mason-Gamer and Kellogg, 1996), the data were combined into a single matrix with six partitions: one partition for each locus of the non-protein-coding loci 18S, 28S and mtSSU, and one partition for each codon position of *RPB2*. The 18S rDNA, 28S rDNA, mtSSU, and *RPB2* partitions included 528, 1443, 284, and 720 characters, respectively, for a combined data matrix of 2975 characters.

Sequences were aligned individually for each locus using MUSCLE (Edgar, 2004). Alignments were visually inspected and ambiguous regions were excluded using PhyDe (Müller et al., 2010). The optimized nucleotide substitution model (GTR + Γ + I) was selected using ModelTest 3.06 (Posada and Crandall, 1998). Maximum Likelihood (ML) phylogenetic trees for each locus were estimated using RAxML 7.0.4 (Stamatakis, 2006; Stamatakis et al., 2008) and/or Garli-1.0 (Zwickl, 2006) whereas Bayesian interference (BI) trees were estimated with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Both Bayesian and ML analyses were run on the CIPRES Science Gateway V. 3.1 (Miller et al., 2010; www.phylo.org) and/or the Duke Shared Cluster Resource (DSCR). ML analyses were conducted using a GTRMIX substitution and rate heterogeneity with unlinked parameters and 1000 ML bootstrap replicates were computed. For Bayesian analyses, the partitions were unlinked with the GTR + Γ + I substitution model used for each partition. Four parallel chains were computed to 100 million generations, with sampling every 1000 generations. Trees were sampled when an equivalent posterior probability plateau was achieved between runs. Posterior probability convergence was examined using Tracer 1.4 (Rambaut and Drummond, 2008), and topological convergence was validated using AWTY (Wilgenbusch et al., 2004). Statistical support was recognized as significant with bootstrap values $\geqslant$70% for ML and $\geqslant$0.95 posterior probability for BI.

An additional multi-gene phylogeny used a subset of 31 *Entomophthoromycota* and the arbuscular mycorrhizal fungus *Glomus* (*Glomales*, *Glomeromycota*) in order to refine the calculation of substitution rates and the estimated divergence time for *Entomophthoromycota* (see Section 2.7).

2.3. Taxon-rich rDNA phylogeny of Entomophthoromycota

A second goal of this study was to generate a phylogeny to include all *Entomophthoromycota* species for which molecular data are available. In order to accomplish this, we utilized a constraint tree based on the multi-gene dataset (see above) but included an additional 32 taxa for which only rDNA was available. The resulting phylogenetic analysis dataset contained 75 fungal taxa, including 63 *Entomophthoromycota* species representing 14 genera. The rDNA dataset was constructed using 110 sequences from the GenBank and AFToL databases as well as 40 sequences generated in this study (Table S3). The alignment consisted of 1827 characters (1343 characters of 18S, and 484 characters of 28S). 18S sequences

were missing for nine taxa (12%) and 28S sequences were missing for eight taxa (10%). Representatives of the genus *Neozygites* were excluded from this study because their sequences were too divergent to align with other *Entomophthoromycota* (White et al., 2006).

2.4. Placement of Entomophthoromycota within the fungal tree of life

In an attempt to place the *Entomophthoromycota* within the fungal tree of life and to identify the closest relatives of this lineage, we constructed a phylogeny based on rDNA (1397 characters of 18S, 324 characters of 28S) from 159 *Zygomycota* s.l. taxa including 31 members of *Entomophthoromycota*. We used 300 sequences from GenBank and AFToL (http://aftol.org/) and 18 newly generated sequences (Table S3).

2.5. Non-molecular phylogeny

Thirty-eight non-molecular characters were selected to generate a data matrix (Table S4) for phylogenetic and ancestral state reconstructions, and for the estimation of the morphological similarities between the main *Entomophthoromycota* lineages.

We used two ecological, 19 morpho-physiological, and 17 ultrastructural characters considered important for the taxonomy of *Entomophthoromycota*. Non-molecular data were collected directly from pertinent literature (e.g. Ben-Ze'ev and Kenneth, 1982; Humber, 1981, 1984; Koval, 2007; Tucker, 1984) as well as our own microscopic observations on species of *Basidiobolus*, *Conidiobolus*, *Entomophthora* and *Zoophthora*.

Three taxa (e.g. *Conidiobolus obscurus*, *C. pseudapiculatus*, and *Schizangiella serpentis* nom. prov.) were excluded from the non-molecular analysis due to lack of data. We included four taxa (*Entomophaga*, *Macrobiotophthora*, *Massospora*, *Strongwellsea*) that were absent in the multi-gene phylogeny but used for the taxon rich constraint-based rDNA phylogeny (Table S4). The non-molecular phylogeny was constructed using maximum parsimony (MP) in PAUP* (Swofford, 2002); 1000 bootstrap replications with 10 random additions per replicate were used as a criterion for clade robustness. We also studied the relatedness between its main lineages based on the number of similarities of main molecular lineages to the core group represented by the type species of this phylum *Entomophthora muscae* (Table 1).

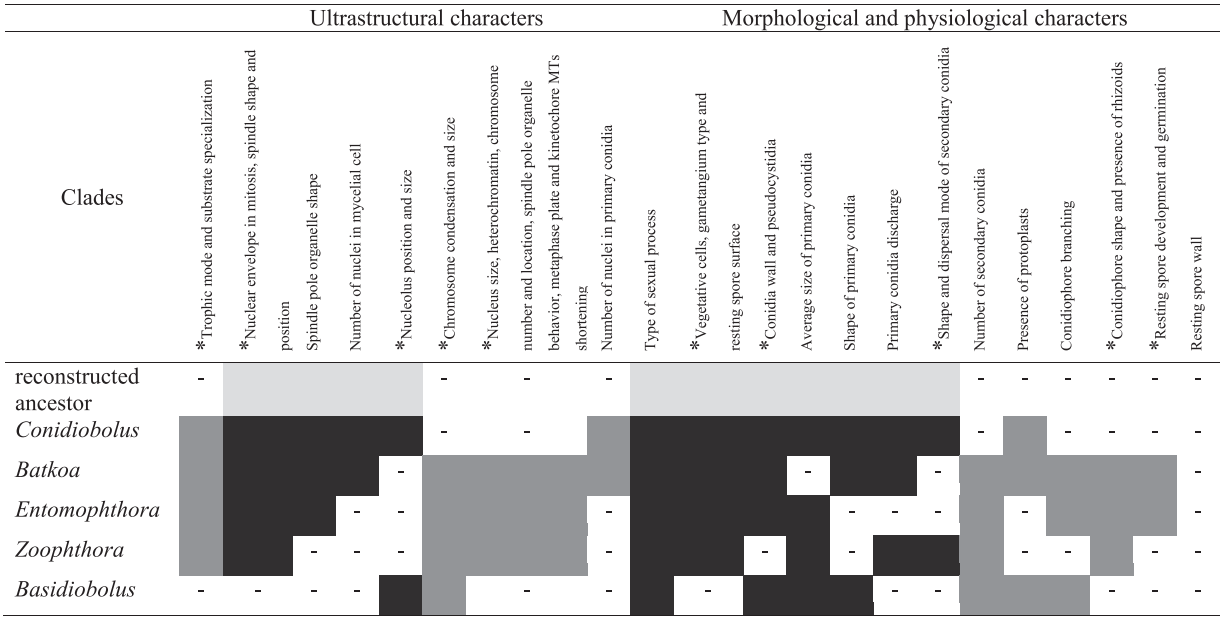
2.6. Ancestral state reconstruction

We calculated the likelihood that each of 38 non-molecular characters was found in the *Entomophthoromycota* ancestor (see Section 2.2, Table S5) over 38,900 trees based on three genes (four loci) using Bayesian analysis. We used ML with the marginal global optimality criterion as implemented with the LASRDisc module in Mesquite 1.05 (Pagel, 1999; Jackson, 2004; Maddison and Maddison, 2009) and option "trace character over trees" and root mode = (0.5, 0.5). An asymmetrical 2-parameter Markov k-state model allowing different rates of gains and losses was chosen based on the likelihood ratio test performed on several random trees from the pool of 38,900 trees. A given ancestral state was assigned to a node when its raw likelihood was >2 log units higher than the likelihood value of the other ancestral state. For these analyses we used 30 taxa that were included in our multi-gene analyses but we excluded *Schizangiella* because many of the non-molecular features were not known for this taxon that has yet to be formally described.

2.7. Estimation of divergence time and differences in substitution rates between lineages

Molecular divergence time analyses were performed using the BEAST v. 1.6.1 software package (Drummond and Rambaut, 2007) with an alignment containing the three concatenated gene regions: 18S + 28S rDNA, mtSSU, and *RPB2*. The reduced dataset consisted of only one sequence per species. There is some debate

Table 1
Shared characters between different lineages of *Entomophthoromycota* and their reconstructed ancestral state.



* - correlated characters,
- reconstructed ancestral character state,
- characters shared between different *Entomophthoromycota* lineages,
- characters shared between different *Entomophthoromycota* lineages and the reconstructed ancestor.

about the time of origin of the sister group, *Glomeromycota*, so we calculated the time of divergence for *Entomophthoromycota* based on two different models. The first model used the first fossil record for *Glomeromycota* (exponential distribution prior with mean of 30 MYA (million years ago) and 46 MYA offset, [Simon et al., 1993]). The second model used an estimated calibration for the root of this fungal group, normal distribution prior with mean 760 MYA and standard deviation of 76 MYA (Berbee and Taylor, 2010).

The three gene regions were assigned to individual partitions in the BEAST analyses, and the partitions were unlinked in the GTR + Γ + I substitution models. The Yule speciation model and uncorrelated lognormal-distributed relaxed clock model were employed (Drummond et al., 2006). The UPGMA algorithm was used to estimate a fully resolved starting tree when the root of the phylogeny was calibrated at 760 MYA, and a random starting tree was used when it was calibrated at 460 MYA. Three independent BEAST analyses were run for 100 million generations, sampling the parameters and trees every 5000 generations. The parameters obtained from the resultant 20,000 generations for each of the three runs were examined for convergence manually using Tracer v. 1.5 (Rambaut and Drummond, 2008). Based on the results of this analysis, after manual inspection, a burn in of 2000 states was removed from each run. The remaining states were used to generate a maximum clade credibility tree with TreeAnnotator v. 1.6.1 (Drummond and Rambaut, 2007) and parameter summary statistics were calculated by Tracer. We obtained the average rates (combined three gene set) for each branch on the relaxed clock model in BEAST (Drummond et al., 2006).

2.8. Computation of absolute and relative substitution rates

The relative substitution rates for each lineage of the multigene tree containing 44 taxa were calculated with PAML4.4c (http://abacus.gene.ucl.ac.uk/software/paml.html) using an independent rates model and local molecular clock (Yang, 1994, 2007) for each of the following lineages: outgroup, *Basidiobolus*, *Conidiobolus*, *Batkoa*, *Entomophthora*, and *Zoophthora*. Absolute rates of substitution were calculated separately for three genes (rDNA, mtSSU, and *RPB2*) using a strict molecular clock. For the calibration, two different time points for the origin of *Glomeromycota* were used independently: a fossil record of 455–460 MYA (Simon et al., 1993; Redecker et al., 2000), and an age estimation of 760 MYA (Berbee and Taylor, 2010).

3. Results

3.1. Phylogeny of Entomophthoromycota

Our most significant finding was that the *Entomophthoromycota* (including *Basidiobolus*) formed a monophyletic lineage that was not closely related to any group of flagellated fungi. The *Entomophthoromycota* was resolved as distinct among the *Zygomycota* s.l. (100% bootstrap (BS) and 0.99 posterior probability (PP), (node A in Figs. 2 and 3) but is most closely related to the partially parasitic *Kickxellomycotina* and primarily parasitic *Zoopagomycotina* (Fig. 2). Similar phylogenetic placement without strong statistical support was obtained using ribosomal DNA from a smaller pool of *Entomophthoromycota* taxa and a full sampling of other *Zygomycota* (Fig. S1). Our analyses suggest that the analyzed set of taxa from *Entomophthoromycota* constitute five major groups (Figs. 2 and 3) that are hereafter referred to as the *Entomophthora*, *Zoophthora*, *Batkoa*, *Conidiobolus* and *Basidiobolus* lineages.

The *Entomophthora*, *Zoophthora*, *Batkoa* lineages fall within the family *Entomophthoraceae* (100% BS and 1.00 PP) and are exclusively insect pathogens (node C in Figs. 2 and 3). Although each of these three lineages was reconstructed with good support in both the ML and BI analyses, the two phylogenies were in disagreement about the placement of the *Entomophthora* lineage. The ML tree depicts the *Entomophthora* lineage grouping with the *Batkoa* lineage (100% BS), whereas the BI tree positions the *Entomophthora* lineage together with the *Zoophthora* lineage with very low support (0.79 PP) (Fig. 3).

The *Entomophthora* lineage (100% BS and 1.00 PP) includes 10 species of the type genus *Entomophthora* and also species from the genera *Entomophaga*, *Eryniopsis* and *Massospora*. Relationships within the genus *Entomophthora* are poorly resolved, which is probably due to partial missing sequence data in all *Entomophthora* species except *E. muscae*.

The *Zoophthora* lineage (100% BS and 1.00 PP) is formed by species of *Zoophthora* s.l., including the genera *Erynia*, *Furia*, *Pandora*, *Strongwellsea* and *Zoophthora* (Humber, 1989). Only the *Zoophthora* group is well resolved and unambiguously separated from the other taxa. The other members of the *Zoophthora* lineage assort into two or three variable and poorly resolved groups in various analyses (Fig. 3).

The insect-pathogenic *Batkoa* lineage is a distinct and well-supported group (100% BS and 1.00 PP).

The *Conidiobolus* lineage, which includes saprobes, facultative invertebrate and vertebrate pathogens, is paraphyletic between the *Basidiobolus* lineage and the core *Entomophthoraceae*. The *Conidiobolus* lineage comprised at least two well resolved groups (100% BS and 1.00 PP) represented by the key species *C. coronatus* and *C. thromboides*.

The well-supported *Basidiobolus* lineage (100% BS and 1.00 PP, Figs. 2 and 3) is the most divergent group within *Entomophthoromycota*. All six *Basidiobolus* species are closely related to each other whereas the undescribed and incompletely characterized snake pathogen *Schizangiella serpentis* nom. prov. is represented by a long branch. In both multi-gene and rDNA phylogenies *Kickxellomycotina* is a closest group to *Entomophthoromycota* (Figs. 2 and S1), however with no significant statistical support based on rDNA analyses.

3.2. Non-molecular phylogeny

Maximum parsimony analysis of biochemical, ultrastructural, and morphological features from the genus-level dataset was consistent with the multi-gene phylogeny (Fig. 3). The non-molecular dataset resolved the same three main lineages of *Entomophthoromycota* found in the molecular dataset: the *Basidiobolus* lineage, the *Conidiobolus* lineage, and the *Entomophthoraceae* (including *Entomophthora*, *Zoophthora*, *Batkoa*, and the other primarily insect-pathogenic genera). These three major lineages received strong bootstrap support and the overall tree topology was similar to the multi-gene phylogeny. We also used the non-molecular characters to examine qualitative differences between the five main lineages of *Entomophthoromycota* (e.g. the *Entomophthora*, *Zoophthora*, *Batkoa*, *Conidiobolus* and *Basidiobolus* lineages). Fig. 4 features some of the key characters of type species *E. muscae*: entomopathogenicity, conidiophores morphology, number of nuclei in primary conidia, hyphal bodies. We also estimated the number of shared and unshared characters between these lineages (Table 1).

The *Basidiobolus* lineage is the most distinct and shares only 10 out of 38 characters with all of the other lineages. The paraphyletic *Conidiobolus* lineage occupies an intermediate position and possesses features similar to both the basal *Basidiobolus* lineage and the *Entomophthoraceae* lineages. The *Conidiobolus* lineage possesses 19 characters similar to at least one of the three lineages in *Entomophthoraceae* and also shares six features in common with the basal *Basidiobolus* lineage. The *Entomophthoraceae* form

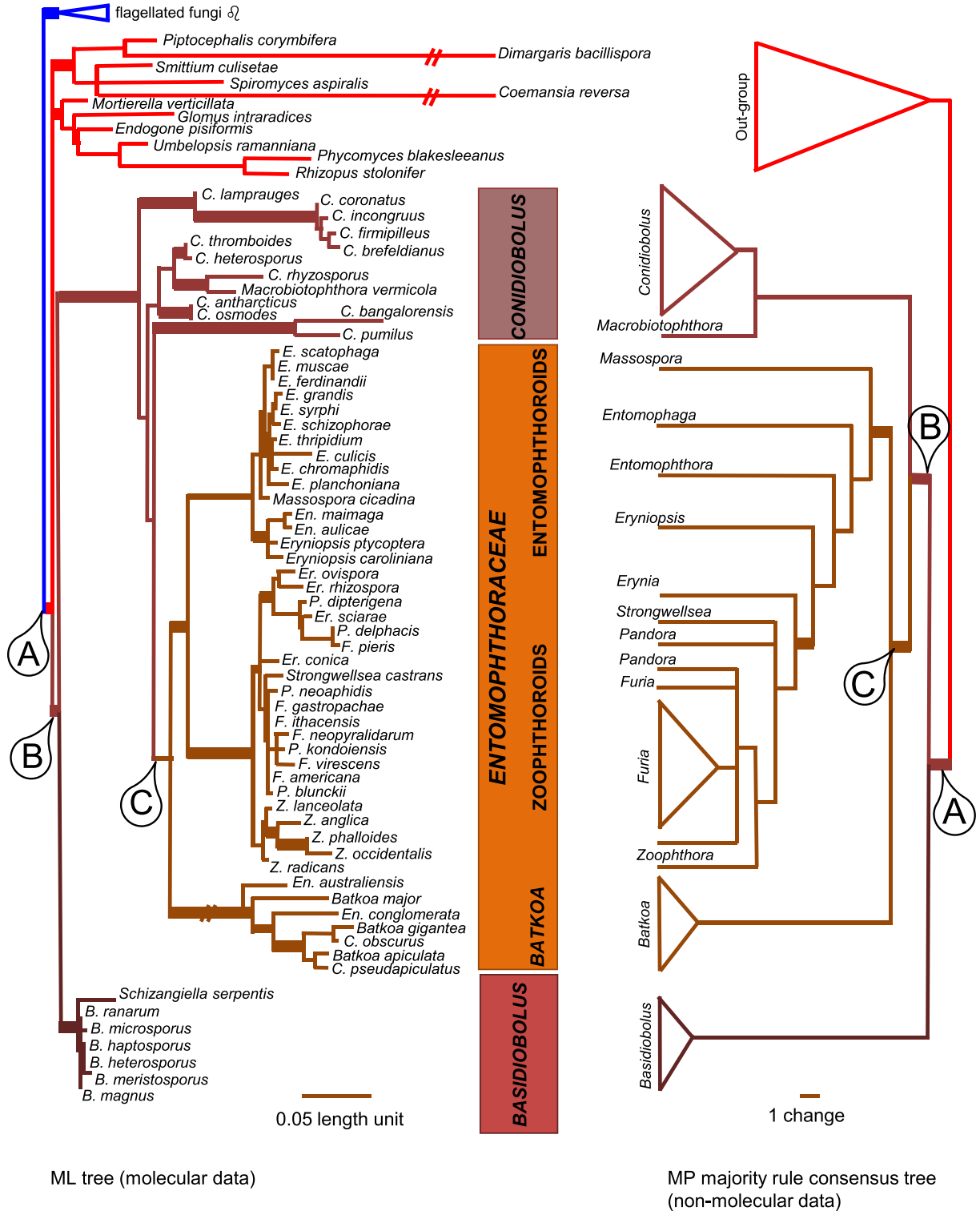


Fig. 2. Maximum Likelihood phylogeny of *Entomophthoromycota* based on three genes (rDNA, mtSSU and *RPB2*). Thickened branches have statistically significant statistical support (ML-16843.24, bootstrap >70%, BI posterior probability >0.95). Blue color indicates flagellated fungi (*Chytridiomycota* and *Blastocladiomycota*), red color indicates *Zygomycota* excluding *Entomophthoromycota*, and the three different shades of brown indicate the three main groups of *Entomophthoromycota* (*Basidiobolus* lineage, *Conidiobolus* lineage, and the three entomopathogenic lineages in *Entomophthoraceae*). Encircled letters designate the main nodes: (A) separation between *Entomophthoromycota* and other *Zygomycota*, (B) separation between the *Basidiobolus* lineage and all other *Entomophthoromycota*, and (C) separation between entomopathogenic *Entomophthoraceae* (the *Batkoa*, *Entomophthora* and *Zoophthora* lineages) and saprotrophic *Entomophthoromycota*. An arrow indicates the unresolved placement of *Entomophthora* clade, which is grouped together with *Zoophthora* s.l. in the ML analysis but together with *Batkoa* in the BI analysis. The micromorphological features of major lineages in *Entomophthoromycota* are on the right: **Cph1-3** – type of conidiophores: **Cph1** – numeral simple, **Cph2** – branched, **Cph3** – single simple, **C1-2** – type of primary conidia: **C1** – unitunicate, **C2** – bitunicate, **2C1-3** – type of secondary conidia: **2C1** – like primary, **2C2** – capilliconidia, **2C3** – microconidia, **RS1-2** – type of resting spores: **RS1** – axially aligned with parental cells, **RS2** – budding from parental cell, **N1-4** – type of nuclear division: **N1** – closed mitosis, tiny fusoid eccentric spindles, chromosomes uncondensed; **N2** – closed mitosis, tiny fusoid eccentric spindles, huge chromosomes; **N3** (in *Neozygites*, not shown) – closed mitosis, fusoid spindles, normal chromosomes; **N4** – open mitosis, barrel-shaped spindle, nucleus-associated organelle, tiny chromosomes. * – Taxa of genus *Batkoa*, including their synonyms.

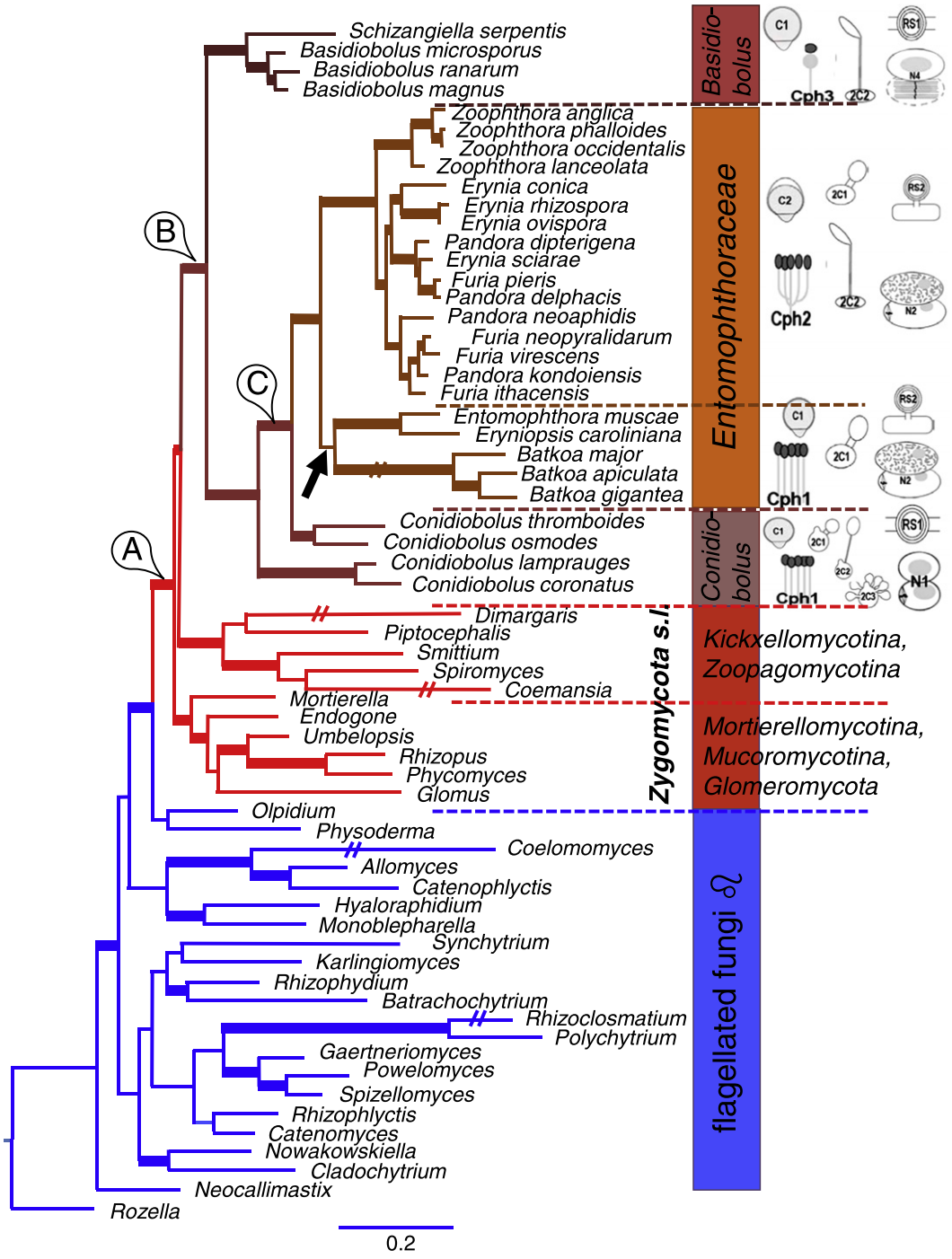


Fig. 3. Maximum Likelihood phylogeny of *Entomophthoromycota* (left side) generated using 18S and 28S rDNA sequences and the multi-gene tree in Fig. 2 to constrain the topology; and Maximum Parsimony phylogeny based on non-molecular characters (75% majority rule consensus phylogeny based on 2305 trees, right side). The thickest branches indicate significant statistical support (ML-16843.23, ML and MP bootstraps >70%, BI posterior probability >0.95). Tailed dots indicate flagellated fungi. Encircled letters designate the important, well-supported nodes in the phylogeny: (A) separation between *Entomophthoromycota* and other *Zygomycota*, (B) separation between the *Basidiobolus* lineage and all other *Entomophthoromycota*, and (C) separation between entomopathogenic *Entomophthoraceae* lineage (the *Batkoa*, *Entomophthora* and *Zoophthora* lineages) and the saprotrophic lineages of *Entomophthoromycota*. Abbreviations for genera of *Entomophthoromycota* are as follows: *B.* = *Basidiobolus*, *C.* = *Conidiobolus*, *E.* = *Entomophthora*, *En.* = *Entomophaga*, *Er.* – *Erynia*, *F.* = *Furia*, *P.* = *Pandora*, *Z.* = *Zoophthora*.

a morphologically uniform group whose three lineages only differ from one another in 6–13 of the 38 characters.

3.3. Nucleotide substitution rates and estimated divergence times

We modeled the putative age of the *Entomophthoromycota* based on two different possible ages of the *Glomeromycota*, a related group of fungi for which both fossils and molecular dating are available. The first model in which *Glomeromycota* arose 455–460 MYA (Redecker et al., 2000), suggests that *Entomophthoromycota* arose ca. 405 ± 90 MYA in the early Devonian period and that the ancestor of *Glomeromycota* and *Entomophthoromycota* lived ca. 483 MYA (with 95% highest posterior density [HPD] 460–530 MYA). Under this scenario, the ancestor of the primarily

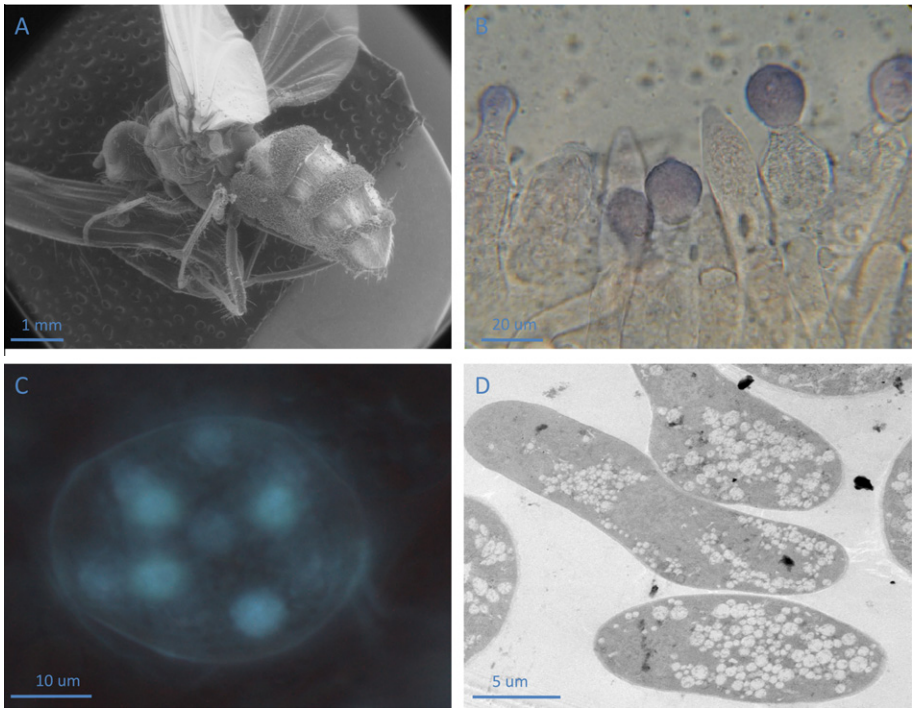


Fig. 4. Microscopy of *Entomophthora muscae*, the type taxon for *Entomophthoromycota*. Infected fly with an abdomen that is partially covered with fungal mycelium, SEM (A). Unbranched conidiophores with conidia, light microscopy (B). Plurinucleate conidium, fluorescence microscopy (C). Fungal hyphal bodies within the fly abdomen, TEM (D).

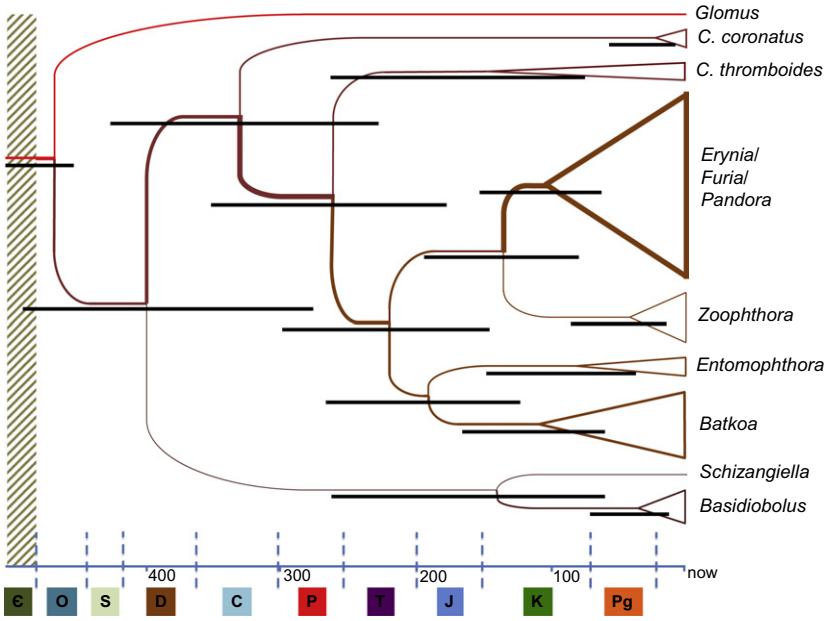


Fig. 5. Estimated divergence time calibrated based on the known fossil date for the outgroup lineage *Glomeromycota* (Redecker et al., 2000). Shaded gray area indicates the major radiation of arthropods during the Cambrian period (Conway-Morris, 2003). Variable thicknesses of the branches indicate substitution rates from 0.0007 (thinnest branches) to 0.005 (thickest branches) substitutions per million years. Є – Cambrian, O – Ordovician, S – Silurian, D – Devonian, C – Carboniferous, P – Permian, T – Triassic, J – Jurassic, K – Cretaceous, Pg – Paleogene.

insect-associated lineages in *Entomophthoraceae* (e.g. the *Entomophthora*, *Zoophthora* and *Batkoa* lineages) appeared ca. 225 ± 75 MYA years ago (Fig. 5).

The second model in which *Glomeromycota* arose 760 MYA (Berbee and Taylor, 2010), suggests that all of the tree nodes shift back to a time before the appearance of land arthropods (ICS, 2004). Under this scenario, the estimated origin of *Entomophthoromycota* was ca. 450 ± 150 MYA and that of the *Glomeromycota-Entomophthoromycota* ancestor was 721 MYA (with 95% HPD 565–870 MYA). The primarily insect-associated lineages in *Entomophthoraceae* were estimated to have originated 387 ± 67 MYA (Fig. S2).

Our attempt to utilize the substitution rates estimated by Berbee and Taylor (2010) for tree calibration (rather than using the time of divergence alone) provided inconsistent results. In particular, the posterior means of the substitution rates did not fit the

Table 2
Absolute substitution rates for each of three genes (A) calculated based on estimated divergence time of the outgroup *Glomeromycota* lineage (ca. 460 MYA, Redecker et al., 2000) and relative substitution rates of different *Entomophthoromycota* lineages (B) calculated relative to the rates for the *Basidiobolus* lineage.

Gene	A. Absolute substitution rates				B. Relative substitution rates			
	Total substutions per million years	*Basidiobolus*	*C. coronatus*	*C. thromboides*	*Batkoa*	*Entomophthora*	*Zoophthora*	Outgroup
rDNA	0.0003	1	0.6004	3.2843	4.9982	1.3901	1.9348	1.1085
mtSSU	0.0005	1	0.7260	1.2126	0.4384	0.9273	4.0915	2.1629
RPB2	0.0020	1	0.7358	1.0447	0.9479	1.0094	1.3776	0.9960

prior boundaries. To identify the cause(s) of this incongruence, we studied relative rates of nucleotide substitution among the different partitions and lineages and identified six groups that differed significantly from each other in their substitution rates: *Basidiobolus*, *Conidiobolus coronatus*, *C. thromboides*, and the three *Entomophthoraceae* lineages (*Entomophthora*, *Batkoa* and *Zoophthora*). Relative substitution rates varied by an order of magnitude among these lineages, ranging from the relatively slow substitution rate in *Batkoa* mtSSU (0.4384 nucleotide substitutions per million years) to the rapid rate in *C. coronatus* rDNA (4.9982). Absolute substitution rates for different genes also varied from 0.003 (rDNA) to 0.020 (*RPB2*) nucleotide substitutions per million years (Table 2). The substitution rates also change along each branch on the phylogenetic tree for each lineage (Fig. 5).

3.4. Ancestral state reconstruction

With a multi-gene phylogenetic tree and a distribution in the terminal taxa of 38 character states (see Section 2.6) we were able to confidently reconstruct the ancestral character state for half of them (Table 1). The major characteristic morphological traits were already present with high probability in the ancestor of all *Entomophthoromycota*. Some of these characters (e.g. forcibly discharged round, smooth conidia) are unique to the *Entomophthoromycota*. Other characters (e.g. sexual reproduction via gametangiogamy and the production of zygospores) are also present in other zygomycete fungi. Based on our analysis, taxa in the *Conidiobolus* lineage share the most features in common with the common ancestor of the *Entomophthoromycota*; this group shared all of the successfully reconstructed characters with the probable ancestor (18 out of 38). In contrast, the *Batkoa* clade shared 13 characters, the *Entomophthora* and *Zoophthora* shared 11 characters, and the *Basidiobolus* lineage shared only seven characters with the probably ancestor.

4. Discussion

Previous phylogenetic analyses of basal fungi using a limited number of genes or minimal taxon sampling have found it difficult to reconstruct the evolutionary history of the *Zygomycota* s.l. (Fig. 1 and references therein). Many species of *Entomophthoromycota* have cryptic lifestyles and unusual life histories, are challenging to obtain in axenic culture, and display wide morphological diversity, making this group of fungi difficult to study. However, our multi-gene phylogeny provides the first unequivocal support for the monophyly of *Entomophthoromycota*, indicating that this is a unique, non-aquatic fungal lineage of *Zygomycota* s.l. that is not closely related to any of the flagellated fungi (*Chytridiomycota* and *Blastocladiomycota*).

According to our results, the *Entomophthoromycota* is a monophyletic group that includes both primarily saprotrophic fungi (e.g. the *Basidiobolus* and *Conidiobolus* lineages) as well as a wide diversity of entomopathogenic fungi in the *Entomophthoraceae* (*Entomophthora*, *Zoophthora* and *Batkoa* lineages). These three main groups (the *Basidiobolus* lineage, the *Conidiobolus* lineage, and the three lineages of *Entomophthoraceae*) were also recovered in our parsimony-based analysis of morphological, ultrastructural and trophic traits. Although the non-molecular analysis lacks the precision of molecular approaches, the congruent patterns in both molecular and non-molecular analyses further increases the confidence in these evolutionary patterns. The multi-gene phylogeny and the constraint-based rDNA phylogeny were also congruent in identifying the same major groups of *Entomophthoromycota* that were previously established in the classical taxonomy of this group (Figs. 2 and 3; Humber, 1984, 1989). Based on the results of both molecular and morphological analyses we concur with Humber (in press) that the *Entomophthoromycota* deserves recognition at the phylum level.

Although our study resolves many of the previously circumscribed lineages within *Entomophthoromycota*, some relationships are still unclear. For example, analyses using different genes, different phylogenetic methods, and different taxa provided inconsistent results about whether the *Entomophthora* lineage is more closely related to the *Zoophthora* lineage or the *Batkoa* lineage (Figs. 2 and 3). The molecular analyses also revealed significant taxonomic problems at the genus and lineage levels, such as the polyphyly of some genera (e.g., *Erynia*, *Furia*, and *Pandora*). Perhaps most striking is the problematic *Conidiobolus* lineage, which is divided into several different groups of species (Ben-Ze'ev and Kenneth, 1982; Humber, 1984). Although *Conidiobolus* is a relatively uniform genus united by similarities in micromorphology and ultrastructure, our molecular analyses indicate that this genus is a polyphyletic assemblage. Moreover, species of the nematode- and tardigrade-parasitic genus *Macrobiotophthora* belong to the *C. thromboides* clade in *Conidiobolus* lineage (Jensen et al., 1998; White et al., 2006, Fig. 3). Together, these results indicate that more taxonomic and phylogenetic research is needed to revise the genus *Conidiobolus* and to clarify relationships among the genera in *Entomophthoraceae.*

Despite these problems, molecular analysis assigned several taxa of uncertain taxonomic position to well-established evolutionary lineages (Figs. 2 and 3). For example, *Schizangiella serpentis* nom. prov., an enigmatic snake pathogen with yeast-like rather than hyphal growth (Ippen, 1980; Kaplan et al., 1983; Dwyer et al., 2006) falls within the *Basidiobolus* lineage. Despite unique morphological features, *Massospora cicadina* is actually closely related to a complex of *Entomophthora* species whereas the morphologically divergent *Strongwellsea castrans* groups with several *Pandora* species. These relationships have also been suggested previously based on traditional character analyses (Humber, 1982, 1984, 1989, in press).

Although previous phylogenetic studies have produced inconsistent results regarding the evolutionary history of *Entomophthoromycota* (Fig. 1), our well-resolved multi-gene phylogeny allowed us to examine patterns and rates of evolution in the group and gain insights on the fungi that may be the closest relatives of *Entomophthoromycota*. Our analysis suggests that phylogenetic difficulties in previous studies of *Entomophthoromycota* may have been due to the widely divergent DNA substitution rates in different genes

and lineages (Table 2). For example, the ribosomal DNA of some groups (e.g. *C. coronatus* and members of the *Batkoa* lineage) is highly distinctive and appears to have changed rapidly compared with other lineages. Despite these difficulties due to varying evolutionary rates, our multi-gene phylogeny (including representatives of *Zygomycota*, *Chytridiomycota,* and *Blastocladiomycota*) places *Entomophthoromycota* with strong statistical support close to but distinctly separated from species in the *Kickxellomycotina* and *Zoopagomycotina* (Fig. 2). It is interesting to note that some species in these latter two subphyla are parasitic on insects (*Kickxellomycotina*) or on amoeba, rotifers, and other microscopic animals and fungi (*Zoopagomycotina*) (Dayal, 1975; Lichtwardt, 1986). In contrast, species in the *Chytridiomycota* and *Blastocladiomycota* as well as the primarily saprotrophic *Mucoromycotina* are resolved as distinct from the *Entomophthoromycota*, *Kickxellomycotina,* and *Zoopagomycotina*. Similar results were obtained with rDNA and the dataset with 159 taxa of *Zygomycota* s.l. (Fig. S1), but without statistical support.

Similar to the difficulties in phylogenetic reconstructions from previous studies, there have also been difficulties in estimating the time of evolutionary divergence and radiation events among basal fungi (Berbee and Taylor, 2001, 2010). Reconstruction of the divergence time for *Entomophthoromycota* remains speculative because fossils suitable for calibration purposes have not been available. To date, only two *Entomophthoromycota* fossils from Dominican amber have been thoroughly described (*Entomophthora*- and *Conidiobolus*-like infection patterns on insects) (Poinar and Thomas, 1982; Poinar and Poinar, 1999). However, the relatively young estimated ages of these fossils (ca. 26–20 MYA) make them far too recent to be useful for phylogenetic calibrations. Another fossil of an *Entomophthora*-like fungus, *Traquairia,* was recently documented from Paleozoic sediments (542–251 MYA) (Krings et al., 2011). However, the exact taxonomic position of this fossil has yet to be clarified and there is not yet a reliable date for this fossil so we opted not to use this fossil in our calibration.

Our dating analyses produced highly variable results but suggest that the ancestor of all *Entomophthoromycota* probably arose between 300 and 600 MYA (Fig. 5). This implies that the ancestor of *Entomophthoromycota* probably existed at the same time as the first arthropods (Budd and Telford, 2009). Most extant species of *Entomophthoromycota* are clearly associated with arthropods. For example, almost all species of *Entomophthoraceae* are obligate insect pathogens whereas several *Conidiobolus* species are facultative pathogens of arthropods but retain some saprotrophic capabilities (Koval, 2007). Although the biology of the *Basidiobolus* lineage is not well characterized, it seems likely that the frogs and reptiles whose guts are colonized by *Basidiobolus* species probably acquire the fungus after ingesting superficially infested arthropods (Manning et al., 2007). Species of *Entomophthoromycota* have certainly benefited from the success and evolutionary radiation of insects and it is possible that the success of arthropods has led to co-divergence of the fungi. However, there is currently only scant evidence for the coevolution of entomopathogenic entomophthoroid genera with their hosts (with the possible exceptions of *Strongwellsea* and *Massospora*; see Humber, 1984, 2008).

In addition to helping elucidate the timing of evolutionary radiations in the *Entomophthoromycota*, the well-resolved phylogeny presented here was also useful for inferring more about the ancestors of this fungal group. Ancestral state reconstruction in combination with the topology of our multi-gene phylogeny suggests that the ancestor of the *Entomophthoromycota* was most morphologically similar to species in the *Conidiobolus* lineage. The reconstructed ancestor shared many features in common with *Conidiobolus* species, including round conidia with a smooth surface, forcible conidia discharge, production of hyphal bodies, and coenocytic (non-septate) mycelium (Table 1). Representatives of the genus *Conidiobolus* also possess all types of secondary conidia known for *Entomophthoromycota*. In contrast, the reconstructed ancestor had fewer traits in common with the earliest diverging *Basidiobolus* lineage or the later diverging *Entomophthoraceae* (*Entomophthora*, *Zoophthora* and *Batkoa* lineages) (Table 1). These results suggest that the ancestors of *Entomophthoromycota* probably evolved as decomposers or weak, facultative pathogens.

Although some species within the early diverging *Conidiobolus* and *Basidiobolus* lineages infect insects (e.g., *Conidiobolus thromboides* on aphids and moths; Hatting et al., 1999; Koval, 2007), most species in these two genera are not dependent on insects to complete their lifecycles (Drechsler, 1956; Humber, 1989). In contrast to species in the *Entomophthora*, *Zoophthora* and *Batkoa* lineages, *Conidiobolus* and *Basidiobolus* species are primarily saprotrophic and can be readily isolated from soil. They are also easily maintained in pure culture on diverse types of nutritive media. Species in the *Basidiobolus* and *Conidiobolus* lineages also may occasionally (but not routinely) act as facultative pathogens of a wide diversity of insects and vertebrates, including humans. Infections by species in these groups (often referred to as basidiobolomycoses or conidiobolomycoses, respectively) are problematic for humans with compromised immune systems throughout the tropical and subtropical regions of the world (Grooters, 2003; James et al., 2006; Prabhu and Patel, 2004). Despite these ecological similarities between members of the *Basidiobolus* and *Conidiobolus* lineages, the *Basidiobolus* lineage represents the earliest evolutionary split on our phylogenetic tree (Figs. 2 and 3) and it is morphologically unique compared to other *Entomophthoromycota*. Our analysis indicates that the *Basidiobolus* lineage shares relatively few characters with the rest of the *Entomophthoromycota* and is also divergent from the reconstructed ancestor of the *Entomophthoromycota* (Table 1), and these differences have been recognized by treating *Basidiobolus* and its relatives in a new class, *Basidiobolomycetes*, within the *Entomophthoromycota* (Humber, in press).

Most species of *Entomophthoromycota* maintain at least some facultative ability to parasitize different animal groups and indeed all species of *Entomophthoraceae* (*Entomophthora*, *Zoophthora* and *Batkoa* lineages) are insect-pathogenic and depend on insect hosts to complete their lifecycles. Members of the *Entomophthoraceae* regularly cause mass infections of diverse arthropod groups (including species known from most orders and families of insects as well as from mites and some spiders) making them important potential biocontrol fungi (Papierok and Hajek, 1997; Steinkraus et al., 2002; Wilding, 1981). Species in the *Entomophthoraceae* clade only grow in association with arthropods, and many are challenging to maintain in axenic laboratory cultures.

5. Conclusion

Our phylogenetic analyses provide the first strong evidence that the newly erected phylum *Entomophthoromycota* is monophyletic and clearly separated from all other *Zygomycota* s.l. Although the exact relationship of *Entomophthoromycota* to other fungal groups needs further study, the probable sister lineages of this group are the *Kickxellomycotina* and *Zoopagomycotina* rather than fungi in *Mucoromycotina*, *Chytridiomycota* or *Blastocladiomycota*. Within the *Entomophthoromycota*, we detected five major lineages: the monophyletic *Basidiobolus* and paraphyletic *Conidiobolus* lineages are saprotrophs and facultative parasites of arthropods and other animals. In contrast, the three most recently diverged lineages (*Batkoa*, *Entomophthora* and *Zoophthora*) constitute a monophyletic family *Entomophthoraceae* that includes only obligate pathogens of insects and arthropods. A parsimony analysis of morphological, physiological, and ultrastructural characters independently reconstructed the same major lineages as the molecular phylogeny and

this reconstruction agrees with general relationships inferred for these fungi from more traditional, non-molecular analyses (Humber, 1984, 1989, in press). Taken together, it seems likely that these inferred relationships reflect the historical patterns of evolutionary divergence within *Entomophthoromycota*. Ancestral trait reconstruction suggests that the ancestor of *Entomophthoromycota* was probably a saprotroph or facultative parasite with morphological characteristics resembling those of extant species of *Conidiobolus*. It was challenging to use molecular clock approaches to determine the time of origin for *Entomophthoromycota* because there are few appropriate fossils for calibration and because there is notable rate hetereogeneity between different genes and between different lineages of *Entomophthoromycota*. Despite these complications, our analysis suggests that the ancestor of *Entomophthoromycota* arose 405 ± 90 MYA and that the insect-pathogenic *Entomophthoraceae* (the *Batkoa*, *Entomophthora* and *Zoophthora* lineages) arose 225 ± 75 MYA.

Acknowledgments

We sincerely thank Blanka Shaw for assistance with the non-molecular database, Katalin Molnar and Bernie Ball with PCR and reagents, Carl Rothfels with PAML4, Nimrod Rubinstein with substitution rates analysis, and Michelle Gignac performed SEM and TEM. We are grateful to Meredith Blackwell, Gregory Bonito, Jason Jackson, Anastasia Litvintseva, François Lutzoni, Marianela Rodrigues-Carres, Sujal Phadke, Elise Van Buskirk, David Swofford and Anne Yoder for essential discussions, Fred Nijhout for insect identification, Tim James and Yan Wang for providing sequences, Nina Gurinovich and Scarlett Geunes-Boyer for manuscript editing.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.07.026.

References

Ben-Ze'ev, I., Kenneth, R.G., 1982. Features-criteria of taxonomic value in the *Entomophthorales*. I. A revision of the batkoan classification. Mycotaxon 11, 393–455.

Berbee, M.L., Taylor, J.W., 2001. Fungal molecular evolution: gene trees and geologic time. In: McLaughlin, D., McLaughlin, E., Lemke, P. (Eds.), The *Mycota*. Springer-Verlag, Berlin, pp. 229–245.

Berbee, M.L., Taylor, J.W., 2010. Dating the molecular clock in fungi – how close are we? Fung. Biol. Rev. 24, 1–16.

Budd, G.E., Telford, M.J., 2009. The origin and evolution of arthropods. Nature 457, 812–817.

Conway-Morris, S., 2003. The Cambrian "explosion" of metazoans and molecular biology: would Darwin be satisfied? Int. J. Dev. Biol. 47, 505–515.

Dayal, R., 1975. Key to Phycomycetes predaceous or parasitic on nematodes or amoebae. I. Zoopagales. Sydowia 27, 293–301.

Drechsler, C., 1956. Supplementary development of *Basidiobolus ranarum* and *Basidiobolus haptosporus*. Mycologia 48, 655–676.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, e214. http://dx.doi.org/10.1186/1471-2148-7-214.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88.

Dwyer, J., Burwell, B., Humber, R.A., McLeod, C., Fleetwood, M., Johnson, T., 2006. *Schizangiella serpentis* infection in a Virginia ratsnake (*Elaphe obsoleta*) [abstract]. Vet. Pathol. 43, 819.

Ebersberger, I., de Matos Simoes, R., Kupczok, A., Gube, M., Kothe, E., Voigt, K., von Haesler, A., 2011. A consistent phylogenetic backbone for the fungi. Mol. Biol. Evol.. http://dx.doi.org/10.1093/molbev/msr285.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Einax, E., Voigt, K., 2003. Oligonucleotide primers for the universal amplification of β-tubulin genes facilitate phylogenetic analyses in the regnum *Fungi*. Org. Divers. Evol. 3, 185–194.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.

Gargas, A., Taylor, J.W., 1992. Polymerase chain reaction (PCR) primers for amplifying and sequencing 18S rDNA from lichenized fungi. Mycologia 84, 589–592.

Grooters, A., 2003. Pythiosis, lagenidiosis, and zygomycosis in small animals. Vet. Clin. North Am. Smalal Anim. Pract. 33, 695–720.

Gryganskyi, A.P., Lee, S.C., Litvintseva, A.P., Smith, M.E., Bonito, G., Porter, T.M., Anishchenko, I.M., Heitman, J., Vilgalys, R., 2010. Structure, function, and phylogeny of the mating locus in the *Rhizopus oryzae* complex. PLoS ONE 5, e15273.

Hatting, J.L., Humber, R.A., Poprawski, T.J., Miller, R.M., 1999. A survey of fungal pathogens of aphids from South Africa, with special reference to cereal aphids. Biol. Control 16, 1–12.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lucking, R., Thorsten, L.H., Lutzoni, F., Matheny, P.B., McLaughlin, D.J., Powell, M.J., Redhead, S., Schoch, C.L., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Koljalg, U., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J., Miadlikowska, J., Miller, A., Moncalvo, J.M., Mozley-Standridge, S., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schussler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.J., Zhang, N., 2007. A higher-level phylogenetic classification of the fungi. Mycol. Res. 111, 509–547.

Hoffmann, K., Voigt, K., Kirk, P.M., 2011. *Mortierellomycotina* subphyl. nov., based on multi-gene genealogies. Mycotaxon 115, 353–363.

Humber, R.A., 1981. An alternative view of certain taxonomic criteria used in the *Entomophthorales*. Mycotaxon 13, 191–240.

Humber, R.A., 1982. *Strongwellsea* vs. *Erynia*: the case for a phylogenetic classification of the *Entomophthorales* (*Zygomycetes*). Mycotaxon 15, 167–184.

Humber, R.A., 1984. Foundation to an evolutionary classification of the *Entomophthorales* (*Zygomycetes*). In: Wheeler, Q., Blackwell, M. (Eds.), Fungus-Insect Relationships. Columbia Press, New York, pp. 144–161.

Humber, R.A., 1989. Synopsis of a revised classification for the *Entomophthorales* (*Zygomycotina*). Mycotaxon 34, 441–460.

Humber, R.A., 2008. Evolution of entomopathogenicity in fungi. J. Invertebr. Pathol. 98, 262–266.

Humber, R.A., in press. *Entomophthoromycota*: a new phylum and reclassification for entomophthoroid fungi. Mycotaxon.

ICS (International Commission of Stratigraphy), 2004. <http://www.stratigraphy.org> (accessed 06.12).

Ippen, R., 1980. Ein Beitrag zu den Mykosen der Schlangen. Milu (Berlin) 5, 386–396.

Jackson, V., 2004. LASRDisc: Likelihood Ancestral State Reconstruction for Discrete Characters, v. 1.01. <http://ceb.csit.fsu.edu/lasrdisc> (accessed 06.12).

James, T., Porter, D., Leander, C.A., Vilgalys, R., Longcore, J.E., 2000. Molecular phylogenetics of the *Chytridiomycota* supports the utility of ultrastructural data in chytrid systematics. Can. J. Bot. 78, 226–350.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.-H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schüßler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lücking, R., Büdel, B., Geiser, D.M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W., Vilgalys, R., 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443, 818–822.

Jensen, A.B., Gargas, A., Eilenberg, J., Rosendahl, S., 1998. Relationships of the insect-pathogenic order *Entomophthorales* (*Zygomycota*, *Fungi*) based on phylogenetic analyses of nuclear small subunit ribosomal DNA sequences (SSU rDNA). Fung. Genet. Biol. 24, 325–334.

Kaplan, W., Chandler, F.W., Padhye, A.A., Hamm Jr., T.E., 1983. A zygomycotic infection in captive snakes. Sabouraudia 21, 85–91.

Kauff, F., Lutzoni, F., 2002. Phylogeny of the *Gyalectales* and *Ostropales* (*Ascomycota*, *Fungi*): among and within order relationships based on nuclear ribosomal RNA small and large subunits. Mol. Phylogenet. Evol. 25, 138–156.

Keeling, P.J., 2003. Congruent evidence from α-tubulin and β-tubulin gene phylogenies for a zygomycete origin of microsporidia. Fung. Genet. Biol. 38, 298–309.

Kirk, P.M., Cannon, P.F., David, J.C., Stalpers, J.A., 2001. Ainsworth & Bisby's Dictionary of the Fungi, ninth ed. CABI Publishing, Wallingford.

Koval, E.Z., 2007. Zygomycetes. Entomophthoralean Fungi. M.G. Kholodny Institute of Botany NAS of Ukraine, Kyiv, Ukraine (in Russian).

Krings, M., Taylor, T.N., White Jr., J.F., 2011. Fungal sporocarps from the Carboniferous: an unusual specimen of *Traquairia*. Rev. Palaeobot. Palynol. 168, 1–6.

Lichtwardt, R.W., 1986. The *Trichomycetes*, Fungal Associates of Arthropods. Springer-Verlag, New York, 343pp.

Liu, X., Voigt, K., 2010. Molecular characters of zygomycetous fungi. In: Gherbawy, Y., Voigt, K. (Eds.), Molecular Identification of Fungi. Springer, Heidelberg, pp. 461–488.

Liu, Y.J., Whelen, S., Hall, B.D., 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol. Biol. Evol. 16, 1799–1808.
Maddison, W.P., Maddison, D.R., 2009. Mesquite: A Modular System for Evolutionary Analysis. Version 2.72 <http://mesquiteproject.org> (accessed 06.12).
Manning, R.J., Waters, S.D., Callaghan, A.A., 2007. Saprotrophy of *Conidiobolus* and *Basidiobolus* in leaf litter. Mycol. Res. 111, 1437–1449.
Mason-Gamer, R.J., Kellogg, E.A., 1996. Testing for phylogenetic conflict among molecular data sets in the Triticeae (Gramineae). Syst. Biol. 45, 524–545.
McCabe, D.E., Humber, R.A., Soper, R.S., 1984. Observation and interpretation of nuclear reductions during maturation and germination of entomophthoralean resting spores. Mycologia 76, 1104–1107.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA, pp. 1–8.
Moreau, F., 1954. Les Champignons. Physiologie, Morphologie, Dévelopment et Systématique. Lechevalier, Paris.
Müller, K., Quandt, D., Müller, J., Neinhuis, C., 2010. PhyDE® 0.9971: Phylogenetic Data Editor. www.phyde.de (accessed 06.12).
Nagahama, T., Sato, H., Shimazu, M., Sugiyama, J., 1995. Phylogenetic divergence of the entomophthoralean fungi: evidence from nuclear 18S ribosomal RNA gene sequences. Mycologia 87, 203–209.
O'Donnell, K., Lutzoni, F.M., Ward, T.J., Benny, G.L., 2001. Evolutionary relationships among mucoralean fungi (*Zygomycota*): evidence for family polyphyly on a large scale. Mycologia 93, 286–296.
Pagel, M., 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst. Biol. 48, 612–622.
Papierok, B., Hajek, A.E., 1997. Fungi: *Entomophthorales*. In: Lacey, L. (Ed.), Manual of Techniques in Insect Pathology. Academic Press, London, pp. 187–212.
Pfitzer, E., 1872. *Ancylistes Closterii,* ein neuer Algen-Parasit aus der Ornung der Phycomyceten. Monatsber. K. Akad. Wiss, Berlin, pp. 379–398.
Poinar Jr., G.O., Poinar, R., 1999. The Amber Forest. Princeton University Press, Princeton, NJ.
Poinar Jr., G.O., Thomas, G.M., 1982. An entomophthoralean fungus from Dominican amber. Mycologia 74, 332–334.
Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Prabhu, R.M., Patel, R., 2004. Mucormycosis and entomophthoramycosis: a review of the clinical manifestations, diagnosis and treatment. Clin. Microbiol. Infect. 10, 31–47.
Rambaut, A., Drummond, A., 2008. Tracer v 1.4.1. Software <http://beast.bio.ed.ac.uk> (accessed 06.12).
Redecker, D., Kodner, R., Graham, R., Graham, L.E., 2000. Glomalean fungi from the Ordovician. Science 289, 1920–1921.
Rehner, S.A., Samuels, G.J., 1994. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycol. Res. 98, 625–634.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Sekimoto, S., Rochon, D.A., Long, J.E., Dee, J.M., Berbee, M.L., 2011. A multigene phylogeny of *Olpidium* and its implications for early fungal evolution. BMC Evol. Biol. 11, 331.
Sharma, N.L., Mahajan, V.K., Singh, P., 2003. Orofacial conidiobolomycosis due to *Conidiobolus incongruous*. Mycoses 46, 137–140.
Simon, L., Bousquet, J., Levesque, R.C., Lalonde, M., 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363, 67–69.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690, <http://sco.h-its.org/exelixis/oldPage/RAxML-Manual.7.0.4.pdf> (accessed 06.12).
Stamatakis, A., Hoover, P., Rougemont, J., 2008. A fast bootstrapping algorithm for the RAxML Web-Servers. Syst. Biol. 57, 758–771.
Steinkraus, D.C., Boys, G.O., Rosenheim, J.A., 2002. Classical biological control of *Aphis gossypii* (*Homoptera*: *Aphidiae*) with *Neozygites frezenii* (*Entomophthorales*: *Neozygitaceae*) in California cotton. Biol. Control 25, 297–304.
Swofford, D.L., 2002. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland.
Tanabe, Y., O'Donnell, K., Saikawa, M., Sugiyama, J., 2000. Molecular phylogeny of parasitic *Zygomycota* (*Dimargaritales*, *Zoopagales*) based on nuclear small subunit ribosomal DNA sequences. Mol. Phylogenet. Evol. 16, 253–262.
Tanabe, Y., Saikawa, M., Watanabe, M.M., Sugiyama, J., 2004. Molecular phylogeny of *Zygomycota* based on EF-1alpha and RPB1 sequences: limitations and utility of alternative markers to rDNA. Mol. Phylogenet. Evol. 30, 438–449.
Tucker, B.E., 1984. Aspects of the Biology and Ultrastructure of the Nematode Destroying Fungus *Macrobiotophthora vermicola* (*Zygomycetes*: *Entomophthorales*). Ph.D. Thesis, University of Washington, Seattle.
Vilgalys, R., Hester, M., 1990. Rapid genetic identification and mapping enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172, 4238–4246.
Voigt, K., Kirk, P., 2011. Recent developments in the taxonomic affiliation and phylogenetic positioning of fungi: impact in applied microbiology and environmental biotechnology. Appl. Microbiol. Biotechnol. 90, 41–57.
White, M.M., James, T.Y., O'Donnell, K., Cafaro, M.J., Tanabe, Y., Sugiyama, J., 2006. Phylogeny of the *Zygomycota* based on nuclear ribosomal sequence data. Mycologia 98, 872–884.
Wilding, N., 1981. Pest control by *Entomophthorales*. In: Burges, H.D. (Ed.), Microbial Control of Pest and Plant Diseases 1970–1980. Academic Press, London, pp. 539–554.
Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2004. AWTY: A System for Graphical Exploration of MCMC Convergence in Bayesian Phylogenetic Inference. <ceb.csit.fsu.edu/awty/> (accessed 11.11).
Yang, Z., 1994. Estimating the pattern of nucleotide substitution. J. Mol. Evol. 39, 105–111.
Yang, Z., 2007. PAML 4: phylogenetic analysis by maximum likelihood. Mol. Biol. Evol. 24, 1586–1591.
Zoller, S., Scheidegger, C., Sperisen, C., 1999. PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming *Ascomycetes*. Lichenologist 31, 511–516.
Zwickl, D.J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. Ph.D. Dissertation, The University of Texas, Austin.

Further reading

Celio, G.J., Padamsee, M., Dentinger, B.T., McLaughin, D.J., 2006. Assembling the Fungal Tree of Life: constructing the structural and biochemical database. Mycologia 98, 850–859.
Schuessler, A., Schwarzott, D., Walker, C., 2001. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. Mycol. Res. 105, 1413–1421.
Voigt, K., Wöstemeyer, J., 2001. Phylogeny and origin of 82 zygomycetes from all 54 genera of the *Mucorales* and *Mortierellales* based on combined analysis of actin and translation elongation factor EF-1[alpha] genes. Gene 270, 113–120.

MYCOLOGICAL RESEARCH 111 (2007) 509–547

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/mycres

A higher-level phylogenetic classification of the *Fungi*

David S. HIBBETT[a,*], *Manfred BINDER*[a], *Joseph F. BISCHOFF*[b], *Meredith BLACKWELL*[c], *Paul F. CANNON*[d], *Ove E. ERIKSSON*[e], *Sabine HUHNDORF*[f], *Timothy JAMES*[g], *Paul M. KIRK*[d], *Robert LÜCKING*[f], *H. THORSTEN LUMBSCH*[f], *François LUTZONI*[g], *P. Brandon MATHENY*[a], *David J. MCLAUGHLIN*[h], *Martha J. POWELL*[i], *Scott REDHEAD*[j], *Conrad L. SCHOCH*[k], *Joseph W. SPATAFORA*[k], *Joost A. STALPERS*[l], *Rytas VILGALYS*[g], *M. Catherine AIME*[m], *André APTROOT*[n], *Robert BAUER*[o], *Dominik BEGEROW*[p], *Gerald L. BENNY*[q], *Lisa A. CASTLEBURY*[m], *Pedro W. CROUS*[l], *Yu-Cheng DAI*[r], *Walter GAMS*[l], *David M. GEISER*[s], *Gareth W. GRIFFITH*[t], *Cécile GUEIDAN*[g], *David L. HAWKSWORTH*[u], *Geir HESTMARK*[v], *Kentaro HOSAKA*[w], *Richard A. HUMBER*[x], *Kevin D. HYDE*[y], *Joseph E. IRONSIDE*[t], *Urmas KÕLJALG*[z], *Cletus P. KURTZMAN*[aa], *Karl-Henrik LARSSON*[ab], *Robert LICHTWARDT*[ac], *Joyce LONGCORE*[ad], *Jolanta MIĄDLIKOWSKA*[g], *Andrew MILLER*[ae], *Jean-Marc MONCALVO*[af], *Sharon MOZLEY-STANDRIDGE*[ag], *Franz OBERWINKLER*[o], *Erast PARMASTO*[ah], *Valérie REEB*[g], *Jack D. ROGERS*[ai], *Claude ROUX*[aj], *Leif RYVARDEN*[ak], *José Paulo SAMPAIO*[al], *Arthur SCHÜßLER*[am], *Junta SUGIYAMA*[an], *R. Greg THORN*[ao], *Leif TIBELL*[ap], *Wendy A. UNTEREINER*[aq], *Christopher WALKER*[ar], *Zheng WANG*[a], *Alex WEIR*[as], *Michael WEISS*[o], *Merlin M. WHITE*[at], *Katarina WINKA*[e], *Yi-Jian YAO*[au], *Ning ZHANG*[av]

[a]*Biology Department, Clark University, Worcester, MA 01610, USA*
[b]*National Library of Medicine, National Center for Biotechnology Information, 45 Center Drive, Bethesda, MD 20892-6510, USA*
[c]*Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA*
[d]*CABI UK, Bakeham Lane, Egham, Surrey TW20 9TY, UK*
[e]*Department of Ecology and Environmental Science, Umeå University, SE-901 87 Umeå, Sweden*
[f]*Department of Botany, The Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605-2496, USA*
[g]*Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA*
[h]*Department of Plant Biology, University of Minnesota, 1445 Gortner Avenue, St Paul, MN 55108-1095, USA*
[i]*Department of Biological Sciences, Box 870344/319 Biology, University of Alabama, Tuscaloosa, AL 35487-0344, USA*
[j]*ECORC, Agriculture and Agri-Food Canada, CEF, Neatby Building, Ottawa, ON K1A 0C6, Canada*
[k]*Department of Botany and Plant Pathology, Oregon State University, 2082 Cordley Hall, Corvallis, OR 97331, USA*
[l]*Centraalbureau voor Schimmelcultures, Uppsalalaan 8, NL-3584 CT Utrecht, The Netherlands*
[m]*USDA ARS Systematic Botany and Mycology Laboratory, Bldg 011A Rm 319 BARC-WEST, 10300 Baltimore Ave, Beltsville, MD 20705 USA*
[n]*ABL Herbarium, Gerrit van der Veenstraat 107, NL-3762 XK Soest, The Netherlands*
[o]*Botanical Institute, University of Tübingen, Auf der Morgenstelle 1, D-72076 Tübingen, Germany*
[p]*Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse, D-35043 Marburg, Germany*
[q]*Department of Plant Pathology, 1453 Fifield Hall, Hull Road, P.O. Box 110680, University of Florida, Gainesville FL 32611-0680, USA*
[r]*Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China*
[s]*Department of Plant Pathology, Pennsylvania State University, University Park, PA 16802, USA*
[t]*Institute of Biological Sciences, University of Wales, Aberystwyth SY23 3DA, UK*
[u]*Departamento de Biología Vegetale II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, Ciudad Universitaria, E-28040 Madrid, Spain*

* Corresponding author.
E-mail address: dhibbett@clarku.edu (D. S. Hibbett).

0953-7562/$ – see front matter
doi:10.1016/j.mycres.2007.03.004

[v]Department of Biology, University of Oslo, P.O. Box 1066, Blindern, N-0316 Oslo, Norway
[w]Department of Botany, The Field Museum, 1400 S Lake Shore Dr., Chicago, IL 60605, USA,
[x]USDA-ARS Plant Protection Research Unit, US Plant, Soil and Nutrition Laboratory, Tower Road, Ithaca, NY 14853-2901, USA
[y]Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity, University of Hong Kong, Pokfulam Road, Hong Kong SAR, China
[z]Institute of Botany and Ecology, Tartu University, 40 Lai Street, Tartu 51005, Estonia
[aa]Microbial Properties Research, NCAUR, ARS, USDA, 1815 N.University Street, Peoria, IL 61604-3999, USA
[ab]Göteborg University, Department of Plant and Environmental Sciences, P. O. Box 461, SE-405 30 Göteborg, Sweden
[ac]Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7534, USA
[ad]Department of Biological Sciences, University of Maine, 5722 Deering Hall, Orono, ME 04469-5722, USA
[ae]Center for Biodiversity, Illinois Natural History Survey, 1816 South Oak Street, Champaign, IL 61820-6970, USA
[af]Royal Ontario Museum, Centre for Biodiversity and Conservation Biology, 100 Queen's Park, Toronto, ON M5S 2C6, Canada
[ag]Division of Natural Sciences, Mathematics, and Engineering, Middle Georgia College, 1100 Second Street, SE, Cochran, GA 31014-1599, USA
[ah]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 181 Riia Street Tartu, 51014, Estonia
[ai]Department of Plant Pathology, Washington State University, P.O. Box 646430, Pullman, WA 99164-6430, USA
[aj]Chemin des Vignes vielles, 84120, Mirabeau, France
[ak]Botanical Institute, University of Oslo, P.O. Box 1066, Blindern, N-0316 Oslo, Norway
[al]CREM, SABT, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[am]Genetics Institute, Department Biology I, Ludwig-Maximilians-University, Maria-Ward-Strasse 1a, D-80638 Munich, Germany
[an]TechnoSuruga Co. Ltd, Tokyo Office, Ogawamachi Kita Building 4F, 1-8-3, Kanda Ogawamachi, Chiyoda-ku, Tokyo 101-0052, Japan
[ao]Department of Biology, University of Western Ontario, 213 Biological and Geological Sciences Building, 1151 Richmond Street North, London, ON, N6A 5B7, Canada
[ap]Department of Systematic Botany, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18 D, SE-752 36, Uppsala, Sweden
[aq]Botany Department, Brandon University, 270-18th Street, Brandon, MB R7A 6A9, Canada
[ar]Royal Botanic Garden Edinburgh, 20A Inverlieth Row, Edinburgh Eh3 5LR, UK
[as]Faculty of Environmental and Forest Biology, SUNY College, 242 Illick Hall, 1 Forestry Drive, Syracuse, NY 13210, USA
[at]Department of Biology, Boise State University, 1910 University Drive, S/N Building, Room 210, Boise, ID 83725-1515, USA
[au]Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, China
[av]Department of Plant Pathology, Cornell University, Geneva, NY 14456, USA

ARTICLE INFO

Article history:
Received 31 January 2007
Received in revised form
22 February 2007
Accepted 2 March 2007
Published online 13 March 2007
Corresponding Editor:
David L. Hawksworth

Keywords:
AFTOL
Eumycota
Lichens
Molecular phylogenetics
Mycota
Nomenclature
Systematics

ABSTRACT

A comprehensive phylogenetic classification of the kingdom *Fungi* is proposed, with reference to recent molecular phylogenetic analyses, and with input from diverse members of the fungal taxonomic community. The classification includes 195 taxa, down to the level of order, of which 16 are described or validated here: *Dikarya* subkingdom nov.; *Chytridiomycota*, *Neocallimastigomycota* phyla nov.; *Monoblepharidomycetes*, *Neocallimastigomycetes* class. nov.; *Eurotiomycetidae*, *Lecanoromycetidae*, *Mycocaliciomycetidae* subclass. nov.; *Acarosporales*, *Corticiales*, *Baeomycetales*, *Candelariales*, *Gloeophyllales*, *Melanosporales*, *Trechisporales*, *Umbilicariales* ords. nov. The clade containing *Ascomycota* and *Basidiomycota* is classified as subkingdom *Dikarya*, reflecting the putative synapomorphy of dikaryotic hyphae. The most dramatic shifts in the classification relative to previous works concern the groups that have traditionally been included in the *Chytridiomycota* and *Zygomycota*. The *Chytridiomycota* is retained in a restricted sense, with *Blastocladiomycota* and *Neocallimastigomycota* representing segregate phyla of flagellated *Fungi*. Taxa traditionally placed in *Zygomycota* are distributed among *Glomeromycota* and several subphyla *incertae sedis*, including *Mucoromycotina*, *Entomophthoromycotina*, *Kickxellomycotina*, and *Zoopagomycotina*. *Microsporidia* are included in the *Fungi*, but no further subdivision of the group is proposed. Several genera of 'basal' *Fungi* of uncertain position are not placed in any higher taxa, including *Basidiobolus*, *Caulochytrium*, *Olpidium*, and *Rozella*.

Introduction

The molecular revolution in fungal taxonomy commenced in the early 1990s, with analyses of PCR-amplified ribosomal RNA genes (White *et al.* 1990). Today, fungal molecular systematics is a mature discipline in which multi-locus datasets, extensive taxon sampling, and rigorous analytical approaches are standard. To gain an overview of the current state of the

science it is only necessary to survey the recent 'Deep Hypha' issue of *Mycologia* [2007 ('2006'); **98**], which contains 21 phylogenetic studies, all of which employ multiple genes to some extent (in some cases, multiple rRNA genes) and that address broad relationships in every major group of *Fungi* (except *Microsporidia*). Another recent milestone is the kingdom-level study of James *et al.* (2006), which used a dataset of six genes (nu-SSU, -LSU, and 5.8S rRNA, *rpb1*, *rpb2* and *tef1*) sampled in nearly 200 species from every major clade of *Fungi* (including *Microsporidia*).

As the broad outlines of fungal phylogeny have come into focus, there have been repeated attempts to summarize the state of knowledge and to restructure higher-level classifications. Two important works that have influenced fungal taxonomy in the 21st century are *Ainsworth & Bisby's Dictionary of the Fungi* (9th edn: Kirk *et al.* 2001), which contains a comprehensive kingdom-wide classification down to the level of genus, and *The Mycota VII* (McLaughlin *et al.* 2001a, 2001b), an edited volume with chapters on all major groups of *Fungi*. These publications represented major advances toward a phylogenetic classification of *Fungi*, but they are already out of date. In the five years since the last edition of the *Dictionary* and the *Mycota VII* appeared, more than 360 articles with the keyword 'phylogen*' were published in *Mycologia* and *Mycological Research* alone, and approximately 80 % of the more than 100 000 fungal rRNA gene sequences now in GenBank were deposited (some by molecular ecologists). Recent publications that survey the entire fungal kingdom based on molecular phylogenies include the chapter by Taylor *et al.* (2004) in *Assembling the Tree of Life* (Cracraft & Donoghue 2004), the 'New Higher Level Classification of Eukaryotes' (Adl *et al.* 2005), and the first large collaborative analysis of the Assembling the Fungal Tree of Life (AFTOL) project (Lutzoni *et al.* 2004). Taxonomic studies on individual groups of *Fungi* are too numerous to list. Two notable highlights include proposals to recognize the phylum *Glomeromycota* (Schüßler *et al.* 2001) and to include the *Microsporidia* within the *Fungi* (Keeling *et al.* 2000).

On-line fungal taxonomies are also proliferating. One of the most important on-line general classifications of *Fungi* is that of GenBank (www.ncbi.nlm.nih.gov/Taxonomy), which serves a diverse community of researchers, including ecologists and molecular biologists. Another highly visible on-line classification is that of the Tree of Life Web Project (tolweb.org/tree), which is widely used by teachers and students. The classification of *Ascomycota* is being updated regularly *via* the on-line Myconet series (www.fieldmuseum.org/myconet), and this has been the basis for recent revisions at GenBank, but there is no comparable on-line resource for other major groups of *Fungi*. It is likely that on-line taxonomies will take on even greater prominence in the future, especially as they become integrated with databases of taxonomic names, particularly Index Fungorum (www.indexfungorum.org), MycoBank (www.mycobank.org), and other global biodiversity informatics resources (e.g. Global Biodiversity Information Facility, www.gbif.org).

Although there is broad agreement regarding the composition of the major clades of *Fungi*, there is considerable variation in the names that have been applied to these groups. For example, the clade that is called *Basidiomycetes* in the latest edition of the *Dictionary* is called *Hymenomycetes* at GenBank. Similarly, the clade that is called *Ascomycetes* in the *Dictionary of the Fungi* is called *Pezizomycotina* in Myconet. Such inconsistencies create confusion, especially for students and non-specialists, and they hamper efforts to develop taxonomic databases.

There is consequently a pressing need for the fungal systematics community to adopt a consensus higher-level classification for the *Fungi* that is based on well-supported monophyletic groups, and which can be recommended for general use. This is an opportune moment to create such a classification. With the new multi-locus analyses, many nodes that were not previously resolved are now supported with confidence. The timing is also good because there are multiple projects in progress that seek to create or update broad classifications of the *Fungi*. In particular, a tenth edition of the *Dictionary* is in preparation, as is a fourth edition of an influential textbook of mycology (Alexopoulos *et al.* 1996). The classifications used by GenBank, the Tree of Life Web Project, and Myconet are being revised continuously. If the classifications employed by these and other major taxonomic resources could be unified, it would promote communication and awareness of fungal phylogeny, and provide a framework for future revisions at all taxonomic levels.

This article presents a higher-level classification for all groups of *Fungi*, with reference to recent molecular phylogenetic studies. The authors represent diverse fungal taxonomy projects, including *Ainsworth & Bisby's Dictionary of the Fungi* (Cannon, Kirk, Stalpers), GenBank (Bischoff), Myconet (Eriksson, Lumbsch, Huhndorf), and Alexopoulos' mycology text (Blackwell, Spatafora). Many of the authors are contributors to the *Fungi* pages in the Tree of Life Web Project. Discussions leading to this classification began in 2004, under the auspices of the AFTOL project and the Deep Hypha Research Coordination Network (Blackwell *et al.* 2007), which were supported by the US National Science Foundation. Throughout the development of this classification, every effort has been made to work in a transparent, consultative manner. The first draft classification was presented at the 2005 Deep Hypha meeting (Tucson, AZ) and subsequently was distributed to a group of 100 fungal systematists for comment. The classification was revised based on comments received and was posted on the AFTOL classification project web site (www.clarku.edu/faculty/dhibbett/AFTOL/AFTOL.htm). Additional modifications were made following the 2006 Deep Hypha meeting (Baton Rouge, LA). For example, the classification of the *Pucciniomycotina* was revised to reflect the classification of Bauer *et al.* (2006). The present paper represents a first attempt at a broad-based consensus classification of the *Fungi*. However, the first 20 authors have exercised editorial control and are therefore to be held accountable for errors.

Structure and principles

This classification is restricted to organisms that belong in the monophyletic kingdom *Fungi*, including sexual and asexual forms. It does not consider other organisms formerly included in the kingdom but which are now known not to belong there, even if still studied by mycologists, such as the oomycetes and slime moulds.

The classification adopted here uses a Linnean hierarchy as modified by the *International Code of Botanical Nomenclature* (*Code*) (McNeill *et al.* 2006), and uses seven ranks, including: order (suffix: *-ales*), subclass (*-mycetidae*), class (*-mycetes*), subphylum (*-mycotina*), phylum (*-mycota;* except *Microsporidia*), subkingdom, and kingdom. The rankings of taxa reflect the preferences and past practices of various authors, as well as the need to keep the nested hierarchies of clades and Linnean categories parallel. Taxa placed at the same rank are not necessarily equivalent in age (except sister taxa), number of species, or degree of morphological divergence.

The classification is limited to taxa down to the level of order. In many orders, especially those representing larger groups, such as *Agaricales*, there is still not enough resolution or taxon sampling to structure a comprehensive family-level classification. The challenge of creating family-level classifications is made even more difficult by the *Code* (McNeill *et al.* 2006), which requires that names of taxa at the rank of family or lower follow the principle of priority (which does not apply to higher ranks). Ideally, construction of consensus classifications within many of the orders recognized here will involve the coordinated efforts of groups of taxonomic specialists. It is hoped that the present classification will facilitate those endeavors.

The taxa included here are all supported as monophyletic by at least one published phylogenetic analysis (not applicable to monotypic taxa), with the exception of the *Lahmiales* and *Triblidiales* (*Pezizomycotina*) and *Asellariales* (*Kickxellomycotina*), for which molecular data are not available. Support for the monophyly of each group is summarized in three tables, which list selected phylogenetic studies, the type of data that were analysed, the number of OTUs sampled, and BS frequencies and Bayesian PPs. No attempt has been made to cite all of the relevant studies for each group. The analyses chosen for inclusion in the tables are those that have the greatest numbers of loci or taxa, and that provide the strongest support for monophyly of the clades in question. To supplement the information in the tables, brief comments on synonyms, phylogenetic relationships, and composition are provided below for some taxa, along with bibliographic citations for all taxon names. However, it is beyond the scope of this article to discuss each taxon in detail. For additional literature on the phylogeny and taxonomy of individual taxa, readers should consult the studies listed in the tables and below, and the references therein.

The classification is also presented as a set of three tree diagrams. Taxa of uncertain position are listed as *incertae sedis*, and have been placed at the least inclusive level in the hierarchy where they can be assigned with confidence. There are several nodes resolved in the tree figures that are not reflected in the classification. These unnamed clades, for which there is strong to moderate support in recent studies, include the *Dacrymycetes* plus *Agaricomycetes* (*Basidiomycota*) (Matheny *et al.* 2006, 2007a), *Saccharomycotina* plus *Pezizomycotina* (*Ascomycota*) (James *et al.* 2006; Spatafora *et al.* 2007), and the inoperculate euascomycetes (*Ascomycota*) (e.g. Lumbsch *et al.* 2002). The inoperculate euascomycetes have been recognized as a superclass, the *Leotiomyceta* (Eriksson & Winka 1997; Lumbsch *et al.* 2002), which is a rank that is not employed here, while the *Dacrymycetes* plus *Agaricomycetes* correspond to the subclass *Hymenomycetidae* of Swann & Taylor (1995). The absence of these groups from the present classification should not be interpreted as a judgment on their monophyly. Rather, it reflects a desire to keep the classification simple, and to minimize the number of intercalary ranks (as per the directives of Art. 4.3 of the *Code*). Future revisions to this classification will have to consider how to incorporate additional deep nodes, including those that will be resolved with the application of genome-scale datasets (Galagan *et al.* 2005; Kuramae *et al.* 2006; Robbertse *et al.* 2006). One possibility is to employ an unranked category (with or without a uniform suffix) that could be inserted at any level in the taxonomic hierarchy (Hibbett & Donoghue 1998). For example, an unranked classification was adopted in part by Adl *et al.* (2005).

Overview of the classification

The classification accepts one kingdom, one subkingdom, seven phyla, ten subphyla, 35 classes, 12 subclasses, and 129 orders. Taxa that are described or validated here include *Chytridiomycota*, *Monoblepharidomycetes*, *Neocallimastigomycota*, *Neocallimastigomycetes*, *Dikarya*, *Acarosporales*, *Baeomycetales*, *Candelariales*, *Umbilicariales*, *Lecanoromycetidae*, *Eurotiomycetidae*, *Mycocaliciomycetidae*, *Melanosporales*, *Corticiales*, *Gloeophyllales*, and *Trechisporales*. Thus, about 90 % of the 195 taxon names employed in the present classification have been validly published previously. The clade containing the *Ascomycota* and *Basidiomycota* is classified as the subkingdom *Dikarya* (as used in James *et al.* 2006), reflecting the putative synapomorphy of dikaryotic hyphae (Tehler 1988). All of the other new names are based on automatically typified teleomorphic names. The classification of *Ascomycota* largely parallels that of the Myconet classification, including recent changes that will be adopted in the forthcoming 2007 'Outline of the Ascomycota'. In *Basidiomycota*, the clades formerly called *Basidiomycetes*, *Urediniomycetes*, and *Ustilaginomycetes* in the last edition of *Ainsworth & Bisby's Dictionary of the Fungi* are called the *Agaricomycotina*, *Pucciniomycotina*, and *Ustilaginomycotina*, respectively, as in Bauer *et al.* (2006). This is done to minimize confusion between taxon names and informal terms (basidiomycetes is a commonly used informal term for all *Basidiomycota*) and to refer to the included genera *Agaricus* (including the cultivated button mushroom) and *Puccinia* (which includes barberry-wheat rust). Another significant change in the *Basidiomycota* classification is the inclusion of the *Wallemiomycetes* and *Entorrhizomycetes* as classes *incertae sedis* within the phylum, reflecting ambiguity about their higher-level placements (Matheny *et al.* 2007b).

The most dramatic changes in the classification concern the 'basal fungal lineages', which include the taxa that have traditionally been placed in the *Zygomycota* and *Chytridiomycota*. These groups have long been recognized to be polyphyletic, based on analyses of rRNA, *tef1*, and *rpb1* (James *et al.* 2000; Nagahama *et al.* 1995; Tanabe *et al.* 2004, 2005). The recent multilocus analyses of James *et al.* (2006) and others now provide the sampling, resolution, and support necessary to structure new classifications of these early-diverging groups, although significant questions remain. The *Chytridiomycota* is retained in a highly restricted sense, including

Chytridiomycetes and *Monoblepharidomycetes*. The *Blastocladiales*, a traditional member of the *Chytridiomycota*, is here treated as a phylum, the *Blastocladiomycota*, as in James *et al.* (2007). The *Neocallimastigales*, whose distinctiveness from other chytrids has long been recognized, is also elevated to phylum, based on both morphology and molecular phylogeny. The genera *Caulochytrium*, *Olpidium*, and *Rozella*, which have traditionally been placed in the *Chytridiomycota*, and *Basidiobolus*, which has been classified in the *Zygomycota* (*Entomophthorales*), are not included in any higher taxa in this classification, pending more definitive resolutions of their placements.

The phylum *Zygomycota* is not accepted in this classification, pending resolution of relationships among the clades that have traditionally been placed in the *Zygomycota* (see discussion under *Mucoromycotina*). The traditional *Zygomycota* are here distributed among the phylum *Glomeromycota* and four subphyla *incertae sedis*, including *Mucoromycotina*, *Kickxellomycotina*, *Zoopagomycotina* and *Entomophthoromycotina*. A clade containing the *Glomeromycota* and the *Dikarya* was resolved previously based on ribosomal RNA genes and was classified as the *Symbiomycota* (Tehler *et al.* 2003). That taxon is not included here, because there was not strong support for the clade in the analyses of James *et al.* (2006) or Liu *et al.* (2006). If the *Symbiomycota* is added to this classification, it will need to be assigned a rank between kingdom and subkingdom, or perhaps be classified as an unranked taxon.

Microsporidia, unicellular parasites of animals and protists with highly reduced mitochondria (Germot *et al.* 1997; Hirt *et al.* 1997; Peyretaillade *et al.* 1998), are included here as a phylum of the *Fungi*, based on analyses by Keeling *et al.* (2000), Gill & Fast (2006), James *et al.* (2006), and Liu *et al.* (2006). The latter study concluded that *Microsporidia* are the sister group of the rest of the *Fungi* and should not be classified as true *Fungi*, but that topology does not conflict with the delimitation of the monophyletic *Fungi* as proposed here. The analysis of James *et al.* (2006) suggested that *Rozella*, which was not sampled by Liu *et al.* (2006), is the sister group of the *Microsporidia*. No subdivision of the *Microsporidia* is proposed, owing to a lack of well-sampled multilocus analyses of this group (but see Vossbrinck & Debrunner-Vossbrinck 2005, for an analysis using SSU rRNA genes).

Phylogenetic classification of *Fungi*

Many of the citations and authorities in the list below were obtained from the Index Fungorum databases (www.indexfungorum.org). A brief list of exemplar genera, including the type for automatically typified names, is given for each order (for small orders, all included genera are listed). A number of the genera listed are used in a modern, restricted sense, and readers are urged to consult the primary literature cited below and in the tables for information about current generic concepts. Comprehensive lists of genera and families included in each order will be forthcoming in the *Dictionary of the Fungi* (10th edn; listing on-line at www.indexfungorum.org) and in the next revision of Myconet (for *Ascomycota*). Further information on the names of fungi (not only kingdom *Fungi*) above the rank of order and their places of publication may be found in the preliminary catalogue compiled by David (2002).

In accordance with the practice in recent editions of the *Code*, all scientific names regardless of rank are placed in italic type here except in the first line of the treatment of each accepted taxon where they are given in bold Roman type to make them stand out. When these names are used by other mycologists in their own publications, we wish to encourage the practice of the use of italics as recommended in the Preface to the current *Code* (McNeill *et al.* 2006).

Kingdom: **Fungi** R. T. Moore, *Bot. Mar.* **23**: 371 (1980).
Synonym: *Fungi* T. L. Jahn & F. F. Jahn, *How to Know the Protozoa*: 7 (1949), *nomen invalidum*. (Table 1, Fig 1)

The concept of the *Fungi* as one of six kingdoms of life was introduced by Jahn & Jahn (1949), and a five kingdom system was advanced by Whittaker (1959), but neither of these works included a Latin diagnosis and the name was therefore invalid under the *Code* until the required Latin was provided by Moore (1980). Although Moore did not make a specific reference to Jahn & Jahn's book, he was well aware that the name was in widespread use in the rank of kingdom. Under the current *Code*, Jahn & Jahn are not to be included in the author citation. However, a proposal to change this provision in the *Code* will be made at the next International Botanical Congress (D. L. Hawksworth, unpubl.). If it is approved, the correct citation would be *Fungi* T. L. Jahn & F. F. Jahn ex R. T. Moore (this rule change would also affect the citations of *Ascomycota* and *Basidiomycota*).

Phylum: **Chytridiomycota** M. J. Powell, **phylum nov**.
MycoBank no.: MB 501278
Synonyms: *Archemycota* Caval.-Sm., *Biol. Rev.* **73**: 246 (1998), *pro parte*.

Thallus monocentricus vel polycentricus vel filamentosus; propagatio asexualis zoosporis, flagello retrorsum inserto, kinetosomate et centriolo supervacaneo praeditis, 9 munimentis flagelli, et complexu ''microbody-corpore lipideo'' descriptis; propagatio sexualis meiosi post copulationem perfecta; apparatus Golgi e cisternis superimpositis constans; tegumentum nuclei mitosi procedente circum polos fenestratum.

Typus: *Chytridium* A. Braun 1851.

Thallus monocentric, polycentric, or filamentous; asexual reproduction by zoospores with a single posteriorly-directed flagellum, both a kinetosome and non-functional centriole, nine flagellar props, and a microbody-lipid globule complex; sexual reproduction with zygotic meiosis where known; Golgi apparatus with stacked cisternae; nuclear envelope fenestrated at poles during mitosis.

Used as a phylum name without Latin diagnosis or description among others by von Arx (1967) and Margulis *et al.* (1990). Equivalent to euchytrids of James *et al.* 2006, the 'core chytrid clade' of James *et al.* (2007), or the 'core chytrid clade' plus the *Monoblepharidales* of James *et al.* (2000). Earlier usages are not indicated in the author citation of the name, because the circumscription adopted here differs significantly from that of those authors.

Table 1 – Support for major groups of *Fungi* in selected phylogenetic studies: basal fungi and *Dikarya*

Rank	Taxon	Reference	Data[a]	OTUs[b]	Support[c]
Kingdom	*FUNGI*	Keeling (2003)	*α-tub*, β-*tub*	38	MLBS = 98 NJBS = 94
		Baldauf *et al.* (2000)	*act*, *α-tub*, β-*tub*, *tef1*	12	MLBS = 85 MPBS = 95
Phylum	*CHYTRIDIOMYCOTA*	James *et al.* (2007)	LSU, SSU, 5.8S	84	BPP ≥ 0.95
		Seif *et al.* (2005)	mt-genome	5	BPP = 1 MLBS = 100
Class	*Chytridiomycetes*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	8	BPP ≥ 0.95 MLBS ≥ 70
		James *et al.* (2007)	LSU, SSU, 5.8S	75	BPP ≥ 0.95 MLBS ≥ 70
		Keeling (2003)	*α-tub*, β-*tub*	5	MLBS = 90 NJBS = 95
Order	*Chytridiales*	James *et al.* (unpublished)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*, *atp6*	9	MLBS = 98
Order	*Rhizophydiales*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	2	BPP ≥ 0.95 MLBS ≥ 70
		Letcher *et al.* (2006)	LSU, 5.8S	96	MPBS = 100 BPP = 1
Order	*Spizellomycetales*	James *et al.* (2007)	LSU, SSU, 5.8S	9	MPBS = 100
Class/Order	*Monoblepharidomycetes*, *Monoblepharidales*	James *et al.* (2007)	LSU, SSU, 5.8S	9	BPP ≥ 0.95 MLBS ≥ 70 MPBS ≥ 70
		Bullerwell *et al.* (2003)	*cox* 1,2,3; *cob*, *atp6*,9; *nad* 1,2,3,4, 4L,6	4	MLBS = 100
Phylum/Class/Order	*NEOCALLIMASTIGOMYCOTA*, *Neocallimastigomycetes*, *Neocallimastigales*	James *et al.* (2007)	LSU, SSU, 5.8S	6	BPP ≥ 0.95 MLBS ≥ 70 MPBS ≥ 70
Phylum/Class/Order	*BLASTOCLADIOMYCOTA*, *Blastocladiomycetes*, *Blastocladiales*	James *et al.* (2007)	LSU, SSU, 5.8S	10	BPP ≥ 0.95
		Liu *et al.* (2006)	*rpb1*, *rpb2*	3	BPP = 1 MPBS = 100
Phylum	*MICROSPORIDIA*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	2	BPP ≥ 0.95 MLBS ≥ 70
		Keeling (2003)	*α-tub*, β-*tub*	6	MLBS = 100 NJBS = 97
Phylum/Class	*GLOMEROMYCOTA*, *Glomeromycetes*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	5	BPP ≥ 0.95 MLBS ≥ 70
		Schüßler *et al.* (2001)	SSU	72	NJBS ≥ 90
Order	*Archaeosporales*	Schüßler *et al.* (2001)	SSU	5	NJBS ≥ 95
Order	*Diversisporales*	Schüßler *et al.* (2001)	SSU	32	NJBS ≥ 95
Order	*Glomerales*	Schüßler *et al.* (2001)	SSU	32	NJBS ≥ 95
Order	*Paraglomerales*	Schüßler *et al.* (2001)	SSU	3	NJBS ≥ 95
	Subphyla incertae sedis (*not placed in any phylum*)				
Subphylum	*Mucoromycotina*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	11	BPP = 1
		Tanabe *et al.* (2004)	*rpb1*	4	NJBS = 82
Order	*Mucorales*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	3	BPP ≥ 0.95 MLBS ≥ 70
		Tanabe *et al.* (2004)	*rpb1*	3	NJBS = 100
		Keeling (2003)	*α-tub*, β-*tub*	4	MLBS = 96 NJBS = 98
		White *et al.* (2007)	LSU, SSU, 5.8S	28	BPP = 1 MPBS ≥ 70
Order	*Endogonales*	White *et al.* (2007)	LSU, SSU, 5.8S	2	BPP = 1 MPBS ≥ 70
Order	*Mortierellales*	White *et al.* (2007)	LSU, SSU, 5.8S	6	BPP = 1 MPBS ≥ 70
Subphylum/Order	*Entomophthoromycotina*, *Entomophthorales*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	2	BPP ≥ 0.95 MLBS ≥ 70
Subphylum/Order	*Zoopagomycotina*, *Zoopagales*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	2	BPP ≥ 0.95 MLBS ≥ 70
		Tanabe *et al.* (2004)	*rpb1*	3	NJBS = 86
Subphylum	*Kickxellomycotina*	Tanabe *et al.* (2004)	*rpb1*	6	NJBS = 84
Order	*Kickxellales*	O'Donnell *et al.* (1998)	SSU	7	MPBS = 100
Order	*Dimargaritales*	Tanabe *et al.* (2000)	SSU	3	NJBS = 100

Table 1 (*continued*)

Rank	Taxon	Reference	Data[a]	OTUs[b]	Support[c]
Order	*Harpellales*	Tanabe *et al.* (2004)	*rpb1*	3	NJBS = 98
		O'Donnell *et al.* (1998)	SSU	4	MPBS = 100
Order	*Asellariales*	—	—	—	—
Subkingdom	*DIKARYA*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	161	BPP = 1 MLBS = 71
		Steenkamp *et al.* (2006)	act, *α-tub*, *β-tub*, *tef1*	10	BPP = 1 MLBS = 84 MPBS = 82 NJBS = 96
		Seif *et al.* (2005)	mt-genome	10	BPP = 1 MLBS = 100
		Liu *et al.* (2006)	*rpb1*, *rpb2*	27	BPP = 1 MPBS = 100

Taxa with only one subsidiary taxon included (i.e. redundant taxa) are listed on a single line, with rank abbreviations divided by a slash (e.g. the class *Agaricostilbomycetes*, which contains a single order, *Agaricostilbales*, is indicated as Class/Order).
a LSU, SSU, and 5.8S refer to nuclear rRNA genes, whereas mt-LSU and mt-SSU refer to mitochondrial rRNA genes, other genes follow standard abbreviations. Some datasets contain missing sequences.
b Indicates the number of OTUs in the specified clade, not the total number of OTUs in the dataset.
c BS, bootstrap %, jk, jackknife %,WP = weighted parsimony, RML = RaxML, PML = PhyML, ME = minimum evolution, BPP, Bayesian posterior probability, NA, not applicable because the group is monotypic, or only a single species was sampled in the reference study.

Class: **Chytridiomycetes** Caval.-Sm., *Biol. Rev.* **73:** 246 (1998).
Synonym: *Archimycetes* A. Fisch. (Fischer 1892) *pro parte* (included *Olpidiopsis*, *Hypochytrium*).
Type: Chytridium A. Braun 1851.

Reproducing asexually by zoospores bearing a single posteriorly-directed flagellum; zoospores containing a kinetosome and a non-flagellated centriole; thallus monocentric or rhizomycelial polycentric; sexual reproduction not oogamous.

Cavalier-Smith (1998) provided a brief, four-word, Latin description that was not diagnostic for phyla of uniflagellate fungi, and has been revised above. The name *Chytridiomycetes* was also used by Serbinow (1907), Cejp (1957), Sparrow (1958), and Alexopoulos *et al.* (1996). For further discussion of the nomenclatural history of the name, see David (2002).

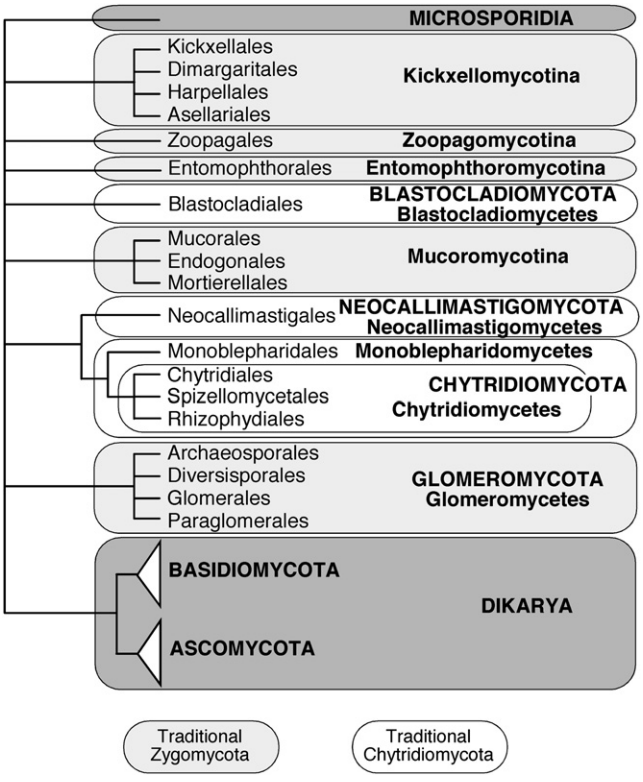


Fig 1 – Phylogeny and classification of *Fungi*. Basal *Fungi* and *Dikarya*. Branch lengths are not proportional to genetic distances. See Table 1 for support values for clades.

Order: **Chytridiales** Cohn, *Jber. schles. Ges. vaterl. Kultur* **57**: 279 (1879).
Emend. Schröter (as '*Chytridineae*') *in* Engler & Prantl, *Nat. Pflanzenfam.* **1**: 64 (1892). *Emend.* Barr, *Can. J. Bot.* **58**: 2384 (1980). *Emend.* Letcher & Powell, *Mycol. Res.* **110**: 907 (2006).
Type: Chytridium A. Braun 1851.

Thallus monocentric or polycentric rhizomycelial; zoospores typically with flagellar base containing an electron-opaque plug, microtubules extending from one side of the kinetosome in a parallel array, ribosomes aggregated near the nucleus, kinetosome parallel to non-flagellated centriole and connected to it by fibrous material, nucleus not associated with kinetosome, fenestrated cisterna (rumposome) adjacent to lipid globule.

Exemplar genera: *Chytridium* A. Braun 1851, *Chytriomyces* Karling 1945, *Nowakowskiella* J. Schröt. 1893.

An emmended description is presented above to conform to the circumscription adopted here. Monophyly of this group, as currently delimited, is not certain; *Polychytrium* Ajello 1942 and its allies and *Chytriomyces angularis* Longcore 1992 and its allies may eventually be segregated from *Chytridiales s. str.*

Order: **Rhizophydiales** Letcher, *in* Letcher *et al.*, *Mycol. Res.* **110**: 908 (2006).
Exemplar genera: *Rhizophydium* Schenk 1858, *Kappamyces* Letcher & M.J. Powell 2005, *Terramyces* Letcher 2006,

Boothiomyces Letcher 2006; *Batrachochytrium* Longcore, Pessier & D.K. Nichols 1999 is on a long branch in this clade with no near relatives.

Order: **Spizellomycetales** D. J. S. Barr, *Can. J. Bot.* **58**: 2384 (1980).
Exemplar genera: *Spizellomyces* D. J. S. Barr 1980, *Powellomyces* Longcore, D. J. S. Barr & Désauln. 1995, *Kochiomyces* D. J. S. Barr 1980.

This classification does not include *Caulochytrium*, *Olpidium*, *Rozella*, or the *Rhizophlyctis rosea* clade, which are considered *incertae sedis*.

Class: **Monoblepharidomycetes** J. H. Schaffn., *Ohio Nat.* **9**: 449 (1909), as '*Monoblepharideae*'.
Type: *Monoblepharis* Cornu 1871.

Thallus filamentous, either extensive or a simple unbranched thallus, often with a basal holdfast; asexual reproduction by zoospores or autospores; zoospores containing a kinetosome parallel to a non-flagellated centriole, a striated disk partially extending around the kinetosome, microtubules radiating anteriorly from the striated disk, a ribosomal aggregation, and rumposome (fenestrated cisterna) adjacent to a microbody; sexual reproduction oogamous by means of posteriorly uniflagellate antherozoids borne in antheridia and nonflagellate female gametes borne in oogonia.

Schaffner (1909) used the name '*Monoblepharideae*' as a class but with the ending of a suborder; this must be changed without change of authorship or date of publication (*Code*, Art. 16.3).

Order: **Monoblepharidales** J. Schröt., *in* Engler & Prantl, *Nat. Pflanzenfam.* **1**: 106 (1893), as '*Monoblepharidineae*'.
Emend. Sparrow, *Aquatic Phycomycetes*: 458 (1943).
Emended description as for *Monoblepharidomycetes*.
Exemplar genera: *Monoblepharis* Cornu 1871, *Harpochytrium* Lagerh. 1890, *Oedogoniomyces* Tak. Kobay. & M. Ôkubo 1954.

Phylum: **Neocallimastigomycota** M. J. Powell, **phylum nov**.
MycoBank no.: MB 501279

Thallus monocentricus vel polycentricus; fungi anaerobici, intra tractum digestivum animalium herbivororum vel fortasse in substratis anaerobicis terrestribus vel limnicis; mitochondriis carentes sed hydrogenosomatibus praediti; zoosporae retrorsum uni- vel multiflagellatae, kinetosoma praesens sed centriolum supervacaneum absens; complexus kinetosomati affixus e radio marginali et annulo circumflagellari compositus; microtubuli e radio entendentes circum nucleum radiantes et flabellum posterius formantes; munimenta flagelli absentia; tegumentum nuclei mitosi procedente integrum remanens.
Typus: *Neocallimastix* Vavra & Joyon ex I. B. Heath 1983.

Thallus monocentric or polycentric; anaerobic, found in digestive system of larger herbivorous mammals and possibly in other terrestrial and aquatic anaerobic environments; lacks mitochondria but contains hydrogenosomes of mitochondrial origin; zoospores posteriorly unflagellate or polyflagellate, kinetosome present but non-functional centriole absent, kinetosome-associated complex composed of a skirt, strut, spur and circumflagellar ring, microtubules extend from spur and radiate around nucleus, forming a posterior fan, flagellar props absent; nuclear envelope remains intact throughout mitosis.

Class: **Neocallimastigomycetes** M. J. Powell, **class. nov**.
MycoBank no.: MB 501280
Diagnosis latina ut in *Neocallimastigomycota* (vide supra).
Typus: *Neocallimastix* Vavra & Joyon ex I.B. Heath 1983.

Order: **Neocallimastigales** J. L. Li, I. B. Heath & L. Packer, *Can. J. Bot.* **71**: 403 (1993).
Exemplar genera: *Neocallimastix* Vavra & Joyon ex I.B. Heath 1983, *Caecomyces* J.J. Gold 1988, *Orpinomyces* D.J.S. Barr, H. Kudo, Jakober & K.J. Cheng 1989.

Phylum: **Blastocladiomycota** T. Y. James, *Mycologia* **98**: 867 (2007) ['2006'].
Synonym: *Allomycota* Caval.-Sm., *BioSystems* 14: 465 (1981).

This phylum was proposed to reflect phylogenetic information from a number of molecular studies (James *et al.* 2007; Liu *et al.* 2006).

Class: **Blastocladiomycetes** T. Y. James, *Mycologia* **98**: 868 (2007) ['2006'].
Synonym: *Allomycetes* Caval.-Sm., *Biol. Rev.* **73**: 246 (1998), based on *Allomyces* E. J. Butler 1911.

Cavalier-Smith provided a brief, five-word Latin description for *Allomycetes* that is not diagnostic from other uniflagellate fungi. The name *Allomycetes* was not taken up, because it is appropriate to have a class name based on the same genus as an included ordinal name, and because Cavalier-Smith's 'diagnosis' was vague.

Order: **Blastocladiales** 1910, H. E. Petersen, *Bot. Tidsskr.* **29**: 357 (1909) ('Blastocladiineae').
Exemplar genera: *Allomyces* E. J. Butler 1911, *Blastocladia* Reinsch 1877, *Coelomomyces* Keilin. 1921.

Phylum: **Microsporidia** Balbiani, *C. R. Acad. Sci. Paris* **95**: 1168 (1882).

The nomenclatural status of *Microsporidia* is ambiguous. It has been treated as a phylum under the zoological *Code* (International Commission on Zoological Nomenclature 1999), but there is disagreement about the correct author citation (Larsson 2000; Sprague & Becnel 1998), and it is uncertain if the name would be valid under the botanical *Code*. This uncertainty arises as *Microsporidium* Balbiani 1884 appears to be a later synonym of *Nosema* Naegeli 1857. The present work follows the recommendation of Sprague & Becnel (1998) in attributing *Microsporidia* to Balbiani (1882), but this must be regarded as provisional. Before the status of the *Microsporidia* can be resolved, it will be necessary to decide whether the nomenclature of the group as a whole should be governed by the zoological or the botanical *Code* although the latter now allows names of fungi described under the zoological *Code* to be accepted. The final decision will require input from the community of scientists who study *Microsporidia*.

No subdivision of the group is proposed here, owing to the lack of well-sampled multi-gene phylogenies within the group. However, Vossbrinck & Debrunner-Vossbrinck (2005) proposed a class-level classification of microsporidia, based on small-subunit rRNA gene sequences.

Phylum: **Glomeromycota** C. Walker & A. Schuessler, *in* Schüßler *et al.*, *Mycol. Res.* **105**: 1416 (2001).

Class: **Glomeromycetes** Caval.-Sm., *Biol. Rev.* **73**: 246 (1998), as '*Glomomycetes*'.
Synonym: *Geomycetes* Caval.-Sm., *Biol. Rev.* **73: 247** (1998).

Order: **Archaeosporales** C. Walker & A. Schuessler, *in* Schüßler *et al.*, *Mycol. Res.* **105**: 1418 (2001).
Synonym: *Geosiphonales* Caval.-Sm., *Biol. Rev.* **73**: **247** (1998).
Exemplar genera: *Archaeospora* J.B. Morton & D. Redecker 2001, *Geosiphon* F. Wettst. 1915.

Order: **Diversisporales** C. Walker & A. Schuessler, *Mycol. Res.* **108**: 981 (2004).
Exemplar genera: *Acaulospora* Gerd. & Trappe 1974, *Diversispora* C. Walker & A. Schüßler 2004, *Gigaspora* Gerd. & Trappe 1974, *Pacispora* Oehl & Sieverd. 2004.

Order: **Glomerales** J. B. Morton & Benny, *Mycotaxon* **37**: 473 (1990), as '*Glomales*'.
Exemplar genus: *Glomus* Tul. & C. Tul. 1845.

Order: **Paraglomerales** C. Walker & A. Schuessler, *in* Schüßler *et al.*, *Mycol. Res.* **105**: 1418 (2001).
Exemplar genus: *Paraglomus* J. B. Morton & D. Redecker 2001.

***Subphyla* incertae sedis (*not assigned to any phylum*):**

Subphylum: **Mucoromycotina** Benny, **subphylum nov**.
MycoBank no.: MB 501281
Fungi saprotrophici vel raro mycoparasiti facultativi, gallas facientes, haustoriis carentes, raro ectomycorrhizam facientes. Mycelium ramosum, juvene coenocyticum, maturum aliquando septis microporosis divisum. Reproductio asexualis sporangiis vel sporangiolis vel merosporangiis, raro chlamydosporis vel arthrosporis vel blastosporis effecta. Reproductio sexualis zygosporis plus minusve globosis e suspensoribus oppositis vel appositis formatis effecta.
Typus: *Mucor* Fresen. 1850.

Fungi saprobes, or rarely gall-forming, nonhaustorial, facultative mycoparasites, or forming ectomycorrhiza. Mycelium branched, coenocytic when young, sometimes producing septa that contain micropores at maturity. Asexual reproduction by sporangia, sporangiola, or merosporangia, or rarely by chlamydospores, arthrospores, or blastospores. Sexual reproduction by more or less globose zygospores formed on opposed or apposed suspensors.

This group includes the *Mucorales*, which is the core group of the traditional *Zygomycota*. Monophyly of the traditional *Zygomycota* (including *Mucorales*, *Glomerales*, *Entomophthorales* and *Harpellales*) was suggested by a recent study by Liu *et al.* (2006) using *rpb1* and *rpb2*, but that finding conflicts with results of analyses that included additional loci and taxa, which suggested that the traditional *Zygomycota* is polyphyletic (James *et al.* 2006).

The name *Zygomycota* was first published without a Latin diagnosis by Moreau (1954) and is therefore invalid. At present, this classification does not include *Zygomycota*. When relationships among basal fungal lineages are more clearly resolved, it may be appropriate to resurrect and validate *Zygomycota*, to include *Mucoromycotina* and perhaps other clades.

Order: **Mucorales** Fr., *Syst. Mycol.* **3** (2): 296 (1832).
Exemplar genera: *Mucor* Fresen. 1850 (*pro parte*), *Parasitella* Bainier 1903, *Phycomyces* Kunze 1823, *Pilobolus* Tode 1784, *Rhizopus* Ehrenb. 1821.

Order: **Endogonales** Moreau ex R. K. Benj., *in* Kendrick (ed.), *Whole Fungus* **2**: 599 (1979).
Emend.: Morton & Benny, *Mycotaxon* **37**: 473 (1990).
Synonym: *Endogonales* Moreau, *Encycl. Mycol.* **23**: 1231 (1954), *nomen invalidum*.
Exemplar genera: *Endogone* Link 1809, *Peridiospora* C. G. Wu & S. J. Lin 1997, *Sclerogone* Warcup 1990, *Youngiomyces* Y. J. Yao 1995.

Order: **Mortierellales** Caval.-Sm., *Biol. Rev.* **73**: 246 (1998).
Exemplar genera: *Mortierella* Coem. 1863, *Dissophora* Thaxt. 1914, *Modicella* Kanouse 1936.

Subphylum: **Entomophthoromycotina** Humber, **subphylum nov.**
MycoBank no.: MB 501282

Fungi pathogenici obligate animalibus (praecipue invertebratis) vel plantis cryptogamicis vel saprotrophici, interdum in animalibus vertebratis parasitici. Status somaticus mycelium coenocyticum vel septatum, pariete circumdatum vel protoplasticum, in hospite culturisve saepe corpora hyphalia multinucleata formans; forma protoplastica hyphoidea vel amoeboidea forma variabilis; cystidia et rhizoidea in aliquot speciebus athropodicolis formata. Characteres nuclei, sicut magnitudo, nucleoli magnitudo et locus, praesentia aut absentia heterochromatini intermitotici, familiis distinguendis iuvant. Conidiophora simplicia ramosave. Sporae primariae conidia vera, uninucleatae vel plurinucleatae vel multinucleatae, variis modis vi propulsae vel passive liberatae, conidia secundaria persaepe formata. Sporae perdurantes crassituncatae, bistratosae velut zygosporae post conjugationem velut azygosporae singulae formatae.
Typus: *Entomophthora* Fresen. 1856.

Obligate pathogens of animals (primarily arthropods), cryptogamic plants, or saprobes; occasionally facultative parasites of vertebrates. Somatic state consisting of a well-defined mycelium, coenocytic or septate, walled or protoplastic, which may fragment to form multinucleate hyphal bodies; protoplasts either hyphoid or amoeboid and changeable in shape; cystidia or rhizoids formed by some taxa. Such nuclear characters as overall size, location and comparative size of nucleoli, presence or absence of granular heterochromatin in chemically unfixed interphasic nuclei, and mitotic patterns are important at the family level. Conidiophores branched or unbranched. Primary spores true conidia, uni-, pluri-, or multinucleate, forcibly

discharged by diverse possible means or passively dispersed; secondary conidia often produced. Resting spores with thick bi-layered walls form as zygospores after conjugations of undifferentiated gametangia from different or the same hyphal bodies or hypha or as azygospores arising without prior gametangial conjugations.

Order: **Entomophthorales** G. Winter, *Rabenh. Krypt.-Fl.* **1**: 74 (1880).

Exemplar genera: *Entomophthora* Fresen. 1856, *Ballocephala* Drechsler 1951, *Conidiobolus* Bref. 1884, *Entomophaga* Batko 1964, *Neozygites* Witlaczil 1885.

Subphylum: **Zoopagomycotina** Benny, **subphylum nov.**
MycoBank no.: MB 501283

Fungi endo- vel ectoparasitici microanimalium vel fungorum. Corpus vegetativum ex thallo simplici ramoso vel nonramoso vel mycelio nonseptato plus minusve extense ramoso constans. Ectoparasitae haustoria intra hospitem formantes. Reproductio asexualis arthrosporis, chlamydosporis vel sporangiolis uni- vel multisporis perfecta; sporangiosporae sporangiolorum multisporum in catenenis (merosporangiis) simplicibus vel ramosis dispositae. Reproductio sexualis zygosporis paene globosis perficitur; hyphae sexuales hyphis vegetativis similes vel plus minusve ampliatae.

Typus: *Zoopage* Drechsler 1935.

Endo- or ectoparasites of microanimals and fungi. Vegetative body consisting of a simple, branched or unbranched thallus or more of less extensively branched mycelium. Ectoparasites forming haustoria inside the host. Asexual reproduction by arthrospores, chlamydospores or uni- or multispored sporangiola; sporangiospores of multispored sporangiola formed in simple or branched chains (merosporangia). Sexual reproduction by nearly globose zygospores; sexual hyphae similar to the vegetative hyphae or more or less enlarged.

The description of this group is based mostly on the validating description for the *Zoopagales* by Benjamin (1979), except that arthrospores have been added, based on Barron's (1975) report of arthrospores in *Helicocephalum* Thaxt. 1891.

Order: **Zoopagales** Bessey ex R.K. Benj., *in* Kendrick (ed.), *Whole Fungus* **2**: 590 (1979).

Synonym: *Zoopagales* Bessey, *Morph. Tax. Fungi* : 177 (1950), *nomen invalidum*.

Exemplar genera: *Cochlonema* Drechsler 1935, *Rhopalomyces* Corda 1839, *Piptocephalis* de Bary 1865, *Sigmoideomyces* Thaxt. 1891, *Syncephalis* Tiegh. & G. Le Monn. 1873, *Zoopage* Drechsler 1935.

Subphylum: **Kickxellomycotina** Benny, **subphylum nov**.
MycoBank no.: MB 501284

Fungi saprotrophici vel mycoparasitici vel obligate symbiotici. Thallus in nonnullis generibus e tenaculo fungos alios parasitans et haustoriis penetrans; mycelium septatum, ramosum vel simplex; septa in medio excavata et obturata. Reproductio asexualis merosporangiis uni- vel bisporis vel trichosporis vel arthrosporis effecta. Reproductio sexualis zygosporis globosis, biconicis vel allantoideis circinatis effecta.

Typus: *Kickxella* Coem. 1862.

Fungi saprobes, mycoparasites, or obligate symbionts. Thallus arising from a holdfast on other fungi as a haustorial parasite, or branched, septate, subaerial hyphae. Mycelium branched or unbranched, regularly septate. Septa with median, disciform cavities containing plugs. Asexual production by 1- or 2-spored merosporangia, trichospores, or arthrospores. Sexual reproduction by zygospores that are globose, biconical, or allantoid and coiled.

Order: **Kickxellales** Kreisel ex R. K. Benj., *in* Kendrick (ed.), *Whole Fungus* 2: 610 (1979).

Synonym: *Kickxellales* Kreisel, *Grundz. nat. Syst. Pilze*: 65 (1969), *nomen invalidum*.

Exemplar genera: *Kickxella* Coem. 1862, *Coemansia* Tiegh. & G. Le Monn. 1873, *Linderina* Raper & Fennell 1952, *Spirodactylon* R. K. Benj. 1959.

Order: **Dimargaritales** R. K. Benj., *in* Kendrick (ed.), *Whole Fungus* **2**: 607 (1979).

Exemplar genera: *Dimargaris* Tiegh. 1875, *Dispira* Tiegh. 1875, *Tieghemiomyces* R. K. Benj. 1959.

Order: **Harpellales** Lichtw. & Manier, *Mycotaxon* **7**: 441 (1978).

The taxa in this order have been referred to as '*Trichomycetes*'. However, *Trichomycetes* is no longer a useful phylogenetic taxon because it describes a polyphyletic group. The use of the term should be restricted to ecological rather than phylogenetic groupings, and not capitalized or italicized, i.e. as 'trichomycetes'.

Exemplar genera: *Harpella* L. Léger & Duboscq 1929, *Furculomyces* Lichtw. & M. C. Williams 1992, *Legeriomyces* Pouzar 1972, *Smittium* R. Poiss. 1937.

Order: **Asellariales** Manier ex Manier & Lichtw., *Mycotaxon* **7**: 442 (1978).

Exemplar genera: *Asellaria* R. Poiss. 1932, *Orchesellaria* Manier ex Manier & Lichtw. 1968.

Asellariales are retained in the *Fungi* here due to their ultrastructural characteristics (Benny & White 2001; Manier 1973; Moss 1975; Saikawa *et al.* 1997). Unpublished *rpb1* and *rpb2* data also support their placement in the *Kickxellomycotina* (T. Y. James & M. M. White, unpubl.).

Subkingdom: **Dikarya** Hibbett, T. Y. James & Vilgalys, **subregnum nov**.
MycoBank no.: MB 501285
Synonyms: *Neomycota* Caval.-Sm., *Rev. Biol.* **73**: 209 (1998).
Carpomycetaceae Bessey, Univ. Studies, Univ. Nebr. **7**: 294 (1907).

Fungi unicellulares vel filamentosi, flagellis carentes, saepe stadium dikaryoticum includentes. *Ascomycota* et *Basidiomycota* complectens.

Unicellular or filamentous *Fungi*, lacking flagella, often with a dikaryotic state. The least-inclusive clade that contains *Ascomycota* and *Basidiomycota*.

The name alludes to the putative synapomorphy of dikaryotic hyphae (Tehler 1988) and was applied by James *et al.* (2006) without formal description. Kendrick (1985) and Tehler *et al.* (2003) referred to this group as the *Dikaryomycota*, but the termination '*–mycota*' denotes the rank of phylum under the *Code*. Cavalier-Smith (1998) referred to this group as *Neomycota*. *Dikarya* is used here, because it is more descriptive and is consistent with recent use (James *et al.* 2006; Tehler *et al.* 2003; Kendrick 1985).

Phylum: **Ascomycota** Caval.-Sm., *Biol. Rev.* 73: 247 (1998), as '*Ascomycota* Berk. 1857. stat. nov.'
Synonyms: *Ascomycetes* Berk., *Intr. Crypt. Bot.*: 270 (1857), rank uncertain; Whittaker (1959: 220).
Ascomycota Bold, *Morph. Pl.*: 7, 180 (1958), *nomen invalidum*; Hawksworth *et al.* (1995: 30), Eriksson & Winka (1997: 4), etc, *nomina nuda*.
Basic type: *Peziza* Fr. 1822.

(Table 2, Fig 2) Cavalier-Smith was not the first to propose the phylum name *Ascomycota*. It appears to have been used first by Bold (1957: 7, 180), but without a Latin diagnosis. The name was in widespread use before its validation by Cavalier-Smith, and its usage was popularized by its employment in the eighth edition of the *Dictionary*, which is listed in Cavalier-Smith's (1998) bibliography. The Latin diagnosis provided by Cavalier-Smith consisted of only two words: 'sporae intracellulares'. It is questionable whether this description is diagnostic for the *Ascomycota*, but as a validating diagnosis it is acceptable under the *Code*. No detailed reference to the basionym was given, but is provided here. We also propose a basic type, *Peziza*, as we can not be sure that the phylum will not be split in the future when more molecular data and material of ascomycetes and basidiomycetes have been sequenced. Hawksworth *et al.* (1995) and Eriksson & Winka (1997: 4) used the phylum names *Ascomycota* and *Basidiomycota*; the latter authors listed 31 nucleotide signatures in the nSSU rDNA genes in *Basidiomycota*. Since then many more sequences have become available, also from many other genes that support monophyly of *Ascomycota* and *Basidiomycota*.

The subdivision of *Ascomycota* used in the present paper is based on the system of Eriksson & Winka (1997), which differs in many respects from that of Cavalier-Smith (1998).

Subphylum: **Taphrinomycotina** O. E. Erikss. & Winka, *Myconet* **1**: 11 (1997).

Class: **Taphrinomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 11 (1997).

Order: **Taphrinales** Gäum. & C. W. Dodge, *Comp. morph. fun.*: 159 (1928).
Exemplar genera: *Taphrina* Fr. 1815, *Protomyces* Unger 1832.

Class: **Neolectomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 8 (1997).

Order: **Neolectales** Landvik, O. E. Erikss, Gargas & P. Gustafss., *Syst. Ascom.* **11**: 114 (1993).
Exemplar genus: *Neolecta* Speg. 1881.

Class: **Pneumocystidomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 9 (1997).

Order: **Pneumocystidales** O. E. Erikss., *Syst. Ascom.* **13**: 170 (1994).
Exemplar genus: *Pneumocystis* P. Delanoë & Delanoë 1912.

Class: **Schizosaccharomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 10 (1997).

Order: **Schizosaccharomycetales** O. E. Erikss., Svedskog & Landvik, *Syst. Ascom.* **11**: 146 (1993).
Exemplar genus: *Schizosaccharomyces* Linder 1893.

Subphylum: **Saccharomycotina** O. E. Erikss. & Winka, *Myconet* **1**: 10 (1997).

Class: **Saccharomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 10 (1997).

Order: **Saccharomycetales** Kudryavtsev, *System Hefen*: 270 (1960).

Growth usually by individual yeast cells, often accompanied by pseudohyphae and/or true hyphae. Cell walls predominately of β-glucan. Ascomata not formed; one to many ascospores formed in asci that often are converted from individual cells or borne on simple ascophores. Mitotic and meiotic nuclear divisions within an intact nuclear membrane. Enveloping membrane system in ascospore delimitation associated independently with postmeiotic nuclei. Asexual reproduction by holoblastic budding, conidia or fission (arthrospores).
Exemplar genera: *Saccharomyces* Meyen ex E. C. Hansen 1838, *Candida* Berkhout 1923, *Dipodascopsis* L. R. Batra & Millner 1978, *Metschnikowia* T. Kamieński 1899.

Subphylum: **Pezizomycotina** O. E. Erikss. & Winka, *Myconet* **1**: 9 (1997).

Class: **Arthoniomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 4 (1997).

Order: **Arthoniales** Henssen & Jahns ex D. Hawksw. & O. E. Erikss, *Syst. Ascom.* **5**: 177 (1986).
Synonym: *Arthoniales* Henssen & Jahns, *Lichenes*: 123 (1973) ['1974'], *nomen invalidum*.

Hawkworth & Eriksson (*loc. cit.*) listed only Henssen, but cited the book by Henssen & Jahns (*loc. cit.*) as place for the original but invalid description so both should be cited although Henssen contributed the taxonomic system to the book.
Exemplar genera: *Arthonia* Ach. 1806, *Chrysothrix* Mont. 1852, *Dirina* Fr. 1825, *Roccella* DC. 1805.

Class: **Dothideomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 5 (1997).

Table 2 – Support for major groups of *Fungi* in selected phylogenetic studies: *Ascomycota*

Rank	Taxon	Reference	Data	OTUs	Support
Phylum	*ASCOMYCOTA*	James *et al.* (2006, fig. 1)	SSU, LSU, 5.8S, *rpb1*, *rpb2*, *tef1*	111	MLBS = 94 BPP = 1
		Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	177	WPBS =< 50 MLBS = 100 BPP = 1
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	276	NJBS = 67 BPP = 1
Subphylum	*Taphrinomycotina*	James *et al.* (2006, fig. 2)	SSU, LSU, 5.8S, *rpb1*, *rpb2*, *tef1*	4	MLBS = 98 BPP = 1
		Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	8	WPBS =< 50 MLBS = 98 BPP = 1
		Liu *et al.* (2006, fig. 3)	*rpb1*, *rpb2*	3	BPP = 1
		Sugiyama *et al.* (2007, fig. 2)	LSU, SSU *rpb2*, β-*tub*	11	BPP = 1
		Kurtzman & Sugiyama (2001, fig. 7)	SSU	8	NJBS = 54
Class/Order	*Taphrinomycetes*, *Taphrinales*	Sugiyama *et al.* (2007, fig. 2)	LSU, SSU *rpb2*, β-*tub*	6	BPP = 1
		Kurtzman & Sugiyama (2001, fig. 7)	SSU	4	NJBS = 100
		Nishida & Sugiyama (1994, fig. 1)	SSU	5	NJBS = 100
Class/Order	*Neolectomycetes*, *Neolectales*	Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	1	NA
		Sugiyama *et al.* (2007, fig. 2)	LSU, SSU, β-*tub*, *rpb2*	2	BPP = 1
		Landvik *et al.* (2001, fig. 1)	β-*tub*	2	MPBS = 100
Class/Order	*Pneumocystidomycetes*, *Pneumocystidales*	Sugiyama *et al.* (2007, fig. 2)	LSU, SSU, β-*tub*, *rpb2*	1	NA
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	1	NA
Class/Order	*Schizosaccharomycetes*, *Schizosaccharomycetales*	Sugiyama *et al.* (2007, fig. 2)	SSU, LSU, *rpb2*, β-*tub*	1	NA
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	2	BPP = 1.0 NJBS = 100
	Taphrinomycotina incertae sedis (*not placed in any subphylum*)				
Genus	*Saitoella*	Sugiyama *et al.* (2007, fig. 2)	SSU, LSU, *rpb2*, β-*tub*	1	NA
		Nishida & Sugiyama (1994, fig. 1)	SSU	1	NA
Subphylum/Class/Order	*Saccharomycotina*, *Saccharomycetes*, *Saccharomycetales*	Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	12	WPBS = 55 MLBS = 100 BPP = 1
		Suh *et al.* (2007, fig. 2)	LSU, SSU	87	MPBS = 99 BPP = 1
Subphylum	*Pezizomycotina*	James *et al.* (2006, fig. 1)	SSU, LSU, 5.8S, *rpb1*, *rpb2*, *tef1*	46	BPP = 1 MLBS = 94
		Robbertse *et al.* (2006, figs. 4,5,6)	Genomes	11	MPBS = 94-100 NJBS = 100 MLBS = 100
		Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	157	WPBS = 100 MLBS = 97 BPP = 1
Class/Order	*Arthoniomycetes*, *Arthoniales*	Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	4	WPBS = 100 MLBS = 100 BPP = 1
		Lumbsch *et al.* (2005, fig. 1)	LSU, SSU, mt-SSU, mt-LSU	6	MPBS = 100 BPP = 1.0
Class	*Dothideomycetes*	Schoch *et al.* (2007, fig.1)	LSU, SSU, *rpb2*, *tef1*	96	BPP = 1 MPBS < 50 MLBS = 70
		Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	17	WPBS < 50 MLBS = 84 BPP = 1
		Kruys *et al.* (2006, fig. 1)	LSU, SSU, mt-SSU	51	BPP > 0.95 MPBS < 50
Subclass	*Dothideomycetidae*	Schoch *et al.* (2007, fig. 1)	LSU, SSU, *rpb2*, *tef1*	26	BPP = 1 MPBS > 50 MLBS > 0.7

Table 2 (*continued*)

Rank	Taxon	Reference	Data	OTUs	Support
		Kruys *et al.* (2006, fig. 1)	LSU, SSU, mt-SSU	11	BPP > 0.95 MPBS < 50
Order	*Capnodiales*	Schoch *et al.* (2007, fig. 1)	LSU, SSU, *rpb2*, *tef1*	11	BPP = 1 MPBS > 70 MLBS > 70
Order	*Dothideales*	Schoch *et al.* (2007, fig. 1)	LSU, SSU, *rpb2*, *tef1*	9	BPP = 1 MPBS > 70 MLBS > 70
		Kruys *et al.* (2006, fig. 1)	LSU, SSU, mt-SSU	4	BPP > 0.95 MPBS = 100
		Lindemuth *et al.* (2001)	LSU, SSU	6	MLBS = 91 NJBS = 100
Order	*Myriangiales*	Schoch *et al.* (2007, fig. 1)	LSU, SSU, *rpb2*, *tef1*	5	BPP = 1 MPBS > 70 MLBS > 70
Subclass/Order	*Pleosporomycetidae*, *Pleosporales*	Schoch *et al.* (2007, fig. 1)	LSU, SSU, *rpb2*, *tef1*	48	BPP = 1 MPBS > 70 MLBS > 70
		Kruys *et al.* (2006, fig. 1)	LSU, SSU, mt-SSU	35	BPP = 1 MPBS = 100
	Dothideomycetes incertae sedis (*not placed in any subclass*)				
Order	*Botryosphaeriales*	Schoch *et al.* (2007, fig. 1)	LSU, SSU, *rpb2*, *tef1*	8	BPP = 1 MPBS > 70 MLBS > 70
Order	*Hysteriales*	Schoch *et al.* (2007, fig. 1)	LSU, SSU, *rpb2*, *tef1*	3	BPP = 1 MPBS > 70 MLBS > 70
Order	*Patellariales*	Pang *et al.* (2002, fig. 26)	SSU	1	NA
		Inderbitzin *et al.* (2001, fig. 18)	SSU	1	NA
Order	*Jahnulales*	Pang *et al.* (2002, fig. 26)	SSU	6	MPBS = 100
Class	*Eurotiomycetes*	Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	11	WPBS = 89 MLBS = 84 BPP = 1
		Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	49	BPP = 1 MPBS = 100 WPBS = 100 MLBS = 100
		Ekman & Tønsberg (2002, fig. 1)	SSU	13	BPP = 0.99
		Del Prado *et al.* (2006, fig. 1)	LSU, mt-SSU	15	BPP = 1
		Lumbsch *et al.* (2005, fig. 1)	LSU, SSU, mt-SSU, mt-LSU	11	BPP > 0.95 MPBS > 70
		Lutzoni *et al.* (2004, fig. 5)	LSU, SSU, mt-SSU, *rpb2*	8	BPP = 1 BBS = 61
		Reeb *et al.* (2004, fig. 1)	SSU, LSU, *rpb2*	7	BPP = 1 BBS = 89
Subclass	*Chaetothyriomycetidae*	Reeb *et al.* (2004, fig. 1)	SSU, LSU, *rpb2*	5	BPP = 1 BBS = 100 MLBS = 100
		Lutzoni *et al.* (2004, fig. 5)	LSU, SSU, mt-SSUSSU, *rpb2*	5	BPP = 1 BBS = 100 NJBS = 99 MPBS = 98
		Del Prado *et al.* (2006, fig. 1)	LSU, mt-SSU	11	BPP = 1
		Spatafora *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	6	BPP = 1 MLBS = 100 WPBS > 70
		Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	21	BPP = 1 MPBS = 100 WPBBS = 100 MLBS = 100
Order	*Chaetothyriales*	Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	5	BPP = 1 NJBS = 94

(*continued on next page*)

Table 2 (continued)

Rank	Taxon	Reference	Data	OTUs	Support
		Liu & Hall (2004, fig. 3)	*rpb2*	5	BPP = 1 MPBS = 96
		Spatafora *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	4	BPP = 1 MLBS = 100 WPBS > 70
		Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	9	BPP = 1 MPBS = 100 WPBBS = 100 MLBS = 100
Order	*Pyrenulales*	Lutzoni *et al.* (2004, fig. 8)	LSU, SSU, mt-SSU, *rpb2*	2	BPP = 1 NJBS = 100 WPBS = 100
		Reeb *et al.* (2004, fig. 1)	LSU, SSU, *rpb2*	2	BPP = 1 BBS = 100 MLBS = 100
		Schmitt *et al.* (2004, fig. 1)	LSU, mt-SSU	2	BPP = 1
		Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	5	BPP = 1 MPBS = 100 WPBBS = 100 MLBS = 100
Order	*Verrucariales*	Wedin *et al.* (2006, fig. 1)	LSU, mt-SSU	3	BPP = 1 MPjk = 100
		Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	7	BPP = 1 MPBS = 100 WPBBS = 100 MLBS = 100
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	3	BPP = 1 NJBS = 98
		Gueidan *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*	83	BPP = 1 MLBS = 100 MPBS = 100
Subclass	*Eurotiomycetidae*	Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	24	BPP = 1 MPBS = 100 WPBBS = 98 MLBS = 100
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	11	NJBS = 96 BPP = 1
Order	*Coryneliales*	Winka (2000, fig. 1)	SSU	2	MPBS = 100 NJBS = 100
		Inderbitzin *et al.* (2004, fig. 14)	SSU	1	NA
		Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	3	BPP = 1 MPBS = 100 WPBBS = 100 MLBS = 100
Order	*Eurotiales*	Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	9	BPP = 1 MPBS = 100 WPBBS = 100 MLBS = 100
Order	*Onygenales*	Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	12	BPP = 1 MPBS = 65 WPBBS = 68 MLBS = 88
Subclass/Order	*Mycocaliciomycetidae*, *Mycocaliciales*	Tibell & Vinuesa (2005, fig. 1)	LSU	20	BPP = 1
		Geiser *et al.* (2007, fig. 1)	SSU, LSU, *rpb1*, *rpb2*, *tef*	4	BPP = 1 MPBS = 100 WPBBS = 100 MLBS = 100
		Ekman & Tønsberg (2002, fig. 1)	SSU	4	BPP = 1
Class	*Laboulbeniomycetes*	Weir & Blackwell (2001, fig. 2)	SSU	4	MPBS = 100
		Henk *et al.* (2003, fig. 1)	SSU	6	MPBS = 100
Order	*Laboulbeniales*	Weir & Blackwell (2001, fig. 1)	SSU	3	MPBS = 100
		Henk *et al.* (2003, fig. 2)	SSU	3	MPBS = 57
Order	*Pyxidiophorales*	Weir & Blackwell (2001, fig. 2)	SSU	1	NA
		Henk *et al.* (2003, fig. 2)	SSU	2	MPBS = 99

Table 2 (*continued*)

Rank	Taxon	Reference	Data	OTUs	Support
Class	*Lecanoromycetes*	Lutzoni *et al.* (2004, fig. 5)	LSU, SSU, *rpb2*, mt-SSU	34	BPP = 1 BBS = 56
		Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	38	WPBS < 50 MLBS = 93 BPP = 1
		Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	264	RMLBS > 70 BS BPP > 0.95
		Hofstetter *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	82	RMLBS > 70 BPP > 0.95
Subclass/Order	*Acarosporomycetidae*, *Acarosporales*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	15	RMLBS > 70 % PMLBS > 70 % BPP > 0.95
		Reeb *et al.* (2004, fig. 1)	LSU, SSU, *rpb2*	14	MLBS = 100 BPP = 100
		Lutzoni *et al.* (2004, fig. 4)	LSU, SSU, *rpb2*	14	BPP = 1 NJBS = 100 MPBS = 100
Subclass	*Lecanoromycetidae*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	71	RMLBS > 70 % PMLBS > 70 % BPP > 0.95
		Hofstetter *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	54	RMLBS > 70 BPP > 0.95
		Reeb *et al.* (2004, fig. 1)	LSU, SSU, *rpb2*	14	MLBS = 73 BPP = 100
Order	*Lecanorales*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	86	RMLBS > 70 BS BPP > 0.95
		Hofstetter *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	30	RMLBS > 70 BPP > 0.95
		Lumbsch *et al.* (2004, fig. 1)	LSU, mt-SSU	14	BPP = 1
		Lücking *et al.* (2004, fig. 3)	LSU, mt-SSU	8	BPP = 1
Order	*Peltigerales*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	46	RMLBS > 70 BSBPP > 0.95
		Miądlikowska & Lutzoni (2004, fig. 1)	LSU, SSU	59	MPBS < 70 BPP = 0.92
		Wilklund & Wedin (2003, fig. 1)	LSU, SSU	31	Bjk = 99
Order	*Teloschistales*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	13	RMLBS > 70 BPP > 0.95
Subclass	*Ostropomycetidae*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	58	RMLBS > 70 BS BPP > 0.95
		Grube *et al.* (2004, fig. 1)	mt-SSU	30	BPP > 0.95
		Reeb *et al.* (2004, fig. 1)	LSU, SSU, *rpb2*	16	MLBS = 100 BPP = 100
Order	*Agyriales*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	8	RMLBS > 70 BS BPP > 0.95
		Lücking *et al.* (2004, fig. 3)	LSU, mt-SSU	11	BPP = 1
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	4	BPP = 1 NJBS = 100
		Wedin *et al.* (2005, fig. 1)	LSU, mt-SSU	8	MPjk = 83 BPP = 0.99
Order	*Baeomycetales*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	4	RMLBS > 70 PMLBS > 70 BPP > 0.95
		Wedin *et al.* (2005, fig. 1)	LSU, mt-SSU	3	MPjk = 99 BPP = 1.0
Order	*Ostropales s.l.*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	21	RMLBS > 70 BS BPP > 0.95
		Schmitt *et al.* (2005, fig. 1)	LSU, mt-SSU	12	BPP = 1
		Wedin *et al.* (2005, fig. 1)	LSU, mt-SSU	13	Bjk = 94 BPP = 0.97

(*continued on next page*)

Table 2 (continued)

Rank	Taxon	Reference	Data	OTUs	Support
		Lutzoni *et al.* (2004, fig. 4)	LSU, SSU, *rpb2*	10	BPP = 1 NJBS = 74 MPBS = 84
		Reeb *et al.* (2004, fig. 1)	LSU, SSU, *rpb2*	9	MLBS = 99 BPP = 1 BBS = 1
Order	*Pertusariales*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	21	RMLBS > 70 BSBpp > 0.95
		Lücking *et al.* (2004, fig. 3)	LSU, mt-SSU	7	BPP = 1
		Schmitt *et al.* (2005, fig. 1)	LSU, mt-SSU	14	BPP = 1
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	11	BPP = 1
	Lecanoromycetes incertae sedis (*not placed in any subclass*)				
Order	*Candelariales*	Wedin *et al.* (2005, fig. 1)	LSU, mt-SSU	3	Jk = 100 BPP = 0.96
		Hofstetter *et al.* (2007, fig. 1)	LSU, SSU, mt-SSU, *rpb1*, *rpb2*	2	RMLBS > 70 BPP > 0.95
		Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, mt-SSU, *rpb1*, *rpb2*	3	RMLBS > 70 PMLBS > 70 BPP > 0.95
Order	*Umbilicariales*	Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	16	BSBSBPP > 0.95
		Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	9	RMLBS > 70 PMLBS > 70 BPP > 0.95
		Hofstetter *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	8	RMLBS > 70 BPP > 0.95
		Reeb *et al.* (2004, fig. 1)	LSU, SSU, *rpb2*	4	MLBS = 70 BPP = 1 BBS = 88
Class	*Leotiomycetes* (*w/o Geoglossaceae*)	Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	22	WPBS = 100 MLBS = 100 BPP = 1
		Wang *et al.* (2006, fig. 1)	LSU, SSU, 5.8S	50	BPP = 1
		Wang *et al.* (2007, fig. 2)	LSU, SSU, 5.8S	78	MPBS = 61 BPP = 1
Order	*Cyttariales*	Wang *et al.* (2007, fig. 1)	SSU, LSU, 5.8S	1	NA
Order	*Erysiphales*	Rossman *et al.* (2004, fig. 2)	LSU	12	MPBS > 55
		Wang *et al.* (2007, fig. 1)	SSU, LSU, 5.8S	16	MPBS = 63 BPP = 0.97
		Takamatsu (2004, fig. 2)	SSU	10	NJBS = 99
Order	*Helotiales* (*w/o Geoglossaceae*)	Wang *et al.* (2007, fig. 1)	SSU, LSU, 5.8S	40	BPP < 0.90
Order	*Rhytismatales*	Rossman *et al.* (2004, fig. 2)	LSU	4	MPBS > 55
		Wang *et al.* (2007, fig. 1)	SSU, LSU, 5.8S	5	MPBS = 100 BPP = 1
Order	*Thelebolales*	de Hoog *et al.* (2005, fig. 3)	SSU	11	MPBS = 56
Class/Order	*Lichinomycetes*, *Lichinales*	Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	1	NA
		Miądlikowska *et al.* (2007, fig. 1)	LSU, SSU, *rpb1*, *rpb2*, mt-SSU	2	RMLBS > 70 PMLBS > 70 BPP > 0.95
		Reeb *et al.* (2004, fig. 1)	LSU, SSU, *rpb2*	3	MLBS = 100 BBS = 100 BPP = 1
Class/Order	*Orbiliomycetes*, *Orbiliales*	Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	2	WPBS = 100 MLBS = 100 BPP = 1
Class/Order	*Pezizomycetes*, *Pezizales*	Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	14	WPBS = 54 MLBS = 99 BPP = 1
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	21	BPP = 0.96 NJBS = 70
Class	*Sordariomycetes*	Spatafora *et al.* (2007, fig. 2)	LSU, SSU, *rpb1*, *rpb2*, *tef1*	47	WPBS = 100 MLBS = 100 BPP = 1

Table 2 (*continued*)

Rank	Taxon	Reference	Data	OTUs	Support
		Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	106	MPBS = 100 WPBS = 100 MLBS = 100 BPP = 1
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	66	BPP = 1 NJBS = 97
Subclass	*Hypocreomycetidae*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	42	MPBS = 92 WPBS = 96 MLBS = 90 BPP = 1
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	26	NJBS < 50 BPP = 1
		Huhndorf *et al.* (2004b, figs. 38, 39)	LSU	21	MPBS = 67 BPP >= 0.95
Order	*Coronophorales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	2	MPBS < 50 WPBS < 50 MLBS = 96 BPP = 1
		Huhndorf *et al.* (2004b, figs. 38, 39)	LSU	16	WPBS = 99 BPP ≥ 95
		Miller & Huhndorf (2005, fig. 7)	LSU, β-*tub*, *rpb2*	2	WPBS = 100 BPP ≥ 95
Order	*Hypocreales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	21	MPBS = 91 WPBS = 90 MLBS = 72 BPP = 1
		Castlebury *et al.* (2004, fig. 1)	LSU, SSU	31	MPBS = 70 BPP = 1
Order	*Melanosporales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	2	MPBS = 100 WPBS = 100 MLBS = 100 BPP = 1
Order	*Microascales* (*incl. Halosphaeriales*)	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	15	MPBS = 74 WPBS = 86 MLBS = 85 BPP = 1
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	10	NJBS = 80 BPP = 1
		Campbell *et al.* (2003, fig. 3)	LSU, SSU	40	MPBS = 100 BPP = 1
		Kohlmeyer *et al.* (2000, fig. 1)	LSU, SSU	16	MPBS = 97
Subclass	*Sordariomycetidae*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	54	MPBS = 82 WPBS = 85 MLBS = 77 BPP = 1
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	36	NJBS < 50 BPP = 0.97
Order	*Boliniales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	4	MPBS = 100 WPBS = 100 MLBS = 100 BPP = 1
		Huhndorf *et al.* (2004a, fig. 1)	LSU	3	WPBS = 99 BPP < 95
		Miller & Huhndorf (2005, fig. 7)	LSU, β-*tub*, *rpb2*	2	WPBS = 100 BPP ≥ 95
Order	*Chaetosphaeriales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	3	MPBS = 100 WPBS = 100 MLBS = 100 BPP = 1
		Miller & Huhndorf (2005, fig. 7)	LSU, β-*tub*, *rpb2*	2	WPBS = 100 BPP ≥ 95
		Shenoy *et al.* (2006, fig. 3)	LSU, *rpb2*	4	MPBS = 100

(*continued on next page*)

Table 2 (continued)

Rank	Taxon	Reference	Data	OTUs	Support
Order	*Coniochaetales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	3	MPBS = 93 WPBS = 100 MLBS = 87 BPP = 1
		Miller & Huhndorf (2005, fig. 7)	LSU, β-*tub*, *rpb2*	2	WPBS = 100 BPP ≥ 95
		Miller & Huhndorf (2004, fig. 10)	LSU	3	WPBS = 98 BPP ≥ 95
Order	*Diaporthales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	19	MPBS = 95 WPBS = 94 MLBS = 77 BPP = 1
		Castlebury *et al.* (2002, fig. 1)	LSU	82	MPBS = 100 NJBS = 100
		Lutzoni *et al.* (2004, fig. 2)	LSU, SSU	10	NJBS = 100 BPP = 1
		Miller & Huhndorf (2005, fig. 7)	LSU, β-*tub*, *rpb2*	2	WPBS = 100 BPP ≥ 95
		Miller & Huhndorf (2004, fig. 10)	LSU	3	WPBS = 100 BPP =≥ 95
Order	*Ophiostomatales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	3	MPBS = 100 WPBS = 100 MLBS = 100 BPP = 1
		Hausner & Reid (2004, fig. 1)	SSU	3	NJBS = 99
		Wingfield *et al.* (1999, fig. 3)	LSU	4	MPBS = 99
Order	*Sordariales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	17	MPBS = 80 WPBS = 77 MLBS = 84 BPP = 1
		Huhndorf *et al.* (2004a, fig. 1)	LSU	22	WPBS =< 50 BPP < 95
		Miller & Huhndorf (2005, fig. 7)	LSU, β-*tub*, *rpb2*	41	WPBS = 65 BPP ≥ 95
Subclass/Order	*Xylariomycetidae*, *Xylariales*	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	8	MPBS = 98 WPBS = 99 MLBS = 78 BPP = 1
	Sordariomycetes incertae sedis (*not placed in any subclass*)	Shenoy *et al.* (2006, fig. 1)	LSU	16	MPBS = 92
Order	*Calosphaeriales*	Vijaykrishna *et al.* (2004, fig. 1)	SSU	3	MPBS = 100
		Réblová *et al.* (2004, fig. 1)	LSU	6	MPBS = 53
		Réblová (2006, fig. 1)	SSU	2	MPBS = 68
Order	*Lulworthiales* (*incl. Spathulosporales*)	Zhang *et al.* (2007, fig. 2)	LSU, SSU, *rpb2*, *tef1*	2	MPBS = 100 WPBS = 100 MLBS = 100 BPP = 1
		Campbell *et al.* (2005, fig. 1)	LSU, SSU	56	BPP = 1
		Inderbitzin *et al.* (2004, fig. 15)	LSU	15	MPBS = 100 NJBS = 91 BPP = 86
		Kohlmeyer *et al.* (2000, fig. 1)	LSU, SSU	7	MPBS = 100
Order	*Meliolales*	Saenz & Taylor (1999, fig. 1)	LSU	2	MPBS = 100
Order		Vijaykrishna *et al.* (2004, fig. 1)	SSU	2	MPBS < 50
Order	*Phyllachorales*	Inderbitzin *et al.* (2004, fig. 14)	SSU	1	NA
Order	*Trichosphaeriales*	Réblová & Seifert (2004, fig. 1)	LSU	8	MPBS < 50
	Pezizomycotina incertae sedis (*not placed in any class*)				
Order	*Lahmiales*	Eriksson (1986)	—	—	—
Order	*Medeolariales*	Inderbitzin *et al.* (2004, fig. 14)	SSU	1	NA
Order	*Triblidiales*	Eriksson (1992)	—	—	—

See Table 1 for explanation.

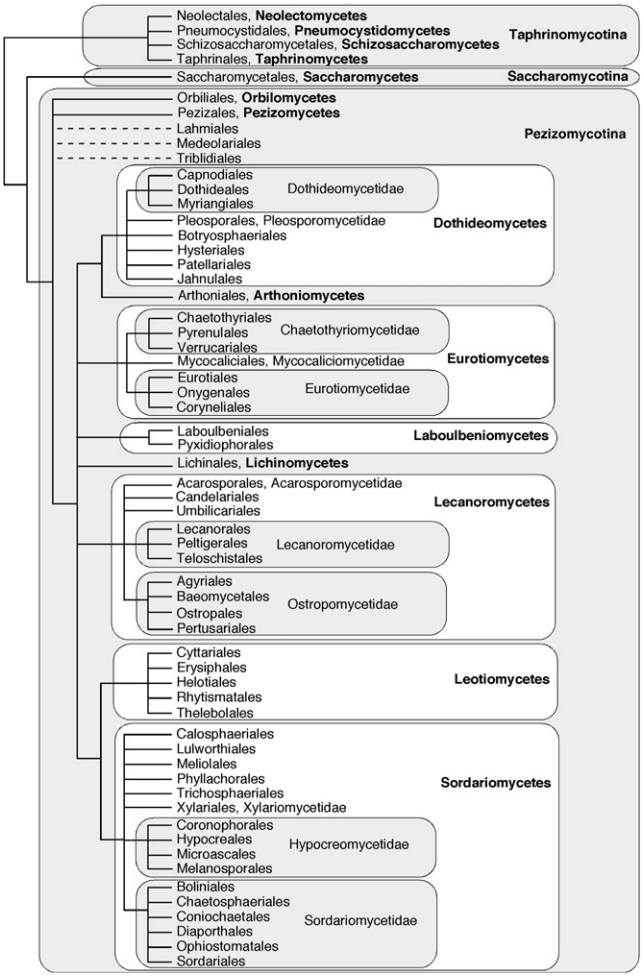


Fig 2 – Phylogeny and classification of *Fungi*. *Ascomycota*. See Table 2 for support values for clades. Dashed lines indicate taxa that are of uncertain placement.

Subclass: **Dothideomycetidae** P. M. Kirk, P. F. Cannon, J. C. David & Stalpers ex Schoch *et al.*, *Mycologia* **98**: 1047 (2007) ['2007'].

Order: **Capnodiales** Woron., *Annls Mycol.* **23**: 177 (1925).
Exemplar genera: *Capnodium* Mont. 1848, *Scorias* Fr. 1825, *Mycosphaerella* Johanson 1884.

Order: **Dothideales** Lindau, *in* Engler & Prantl (eds), *Nat. Pflanzenfam.* **1**(1): 373 (1897).
Exemplar genera: *Dothidea* Fr. 1818, *Dothiora* Fr. 1849, *Sydowia* Bres. 1895, *Stylodothis* Arx & E. Müll. 1975.

Order: **Myriangiales** Starbäck, *K. svenska Vetensk-Akad. Handl., Bih.*, Afd. III **25**: 37 (1899).
Exemplar genera: Myriangium Mont. & Berk. 1845, *Elsinoë* Racib. 1900.

Subclass: **Pleosporomycetidae** C. L. Schoch, Spatafora, Crous & Shoemaker, *Mycologia* **98**: 1049 (2007) ['2006'].

Order: **Pleosporales** Luttr. ex M. E. Barr, *Prodr. Class Loculoasc.*: 67 (1987b).
Synonym: *Pleosporales* Luttr., *Mycologia* **47**: 520 (1955), *nomen invalidum*.
Exemplar genera: *Pleospora* Rabenh. ex Ces. & De Not. 1863, *Phaeosphaeria* I. Miyake 1909, *Lophiostoma* Ces. & De Not. 1863, *Sporormiella* Ellis & Everh.1892, *Montagnula* Berl. 1896.

Dothideomycetes *incertae sedis* (not placed in any subclass)

Order: **Botryosphaeriales** C. L. Schoch, Crous & Shoemaker, *Mycologia* **98**: 1051 (2007) ['2006'].
Exemplar genera: *Botryosphaeria* Ces. & De Not. 1863, *Guignardia* Viala & Ravaz 1892.

Order: **Hysteriales** Lindau *in* Engler & Prantl (eds), *Nat. Pflanzenfam.* **1**: 265 (1896), as '*Hysteriinae*'.
Exemplar genera: *Hysterium* Pers. 1797, *Hysteropatella* Rehm. 1890.

Order: **Patellariales** D. Hawksw. & O. E. Erikss., *Syst. Ascom.* **5**: 181 (1986).
Exemplar genus: *Patellaria* Fr. 1822.

Order: **Jahnulales** Ka-Lai Pang, Abdel-Wahab, El-Shar., E. B. G. Jones & Sivichai, *in* Pang *et al.*, *Mycol. Res.* **106**: 1033 (2002).
Exemplar genera: *Aliquandostipite* Inderb. 2001, *Jahnula* Kirschst. 1936, *Patescospora* Abdel-Wahab & El-Shar. 2002.

Class: **Eurotiomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 6 (1997).

The circumscription of this class and the classification within the *Eurotiomycetes* presented here are derived from the phylogenetic re-delimitation of this class by Ekman & Tønsberg (2002), Lutzoni *et al.* (2004) and Geiser *et al.* (2007), reflecting the inference of shared ancestry between *Eurotiomycetes*, comprising *Coryneliales*, *Onygenales* and *Eurotiales* and *Chaetothyriomycetes*. Three subclasses, *Chaetothyriomycetidae*, *Eurotiomycetidae*, and *Mycocaliciomycetidae*, are defined to represent the major lineages within *Eurotiomycetes*.

Subclass: **Chaetothyriomycetidae** Doweld, *Prosyllabus:* LXXVIII (2001).

Lichenized, parasitic, and saprobic ascomycetes with mostly bitunicate/fissitunicate to evanescent asci, produced in perithecial ascomata arranged superficially or immersed in a thallus. Thalli often produced on the surfaces of rocks, lichens, decaying plant material and other substrata. Ascospores variable, from colourless to pigmented, simple to muriform. Hamathecium, when present, consisting of pseudoparaphyses. Pigments, when present, generally related to melanin. Asexual stages with phialidic and annellidic anamorphs observed in non-lichenized taxa.

Order: **Chaetothyriales** M. E. Barr, *Mycotaxon* **29**: 502 (1987).
Exemplar genera: *Capronia* Sacc. 1883, *Ceramothyrium* Bat. & H. Maia 1956, *Chaetothyrium* Speg. 1888.

Order: **Pyrenulales** Fink ex D. Hawksw. & O. E. Erikss., *Syst. Ascom.* **5**: 182 (1986).
Synonym: *Pyrenulales* Fink, *Ohio St. Univ. Bull.* **19**(28): 107 (1951), *nomen invalidum*.
Exemplar genera: *Pyrenula* Ach. 1814, *Pyrgillus* Nyl. 1858.

Order: **Verrucariales** Mattick ex D. Hawksw. & O. E. Erikss., *Syst. Ascom.* **5**: 183 (1986).
Synonym: *Verrucariales* Mattick, *in* Engler, *Syll. Pflanzenfam.* (12 edn): 208 (1954), *nomen invalidum*.
Exemplar genera: *Agonimia* Zahlbr. 1909, *Dermatocarpon* Eschw. 1824, *Polyblastia* A. Massal. 1852, *Verrucaria* Schrad. 1794.

Subclass: **Eurotiomycetidae** Geiser & Lutzoni, **subclass. nov.**
MycoBank no.: MB 501287

Fungi saprotrophici vel parasitici vel mycorrhizales; asci globosi in toto ascomate sparsi, raro hymenium formantes; asci plerumque evanescentes, nonnumquam bitunicati. Ascosporae plerumque unicellulares, lenticulares, nonnumquam globosae vel ellipsoideae. Ascomata, si formata, plerumque cleistothecialia vel gymnothecialia, saepe textura stromatica circumdata. Structurae hamatheciales absentes. Gametangia plerumque indistincta e glomere hyphali constantia. Fungi saepe laete colorati. Anamorphae variabiles, seu phialidicae seu arthroconidiales.
Typus: *Eurotium* Link 1809.

Saprotrophic, parasitic and mycorrhizal. Ascomata, when present, usually cleistothecial/gymnothecial, globose, often produced in surrounding stromatic tissue and brightly coloured; hamathecial elements lacking; gametangia usually undifferentiated and consisting of hyphal coils. Asci usually evanescent, sometimes bitunicate, scattered throughout the ascoma, rarely from a hymenium. Ascospores usually single-celled, lenticular, sometimes spherical or elliptical. Anamorphs variable, including phialidic and arthroconidial forms.

This name was employed by Lutzoni *et al.* (2004) and Geiser *et al.* (2007), in the same sense as the present classification, but without a formal diagnosis.

Order: **Coryneliales** Seaver & Chardón, *Scient. Surv. P. Rico*: 40 (1926).
Exemplar genera: *Corynelia* Ach. 1823, *Caliciopsis* Peck 1880.

Order: **Eurotiales** G. W. Martin ex Benny & Kimbr., *Mycotaxon* **12**: 23 (1980).
Synonym: *Eurotiales* G. W. Martin, *Std. nat. Hist. Iowa Univ.* **18**(Suppl.): 16 (1941), *nomen invalidum*.
Exemplar genera: *Eurotium* Link 1809, *Emericella* Berk. 1857, *Talaromyces* C. R. Benj. 1955, *Elaphomyces* Nees 1820, *Trichocoma* Jungh. 1838, *Byssochlamys* Westling 1909.

Order: **Onygenales** Cif. ex Benny & Kimbr., *Mycotaxon* **12**: 8 (1980).
Synonym: *Onygenales* Cif., *Atti Ist. Bot. Univ. Pavia, ser.* 5, **14**: 238 (1957), *nomen invalidum*.
Emend. Currah *Mycotaxon* **24**: 13 (1985).
Exemplar genera: *Onygena* Pers. 1799, *Gymnoascus* Baran. 1872, *Arthroderma* Curr. 1860.

Subclass: **Mycocaliciomycetidae** Tibell. **subclass nov.**
MycoBank no.: MB 501288

Parasitae vel commensales in lichenibus vel saprotrophici. Ascomata disciformia, stipitata vel sessilia. Excipulum cupulatum, saltem partim scleroticum hyphis stipitis simile. Dispersio sporarum activa, raro passiva et tum mazedio parce evoluto. Asci unitunicati, cylindrici, vulgo apice distincte incrassato, 8-spori. Ascosporae pallidae ad atrofuscae, ellipsoidales, non-septatae vel transversaliter 1–7-septatae. Paries sporae atrofuscus, laevis vel ornamento intra plasmalemma formato. Derivata acidi vulpinici in speciebus paucis praesentia. Anamorphae coelomycetum et hyphomycetum variae praesentes.
Typus: Mycocalicium Vain. 1890.

Parasites or commensals on lichens or saprobes. Ascomata disciform, stalked or sessile. Excipulum cupulate, and like the stalk hyphae at least in part sclerotized. Spore dispersal active, more rarely passive and ascomata then with a moderately developed mazaedium. Asci unitunicate, cylindrical, mostly with a distinctly thickened apex, 8-spored. Ascospores pale to blackish brown, ellipsoidal or spherical to cuboid, non-septate or transversely 1–7-septate. Spore wall pigmented, smooth or with an ornamentation formed within the plasmalemma. Vulpinic acid derivatives occur in a few species. A variety of coelomycetous and hyphomycetous anamorphs occur.

Order: **Mycocaliciales** Tibell & Wedin, *Mycologia* **92**: 579 (2000).
Exemplar genera: *Mycocalicium* Vain. 1890, *Chaenothecopsis* Vain. 1927, *Stenocybe* (Nyl.) Körb. 1855, *Sphinctrina* Fr. 1825.

Class: **Laboulbeniomycetes** Engl., *Syll. Pflanzenfam.* (2nd edn): 46 (1898).

Order: **Laboulbeniales** Lindau, *in* Engler & Prantl (eds), *Nat. Pflanzenfam.* **1**(1): 491 (1897), as '*Laboulbeniineae*'.
Exemplar genera: *Laboulbenia* Mont. & C.P. Robin 1835, *Rickia* Cavara 1899, *Ceratomyces* Thaxt. 1892.

Order: **Pyxidiophorales** P. F. Cannon, *in* Kirk *et al.*, *Ainsworth & Bisby's Dict. Fungi* (9th edn): xi (2001).
Exemplar genus: *Pyxidiophora* Bref. & Tavel 1891.

Class: **Lecanoromycetes** O. E. Erikss. & Winka, *Myconet* **1**: 7 (1997).

Subclass: **Acarosporomycetidae** Reeb, Lutzoni & Cl. Roux, *Mol. Phylogen. Evol.* **32**: 1053 (2004).

Order: **Acarosporales** Reeb, Lutzoni & Cl. Roux, **ord. nov.**
MycoBank no.: MB 501289

Ascomycetes lichenisati algas virides thallo continentes. Ascomata immersa vel sessilia, disciformia vel perithecioidea. Excipulum hyalinum, annulatum. Hymenium non-amyloideum. Paraphyses mediocriter vel infirme ramosae, septatae, mediocriter vel infirme anastomosantes. Asci unitunicati, non-amyloidei vel satis infirme amyloidei, polyspori. Ascosporae hyalinae, non-septatae, non-halonatae.
Typus: *Acarospora* A. Massal. 1852.

Lichen-forming ascomycetes with chlorococcoid photobiont. Ascomata immersed or sessile, disciform or perithecioid. True exciple hyaline, annulate. Hymenium non-

amyloid. Paraphyses moderately to poorly branched, septate, moderately to poorly anastomosing. Asci functionally unitunicate, lecanoralean, non-amyloid or with slightly amyloid tholi, polyspored, generally with more than 100 ascospores per ascus. Ascospores hyaline, small, non-septate, non-halonate.

The members of this order were formerly classified within the *Lecanorales*, but Reeb *et al.* (2004) and Lutzoni *et al.* (2004) demonstrated that the *Acarosporaceae* diverged earlier than the *Lecanoromycetidae* and *Ostropomycetidae*. This early divergence within the *Lecanoromycetes* was confirmed by Wedin *et al.* (2005) and Miądlikowska *et al.* (2007).

Exemplar genera: *Acarospora* A. Massal. 1852, *Pleopsidium* Körb. 1855, *Sarcogyne* Flot. 1851.

Subclass: **Lecanoromycetidae** P. M. Kirk, P. F. Cannon, J. C. David & Stalpers ex Miądl., Lutzoni & Lumbsch, **subclass. nov.**
MycoBank no.: MB 501290
Synonym: *Lecanoromycetidae* P. M. Kirk, P. F. Cannon, J. C. David & Stalpers, *Ainsworth & Bisby's Dict. Fungi* (9th edn): xi (2001), *nomen invalidum*.

Ascomycetes lichenisati algas virides vel cyanobacteria thallo continentes. Ascomata immersa, sessilia vel elevata, generaliter disciformia. Excipulum hyalinum vel pigmentatum, annulatum vel cupulatum. Hymenium amyloideum vel non-amyloideum. Paraphyses simplices vel ramosae, septatae, anastomosantes vel non-anastomosantes. Asci bitunicati, unitunicati vel prototunicati, non-amyloidei vel amyloidei, generaliter octospori, sed etiam 1- ad multispori. Ascosporae hyalinae vel brunneae, non-septatae, vel septate usque ad muriformes, halonatae vel non-halonatae.

Typus: *Lecanora* Ach. 1809.

Lichen-forming ascomycetes with green algal or cyanobacterial photobiont. Ascomata immersed, sessile or stalked, usually disciform. True exciple hyaline or pigmented, annulate or cupulate. Hymenium amyloid or non-amyloid. Paraphyses simple or moderately to richly branched, septate, anastomosing or not. Asci bitunicate, functionally unitunicate, or prototunicate, lecanoralean, non-amyloid or amyloid, mostly 8-spored, but varying from 1- to poly-spored. Ascospores hyaline or brown, non-septate, trans-septate or muriform, halonate or non-halonate.

This subclass includes the bulk of lichenized discomycetes and corresponds to the phylogenetic circumscription of this subclass by Reeb *et al.* (2004), Lutzoni *et al.* (2004) and Miądlikowska *et al.* (2007). It is in agreement with the *Lecanorales* of Lumbsch *et al.* (2004) and Wiklund & Wedin (2004). The orders *Peltigerales* and *Teloschistales* are here accepted at the ordinal level, following Miądlikowska & Lutzoni (2003) and Miądlikowska *et al.* (2007).

Order: **Lecanorales** Nannf., *Nova Acta R. Soc. Scient. Upsal*, ser. 4 **8**(2): 68 (1932).

Exemplar genera: *Cladonia* Hill. ex P. Browne 1756, *Lecanora* Ach. 1809, *Parmelia* Ach. 1803, *Ramalina* Ach. 1809, *Usnea* Dill. ex Adans. 1763

Order: **Peltigerales** Walt. Watson, *New Phytologist* **28**: 9 (1929).

Exemplar genera: *Coccocarpia* Pers. 1827, *Collema* F. H. Wigg. 1780, *Nephroma* Ach. 1810, *Pannaria* Del. ex Bory 1828, *Peltigera* Willd. 1787.

Order: **Teloschistales** D. Hawksw. & O. E. Erikss., *Syst. Ascom.* **5**: 183 (1986).

Exemplar genera: *Caloplaca* Th. Fr. 1861, *Teloschistes* Norman 1853, *Xanthoria* (Fr.) Th. Fr. 1860.

Subclass: **Ostropomycetidae** Reeb, Lutzoni & Cl. Roux, *Mol. Phylogen. Evol.* **32**: 1055 (2004).

Order: **Agyriales** Clem. & Shear, *Gen. Fungi*: 141 (1931).

Exemplar genera: *Agyrium* Fr. 1822, *Placopsis* (Nyl.) Linds. 1867, *Trapelia* M. Choisy 1929, *Trapeliopsis* Hertel & Gotth. Schneid. 1980.

Order: **Baeomycetales** Lumbsch, Huhndorf & Lutzoni, **ord. nov.**
MycoBank no.: MB 501291

Ascomycetes lichenisati algas virides thallo continentes. Ascomata elevata vel raro sessilia, disciformia. Excipulum hyalinum vel pigmentatum, annulatum vel cupulatum. Hymenium non-amyloideum. Paraphyses ramosae, septatae. Asci unitunicati, non-amyloidei vel satis infirme amyloidei, octospori. Ascosporae hyalinae, non-septatae vel septatae, halonatae vel non-halonatae.

Typus: *Baeomyces* Pers. 1794.

Lichen-forming ascomycetes with chlorococcoid photobiont. Ascomata sessile or rarely stalked, disciform. True exciple hyaline or pigmented, annulate or cupulate. Hymenium non-amyloid. Paraphyses moderately to richly branched, septate. Asci unitunicate, non-amyloid or with slightly amyloid tholi, 8-spored. Ascospores hyaline, non-septate or trans-septate, halonate or non-halonate.

Baeomycetales was shown to differ from *Agyriales* by Kauff & Lutzoni (2002) and this was confirmed by Miądlikowska *et al.* (2007) and Lumbsch *et al.* (2007).

Exemplar genera: *Ainoa* Lumbsch & I. Schmitt 2001, *Baeomyces* Pers. 1794, *Phyllobaeis* Gierl & Kalb 1993.

Order: **Ostropales** Nannf., *Nova Acta R. Soc. Scient. Upsal.*, ser. 4 **8**(2): 68 (1932).

This order includes also taxa formerly classified in separate orders, such as *Gomphillales*, *Graphidales*, *Gyalectales* and *Trichotheliales*.

Exemplar genera: *Ostropa* Fr. 1825, *Stictis* Pers. 1799, *Gyalecta* Ach. 1808, *Gomphillus* Nyl. 1855, *Graphis* Adans. 1763., *Odontotrema* Nyl. 1858, *Porina* Müll. Arg. 1883, *Thelotrema* Ach. 1803.

Order: **Pertusariales** M. Choisy ex D. Hawksw. & O. E. Erikss., *Syst. Ascom.* **5**: 181 (1986).
Synonym: *Pertusariales* M. Choisy, *Bull. mens. Soc. linn. Lyon* **18**: 12 (1949), *nomen invalidum*.

This order may not be monophyletic as currently circumscribed, with *Ochrolechiaceae* and some groups of the heterogeneous *Pertusaria* clustering in a separate clade, but without support. Nonetheless, a cluster of taxa in a 'core' group of *Pertusariales* has been strongly supported as monophyletic in phylogenetic analyses by Miądlikowska *et al.* (2007), Lücking

et al. (2004), Schmitt et al. (2005), Lutzoni et al. (2004), and Grube et al. (2004).

Exemplar genera: *Coccotrema* Müll. Arg. 1888, *Icmadophila* Trevis. 1853, *Ochrolechia* A. Massal. 1852, *Pertusaria* DC. 1805.

Lecanoromycetes *incertae sedis* (not placed in any subclass):

Order: **Candelariales** Miadl., Lutzoni & Lumbsch, **ord. nov.**
MycoBank no.: MB 501292

Ascomycetes lichenisati algas virides thallo continentes. Ascomata sessilia, disciformia. Excipulum hyalinum, annulatum. Hymenium amyloideum. Paraphyses ramosae, septatae. Asci unitunicati, amyloidei, ad typum *Candelariae* dictum pertinentes, octo- vel saepe multispori. Ascosporae hyalinae, non-septatae vel raro 1-septatae.

Typus: *Candelaria* A. Massal. 1853.

Lichen-forming ascomycetes with chlorococcoid photobiont, predominantly nitrophilous. Thallus of various morphology, yellow to orange (pulvinic acid derivatives). Ascomata apothecial, sessile, with or without a distinct margin, yellow to orange. The ascomatal wall formed from densely septate twisted hyphae. paraphyses mostly simple. Excipulum hyaline, hymenium amyloid. Asci unitunicate of *Candelaria*-type with the amyloid lower part of the apical dome and broad apical cushion, often multispored. Ascospores hyaline, aseptate, rarely 1-septate.

Candelariales was shown to differ from *Lecanorales* by Wedin et al. (2005) and this was confirmed by Hofstetter et al. (2007) and Miądlikowska et al. (2007).

Exemplar genera: *Candelaria* A. Massal. 1853, *Candelariella* Müll. Arg. 1894.

Order: **Umbilicariales** Lumbsch, Hestmark & Lutzoni, **ord. nov.**
MycoBank no.: MB 501293

Ascomycetes lichenisati algas virides thallo continentes. Ascomata sessilia, raro immersa usque ad pauce elevata, plerumque atra, irregularia, disciformia. Excipulum pigmentatum, annulatum. Hymenium amyloideum. Paraphyses simplices vel paulum ramosae, septatae. Asci unitunicati, tholo inconspicue amyloideo, 1–8-spori. Ascosporae hyalinae vel brunneae, non-septatae usque ad muriformes.

Typus: *Umbilicaria* Hoffm. 1789.

Lichen-forming ascomycetes with chlorococcoid photobiont. Ascomata sessile, or rarely immersed or stalked, mostly black, irregular, disciform. True exciple pigmented, annulate. Hymenium amyloid. Paraphyses simple or slightly branched, septate, apically thickened. Asci unitunicate, with slightly amyloid tholi, 1–8-spored. Ascospores hyaline or brown, non-septate to muriform.

Exemplar genera: *Lasallia* Mérat 1821, *Umbilicaria* Hoffm. 1789.

Class: **Leotiomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 7 (1997).
Excluding *Geoglossaceae* (Wang et al. 2006).

Order: **Cyttariales** Luttr. ex Gamundí, *Darwiniana* **16**: 502 (1971).
Synonym: *Cyttariales* Luttr., *Univ. Miss. Stud.* **24**(2): 109 (1951), *nomen invalidum*.
Exemplar genus: *Cyttaria* Berk. 1842.

Order: **Erysiphales** H. Gwynne-Vaughan, *Fungi, Ascom., Ustilag., Ured.*: 78 (1922).
Exemplar genera: *Erysiphe* R. Hedw. ex DC. 1805, *Blumeria* Golovin ex Speer 1975, *Uncinula* Lév. 1851.

Order: **Helotiales** Nannf., *Nova Acta R. Soc. Scient. Upsal., ser.* 4 **8**(2): 68 (1932).

Based on current character and taxon sampling (Wang et al. 2006, 2007; Spatafora et al. 2007), the monophyly of *Helotiales s. lat.* is not well supported. There exists a minimum of five helotialean lineages that are intermixed with other leotiomycetan taxa (e.g. *Cyttariales*, *Erysiphales*) resulting in a paraphyletic *Helotiales s. lat.* The interrelationships of these taxa are poorly resolved, however, thus preventing the synthesis of an accurate phylogenetic classification at this time. *Leotiomycetes* represents one of the more undersampled higher taxa among the *Ascomycota*, and it is likely that future sampling will result in a phylogenetic classification of a more restricted *Helotiales* and the recognition of additional orders based on current helotialean families (e.g. *Leotiaceae* or *Helotiaceae*, *Sclerotiniaceae*).

Exemplar genera: *Mitrula* Fr. 1821, *Hymenoscyphus* Gray 1821, *Ascocoryne* J.W. Groves & D.E. Wilson 1967.

Order: **Rhytismatales** M. E. Barr ex Minter, *in* Hawksworth & Eriksson, *Syst. Ascom.* **5**: 182 (1986).
Synonym: *Rhytismatales* M. E. Barr, *Mem. N. Y. Bot.Gdn* **28**: 6 (1976), *nomen invalidum*.
Exemplar genera: *Rhytisma* Fr. 1818, *Lophodermium* Chevall. 1826, *Cudonia* Fr. 1849.

Order: **Thelebolales** P. F. Cannon, *in* Kirk *et al.*, *Ainsworth & Bisby's Dict. Fungi* (9th edn): xi (2001).
Exemplar genera: *Thelebolus* Tode 1790, *Coprotus* Korf ex Korf & Kimbr. 1967, *Ascozonus* (Renny) E.C. Hansen 1876.

Class: **Lichinomycetes** Reeb, Lutzoni & Cl. Roux., *Mol. Phylogen. Evol.* **32**: 1055 (2004).

Order: **Lichinales** Henssen & Büdel, *in* Hawksworth & Eriksson, *Syst. Ascom.* **5**: 138 (1986).
Exemplar genera: *Heppia* Nägeli ex A. Massal. 1854, *Lichina* C. Agardh 1817, *Peltula* Nyl. 1853.

Class: **Orbiliomycetes** O. E. Erikss. & Baral, *in* Eriksson *et al.*, *Myconet* **9**: 96 (2003).

Order: **Orbiliales** Baral, O. E. Erikss., G. Marson & E. Weber, *in* Eriksson *et al.*, *Myconet* **9**: 96 (2003).
Exemplar genera: *Orbilia* Fr. 1849, *Hyalorbilia* Baral & G. Marson 2000.

Class: **Pezizomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 8 (1997).

Order: **Pezizales** J. Schröt., *in* Engler & Prantl (eds), *Nat. Pflanzenfam.* **1**: 173 (1894), as '*Pezizineae*'.

Exemplar genera: *Peziza* Fr. 1822, *Glaziella* Berk. 1880, *Morchella* Dill. ex Pers. 1794, *Pyronema* Carus 1835, *Tuber* F.H. Wigg. 1780.

Glaziella has been described several times, *inter alia* as a zygomycete. Gibson *et al.* (1986) demonstrated it was an ascomycete and proposed a new family and order close to *Pezizales*, but small subunit rRNA gene sequences show that it should be included in *Pezizales* (Landvik & Eriksson 1994).

Class: **Sordariomycetes** O. E. Erikss. & Winka, *Myconet* **1**: 10 (1997).

Subclass: **Hypocreomycetidae** O. E. Erikss. & Winka, *Myconet* **1**: 6 (1997).

Order: **Coronophorales** Nannf., *Nova Acta R. Soc. Scient. Upsal, ser.* 4 **8**: 54 (1932).

Exemplar genera: *Nitschkia* G.H. Otth ex P. Karst. 1873, *Scortechinia* Sacc. 1885, *Bertia* De Not. 1844, *Chaetosphaerella* E. Müll. & C. Booth 1972.

Order: **Hypocreales** Lindau, *in* Engler & Prantl (eds), *Nat. Pflanzenfam.* **1**: 343 (1897).

Exemplar genera: *Hypocrea* Fr. 1825, *Nectria* (Fr.) Fr. 1849, *Cordyceps* (Fr.) Link 1833, *Claviceps* Tul. 1853, *Niesslia* Auersw. 1869.

Order: **Melanosporales** N. Zhang & M. Blackw., **ord. nov.**
MycoBank no.: MB 501294

Ascomata perithecialia vel nonnumquam ostiolo carentia; peridium ascomatis e basi glomeris ascogonialis oriundum, translucidum; centrum pseudoparenchymaticum, paraphysibus absentibus; asci unitunicati, evanescentes; ascosporae fuscae, poro germinationis utrinque praeditae; anamorphae hyphomycetales. Fungi saepe mycoparasitici.

Typus: *Melanospora* Corda 1837.

Ascoma perithecial or secondarily cleistothecial, peridium derived from base of an ascogonial coil, translucent; centrum pseudoparenchymatous, paraphyses absent in development; asci unitunicate, evanescent; ascospores dark, with germ pores at both ends; anamorphs hyphomycetous; often mycoparasitic.

Exemplar genus: *Melanospora* Corda 1837.

Order: **Microascales** Luttr. ex Benny & Kimbr., *Mycotaxon* **12**: 40 (1980).
Synonym: *Microascales* Luttr., *Univ. Miss. Stud.* **24**(2): 108 (1951), *nomen invalidum*.

The group as recognized here includes members of the *Halosphaeriales*. In Zhang *et al.* (2007) and Tang *et al.* (2007), the *Halosphaeriales* were maintained separate from the *Microascales*.

Exemplar genera: *Microascus* Zukal 1885, *Petriella* Curzi 1930, *Halosphaeria* Linder 1944, *Lignincola* Höhnk 1955, *Nimbospora* J. Koch 1982.

Subclass: **Sordariomycetidae** O. E. Erikss. & Winka, *Myconet* **1**: 10 (1997).

Order: **Boliniales** P. F. Cannon, *in* Kirk *et al.*, *Ainsworth & Bisby's Dict. Fungi* (9th edn): x (2001).

Exemplar genera: *Camarops* P. Karst. 1873, *Apiocamarops* Samuels & J. D. Rogers 1987.

Order: **Calosphaeriales** M. E. Barr, *Mycologia* **75**: 11 (1983).

This order has not been placed in a subclass but the work of Réblová *et al.* (2004) shows that it may be related to the *Diaporthales*. Members of this group were not included in Zhang *et al.* (2007) or Tang *et al.* (2007).

Exemplar genera: *Calosphaeria* Tul. & C. Tul. 1863, *Togniniella* Réblová, L. Mostert, W. Gams & Crous 2004, *Pleurostoma* Tul. & C. Tul. 1863.

Order: **Chaetosphaeriales** Huhndorf, A. N. Mill. & F. A. Fernández, *Mycologia* **96**: 378 (2004).

Exemplar genera: *Chaetosphaeria* Tul. & C. Tul. 1863, *Melanochaeta* E. Müll., Harr & Sulmont 1969, *Zignoëlla* Sacc. 1878, *Striatosphaeria* Samuels & E. Müll. 1979.

Order: **Coniochaetales** Huhndorf, A. N. Mill. & F. A. Fernández, *Mycologia* **96**: 378 (2004a).

Exemplar genera: *Coniochaeta* (Sacc.) Cooke 1887, *Coniochaetidium* Malloch & Cain 1971.

Order: **Diaporthales** Nannf., *Nova Acta R. Soc. Scient. upsal., ser.* 4 **8**: 53 (1932).

Exemplar genera: *Diaporthe* Nitschke 1870, *Gnomonia* Ces. & De Not. 1863, *Cryphonectria* (Sacc.) Sacc. & D. Sacc. 1905, *Valsa* Fr. 1849.

Order: **Ophiostomatales** Benny & Kimbr., *Mycotaxon* **12**: 48 (1980).

Exemplar genera: *Ophiostoma* Syd. & P. Syd. 1919, *Fragosphaeria* Shear 1923.

Order: **Sordariales** Chadef. ex D. Hawksw. & O. E. Erikss., *Syst. Ascom.* **5**: 182 (1986).
Synonym: *Sordariales* Chadef., *in* Chadefaud & Emberger, *Traité Bot.* **1**: 594 (1960), *nomen invalidum*.

Exemplar genera: *Sordaria* Ces. & De Not. 1863, *Podospora* Ces. 1856, *Neurospora* Shear & B.O. Dodge 1927, *Lasiosphaeria* Ces. & De Not. 1863, *Chaetomium* Kunze 1817.

Subclass: **Xylariomycetidae** O. E. Erikss. & Winka, *Myconet* **1**: 12 (1997).

Order: **Xylariales** Nannf., *Nova Acta R. Soc. Scient. Upsal., ser.* 4, **8**: 66 (1932).

Exemplar genera: *Xylaria* Hill ex Schrank 1789, *Hypoxylon* Bull. 1791, *Anthostomella* Sacc. 1875, *Diatrype* Fr. 1849, *Graphostroma* Piroz. 1974.

Sordariomycetes *incertae sedis* (not placed in any subclass)

Order: **Lulworthiales** Kohlm., Spatafora & Volkm-Kohlm., *Mycologia* **92**: 456 (2000).
This order includes members formerly placed in the *Spathulosporales*.
Exemplar genera: *Lulworthia* G. K. Sutherl. 1916, *Lindra* I.M. Wilson 1956.

Order: **Meliolales** Gäum. ex D. Hawksw. & O. E. Erikss., *Syst. Ascom.* **5**: 180 (1986).
Synonym: *Meliolales* Gäum., *Pilze* (2nd edn): 158 (1964), *nomen invalidum*.
Exemplar genus: *Meliola* Fr. 1825.

Order: **Phyllachorales** M. E. Barr, *Mycologia* **75**: 10 (1983).
Exemplar genus: *Phyllachora* Nitschke ex Fuckel 1870.

Order: **Trichosphaeriales** M. E. Barr, *Mycologia* **75**: 11 (1983).
Exemplar genus: *Trichosphaeria* Fuckel 1870.

Pezizomycotina *incertae sedis* (not placed in any class)

Order: **Lahmiales** O. E. Erikss., *Mycotaxon* **27**: 357 (1986).
Exemplar genus: *Lahmia* Körb. 1861.

Order: **Medeolariales** Korf, *in* Eriksson *Mycotaxon* **15**: 232 (1982).
Exemplar genus: *Medeolaria* Thaxt. 1922.
Order: **Triblidiales** O. E. Erikss., *Syst. Ascom.* **11**: 9 (1992).
Exemplar genera: *Huangshania* O. E. Erikss. 1992, *Pseudographis* Nyl. 1855, *Triblidium* Rebent. 1804.

Phylum: **Basidiomycota** R. T. Moore, *Bot. Mar.* **23**: 371 (1980).
Synonyms: *Basidiomycota* Bold, *Morph. Pl.*: 7, 198 (1958), *nomen invalidum*;
Basidiomycetes Whittaker (1959: 220), *nomen invalidum*. (Table 3, Fig 3) As in the case of *Fungi*, Moore (1980) validated a name that had already been used by Bold (1957), but he did not cite Bold's work.

Subphylum: **Pucciniomycotina** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 45 (2006).
Equivalent to *Urediniomycetes* (Kirk *et al.* 2001; Swann & Taylor 1995; Swann *et al.* 2001). The classification of *Pucciniomycotina* employed here parallels that of Bauer *et al.* (2006) and Aime *et al.* (2007).

Class: **Pucciniomycetes** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 48 (2006).
Equivalent to *Urediniomycetidae* (Swann *et al.* 2001).

Order: **Septobasidiales** Couch ex Donk, *Persoonia* **3**: 243 (1964).
Synonym: *Septobasidiales* Couch, *Gen. Septobasidium*: 65 (1938), *nomen invalidum*.
Exemplar genera: *Septobasidium* Pat. 1892, *Auriculoscypha* D. A. Reid & Manim. 1985.

Order: **Pachnocybales** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 48 (2006).
Exemplar genus: *Pachnocybe* Berk. 1836.

Order: **Helicobasidiales** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 48 (2006).
Exemplar genera: *Helicobasidium* Pat. 1885, *Tuberculina* Tode ex Sacc. 1880.

Order: **Platygloeales** R. T. Moore, *Mycotaxon* **39**: 247 (1990).
Equivalent to *Platygloeales s. str.* (Swann *et al.* 2001).
Exemplar genera: *Platygloea* J. Schröt. 1887 *s. str.*, *Eocronartium* G.F. Atk. 1902.

Order: **Pucciniales** Clem. & Shear, *Gen. Fungi* (2nd edn): 147 (1931).
Equivalent to *Uredinales*.
Exemplar genera: *Puccinia* Pers. 1801, *Uromyces* (Link) Unger 1832.

Class: **Cystobasidiomycetes** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 46 (2006).
Equivalent to the *Erythrobasidium–Naohidea–Sakaguchia* clade (Swann *et al.* 2001) and *Cystobasidiaceae* lineage (Weiß *et al.* 2004a). Genera of *Cystobasidiomycetes* that are not placed in any order include *Sakaguchia* Y. Yamada, K. Maeda & Mikata 1994, and *Cyrenella* Goch. 1981 (Aime *et al.* 2007; Bauer *et al.* 2006).

Order: **Cystobasidiales** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 46 (2006).
Exemplar genera: *Cystobasidium* (Lagerh.) Neuhoff 1924, *Occultifur* Oberw. 1990, *Rhodotorula* F.C. Harrison 1927 *pro parte*.

Order: **Erythrobasidiales** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 46 (2006).
Exemplar genera: *Erythrobasidium* Hamam. Sugiyama & Komag. 1988, *Rhodotorula* F. C. Harrison 1927 *pro parte*, *Sporobolomyces* Kluyver & C. B. Niel 1924 *pro parte*, *Bannoa* Hamam. 2002.

Order: **Naohideales** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 46 (2006).
Exemplar genus: *Naohidea* Oberw. 1990.

Class: **Agaricostilbomycetes** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 45 (2006).
Equivalent to *Agaricostilbomycetidae* (Swann *et al.* 2001; Weiß *et al.* 2004a).

Order: **Agaricostilbales** Oberw. & R. Bauer, *Sydowia* **41**: 240 (1989).
Exemplar genera: *Agaricostilbum* J. E. Wright 1970 (emend. Wright, Bandoni & Oberw. 1981), *Chionosphaera* D. E. Cox 1976, *Kondoa* Y. Yamada, Nakagawa & I. Banno 1989 (*emend.* Fonseca, Sampaio, Inácio & Fell 2000).

Order: **Spiculogloeales** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 45 (2006).
Equivalent to *Mycogloea* group (Weiß *et al.* 2004a).
Exemplar genera: *Mycogloea* L. S. Olive 1950, *Spiculogloea* P. Roberts 1996, *Sporobolomyces* Kluyver & C. B. Niel 1924 *pro parte*.

Table 3 – Support for major groups of Fungi in selected phylogenetic studies: Basidiomycota

Rank	Taxon	Reference	Data	OTUs	Support
Phylum	*BASIDIOMYCOTA*	James *et al.* (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	50	BPP = 1 MLBS = 80
Subphylum	*Pucciniomycotina*	Matheny *et al.* (2007a, fig. 4)	SSU, LSU, 5.8S, *rpb1*, *rpb2*, *tef1*	17	BPP = 1 MPBS = 100
		Aime *et al.* (2007, fig. 2)	LSU, SSU	109	BPP = 1 MPBS = 100
Class	*Pucciniomycetes*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	7	BPP > 0.95 MPBS > 70
		Matheny *et al.* (2007a, fig. 5)	LSU, SSU, 5.8S	24	BPP = 0.97 MPBS ≥ 70
		Aime *et al.* (2007, fig. 2)	LSU, SSU	19	BPP = 1 MPBS = 100
		Aime *et al.* (2007, fig. 3)	LSU, SSU	41	MPBS = 86
Order	*Septobasidiales*	Arun Kumar *et al.* (2007, fig. 7)	LSU, SSU	4	BPP = 1 MPBS = 100
Order	*Pachnocybales*	Bauer *et al.* (2006, fig. 1)	LSU	1	NA
		Berres *et al.* (1995, fig. 4)	LSU	1	NA
Order	*Helicobasidiales*	Aime *et al.* (2007, fig. 2)	LSU, SSU	2	BPP = 1 MPBS = 96 NJBS = 98
		Aime *et al.* (2007, fig. 3)	LSU, SSU	10	MPBS = 87
Order	*Platygloeales*	Aime *et al.* (2007, fig. 2)	LSU, SSU	4	BPP = 1 MPBS = 100 NJBS = 100
		Aime *et al.* (2007, fig. 3)	LSU, SSU	8	MPBS = 99
		Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	2	BPP > 0.95 MPBS > 70
Order	*Pucciniales*	Aime *et al.* (2007, fig. 2)	LSU, SSU	12	BPP = 1 MPBS = 100 NJBS = 100
		Aime (2006)	LSU	46	MPBS = 99
		Wingfield *et al.* (2004)	SSU	72	MPBS < 50
		Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	5	BPP > 0.95 MPBS > 70
Class	*Cystobasidiomycetes*	Aime *et al.* (2007, fig. 2)	LSU, SSU	27	BPP = 1 MPBS = 100 NJBS = 96
		Sampaio (2004, fig. 1)	LSU	11	BPP = 0.92
		Sampaio (2004, fig. 2)	LSU	26	BPP = 0.98
Order	*Cystobasidiales*	Nagahama *et al.* (2006, fig. 2)	LSU, SSU, 5.8S, *tef1*	9	MLBS = 100
		Aime *et al.* (2007, fig. 2)	LSU, SSU	12	BPP = 1 MPBS = 100 NJBS = 100
		Sampaio (2004, fig. 2)	LSU	8	BPP = 1
Order	*Erythrobasidiales*	Nagahama *et al.* (2006, fig. 2)	LSU, SSU, 5.8S, *tef1*	21	MLBS = 72
		Aime *et al.* (2007, fig. 2)	LSU, SSU	14	BPP = 1 MPBS = 83 NJBS = 91
		Sampaio (2004, fig. 2)	LSU	18	BPP = 1
Order	*Naohideales*	Aime *et al.* (2007, fig. 3)	LSU, SSU	2	MPBS = 98
		Weiß *et al.* (2004)	LSU	3	BPP = 0.94 NJBS < 50
Class	*Agaricostilbomycetes*	Aime *et al.* (2007, fig. 2)	LSU, SSU	25	BPP = 1 MPBS < 70 NJBS < 70
		Bauer *et al.* (2006, fig. 2)	LSU, SSU	4	NJBS = 89
		Matheny *et al.* (2007a, fig. 5)	LSU, SSU, 5.8S	8	BPP = 1 MPBS > 70
Order	*Agaricostilbales*	Aime *et al.* (2007, fig. 2)	LSU, SSU	22	BPP = 1 MPBS = 100 NJBS = 100
		Aime *et al.* (2007, fig. 2)	LSU, SSU	34	MPBS = 98
		Sampaio (2004, fig. 1)	LSU	7	BPP = 1
		Sampaio (2004, fig. 2)	LSU	23	BPP = 1

(*continued on next page*)

Table 3 (continued)

Rank	Taxon	Reference	Data	OTUs	Support
		Fell et al. (2001)	LSU	24	MPBS = 64
Order	*Spiculogloeales*	Aime et al. (2007, fig. 2)	LSU, SSU	3	BPP = 1 MPBS = 100 NJBS = 100
		Aime et al. (2007, fig. 3)	LSU, SSU	7	MPBS = 74
		Bauer et al. (2006, fig. 2)	LSU, SSU	2	NJBS = 90
Class	*Microbotryomycetes*	Aime et al. (2007, fig. 2)	LSU, SSU	31	BPP = 1 MPBS = 100 NJBS = 100
		Aime et al. (2007, fig. 3)	LSU, SSU	60	MPBS = 74
		Matheny et al. (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	6	BPP > 0.95 MPBS > 70
		Sampaio (2004, fig. 2)	LSU	49*	BPP = 0.87
		Fell et al. (2001)	LSU	78	MPBS = 75
Order	*Heterogastridiales*	Bauer et al. (2006, fig. 2)	LSU, SSU	1	NA
Order	*Microbotryales*	Aime et al. (2007, fig. 2)	LSU, SSU	4	BPP = 1 MPBS = 99 NJBS = 94
		Aime et al. (2007, fig. 3)	LSU, SSU	12	MPBS = 82
Order	*Leucosporidiales*	Aime et al. (2007, fig. 2)	LSU, SSU	3	BPP = 0.98 MPBS = 85 NJBS = 100
		Aime et al. (2007, fig. 3)	LSU, SSU	9	MPBS = 67
Order	*Sporidiobolales*	Aime et al. (2007, fig. 2)	LSU, SSU	13	BPP = 1 MPBS = 74 NJBS = 68
		Aime et al. (2007, fig. 3)	LSU, SSU	17	MPBS = 69
		Sampaio (2004, fig. 2)	LSU	20	BPP = 0.98
Class/Order	*Atractiellomycetes*, *Atractiellales*	Aime et al. (2007, fig. 2)	LSU, SSU	4	BPP = 1 MPBS = 80 NJBS = 96
		Aime et al. (2007, fig. 3)	LSU, SSU	8	MPBS = 68
		Bauer et al. (2006, fig. 2)	LSU, SSU	7	NJBS = 68
Class/Order	*Classiculomycetes*, *Classiculales*	Aime et al. (2007, fig. 2)	LSU, SSU	2	BPP = 1 MPBS = 100 NJBS = 100
		Weiß et al. (2004, figs. 1–2)	LSU	2	BPP = 1 NJBS = 99
Class/Order	*Mixiomycetes*, *Mixiales*	Aime et al. (2007, fig. 2)	LSU, SSU	1	NA
		Bauer et al. (2006, fig. 2)	LSU, SSU	1	NA
Class/Order	*Cryptomycocolacomycetes*, *Cryptomycocolacales*	Aime et al. (2007, fig. 3)	LSU, SSU	1	NA
		Bauer et al. (2006, fig. 1)	LSU	2	NJBS = 100
Subphylum	Ustilaginomycotina	Matheny et al. (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	24	BPP = 1 MPBS = 100
		Matheny et al. (2007a, fig. 5)	LSU, SSU, 5.8S	59	BPP = 1 MPBS > 70
Class	Ustilaginomycetes	Bauer et al. (2006, fig. 2)	LSU, SSU	21	NJBS = 100
		Matheny et al. (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	12	BPP > 0.95 MPBS > 70
		Matheny et al. (2007a, fig. 5)	LSU, SSU, 5.8S	25	BPP = 1 MPBS > 70
		Begerow et al. (2007, fig. 1)	LSU, ITS, *atp6*, *βtub*	53	BPP = 1 MPBS = 83 NJBS = 77
		Bauer et al. (2001, figs. 33–34)	LSU	36	MPBS = 79 NJBS = 93
		Fell et al. (2001, fig. 24)	LSU	27	NJBS = 86
Order	*Urocystales*	Begerow et al. (2007, fig. 1)	LSU, ITS, *atp6*, *βtub*	5	BPP = 1 MPBS = 66 NJBS = 96
		Matheny et al. (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	1	NA
		Bauer et al. (2001, figs. 33–34)	LSU	9	MPBS = 95[3] NJBS = 96[3]

Table 3 (*continued*)

Rank	Taxon	Reference	Data	OTUs	Support
Order	*Ustilaginales*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	10	BPP > 0.95 MPBS > 70
		Matheny *et al.* (2007a, fig. 5)	LSU, SSU, 5.8S	23	BPP > 0.95 MPBS > 70
		Begerow *et al.* (2007, fig. 1)	LSU, ITS, *atp6*, βtub	46	BPP = 1 MPBS < 60 NJBS < 60
Class	Exobasidiomycetes	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	12	BPP > 0.95 MPBS < 50
		Begerow *et al.* (2007, fig. 1)	LSU, ITS, *atp6*, βtub	35	BPP < 0.60 MPBS < 60 NJBS < 60
		Bauer *et al.* (2001, figs. 33–34)	LSU	36	MPBS = 85 NJBS = 56
Order	*Doassansiales*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	1	NA
		Matheny *et al.* (2007a, fig. 5)	LSU, SSU, 5.8S	4	BPP > 0.95 MPBS > 70
		Begerow *et al.* (2007, fig. 1)	LSU, ITS, *atp6*, βtub	4	BPP = 1 MPBS = 84 NJBS = 77
		Bauer *et al.* (2001, figs. 33–34)	LSU	5	MPBS = 96 NJBS = 97
Order	*Entylomatales*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	4	BPP > 0.95 MPBS > 70
		Begerow *et al.* (2007, fig. 1)	LSU, ITS, *atp6*, βtub	3	BPP = 1 MPBS < 60 NJBS < 60
		Bauer *et al.* (2001, figs. 33–34)	LSU	9	MPBS = 72 NJBS = 91
Order	*Exobasidiales*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	2	BPP > 0.95 MPBS > 70
		Matheny *et al.* (2007a, fig. 5)	LSU, SSU	6	BPP > 0.95 MPBS > 70
		Begerow *et al.* (2007, fig. 1)	LSU, ITS, *atp6*, βtub	8	BPP = 1 MPBS < 60 NJBS = 61
Order	*Georgefischeriales*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	2	BPP > 0.95 MPBS > 70
		Begerow *et al.* (2007, fig. 1)	LSU, ITS, *atp6*, βtub	5	BPP < 0.60 MPBS < 60 NJBS < 60
		Bauer *et al.* (2001, figs. 33–34)	LSU	9	MPBS = 86 NJBS = 65
Order	*Microstromatales*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	1	NA
		Matheny *et al.* (2007a, fig. 5)	LSU, SSU, 5.8S	3	BPP > 0.95 MPBS > 70
		Begerow *et al.* (2007, fig. 1)	LSU, ITS, *atp6*, βtub	5	BPP = 1 MPBS = 63 NJBS = 67
Order	*Tilletiales*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	2	BPP > 0.95 MPBS > 70
		Matheny *et al.* (2007a, fig. 5)	LSU, SSU, 5.8S	7	BPP > 0.95 MPBS > 70
		Begerow *et al.* (2007, fig. 1)	LSU, ITS, *atp6*, βtub	5	BPP = 1 MPBS = 76 NJBS = 64
	Ustilaginomycotina incertae sedis (*not placed in any class*)				
Order	*Malasseziales*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	1	NA
		Begerow *et al.* (2007, fig. 1)	LSU, ITS, *atp6*, βtub	2	BPP = 1 MPBS = 100 NJBS = 100

(*continued on next page*)

Table 3 (continued)

Rank	Taxon	Reference	Data	OTUs	Support
		Bauer et al. (2001, figs. 33–34)	LSU	4	MPBS = 100 NJBS = 100
Subphylum	*Agaricomycotina*	Matheny et al. (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	125	BPP = 1 MPBS = 95
Class	*Tremellomycetes*	Matheny et al. (2007a, fig. 4)	SSU, LSU, 5.8S, *rpb1*, *rpb2*, *tef1*	5	BPP > 0.95 MPBS = 50-69
		Fell et al. (2001, figs. 19, 22)	LSU	139	MPBS = 100
Order	*Cystofilobasidiales*	Matheny et al. (2007a, fig. 5)	LSU, SSU, 5.8S	5	BPP = 1 MPBS ≥ 70
		Fell & Scorzetti (2004, fig. 1)	LSU	16	BPP = 1 MPBS = 83
Order	*Filobasidiales*	Fell et al. (2001, figs. 19, 22)	LSU	34	MPBS = 96
Order	*Tremellales*	Matheny et al. (2007a, fig. 5)	LSU, SSU, 5.8S	5	BPP ≥ 0.95 MPBS ≥ 70
		Fell et al. (2001, figs. 19, 22)	LSU	89	MPBS = 56
Class/Order	*Dacrymycetes*, *Dacrymycetales*	Matheny et al. (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	4	BPP = 1 MPBS = 100
		Weiß & Oberwinkler (2001, fig. 6)	LSU	9	NJBS = 99
Class	*Agaricomycetes*	Matheny et al. (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	119	BPP = 1 MPBS = 95
		James et al. (2006)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	37	BPP = 1 MLBS = 92
Subclass	*Agaricomycetidae*	Matheny et al. (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	63	BPP = 1 MPBS = 96
		Binder & Hibbett (2007, fig. 2)	LSU, SSU, 5.8S, mt-LSU, *atp6*	47	BPP > 0.98 MLBS = 88
		Binder et al. (2005, fig. 1)	LSU, SSU, mt-LSU, mt-SSU	46	MPBS = 62
Order	*Agaricales*	Matheny et al. (2006, fig. 2)	LSU, SSU, 5.8S	230	BPP = 0.84
		Matheny et al. (2006, fig. 3)	LSU, SSU, 5.8S, *rpb1*, *rpb2*	238	BPP = 1 MPBS = 43
		Matheny et al. (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	41	BPP = 1 MPBS = 76
		Moncalvo et al. (2002, fig. 2)	LSU	786	MPBS < 50
Order	*Atheliales*	Larsson et al. (2004, fig. 1)	LSU	8	MPBS = 97
		Binder et al. (2005, fig. 4)	LSU, SSU, mt-LSU, mt-SSU	3	MPBS = 75
Order	*Boletales*	Matheny et al. (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	11	BPP = 1 MPBS = 100
		Binder & Hibbett (2007, fig. 2)	LSU, SSU, 5.8S, mt-LSU, *atp6*	42	BPP > 0.98 MLBS = 99
		Binder & Hibbett (2007, fig. 3)	LSU	301	BPP > 0.98
Subclass	*Phallomycetidae*	Hosaka et al. (2007, fig. 2)	LSU, mt-SSU, *atp6*, *rpb2*, *tef1*	222	BPP = 1 MPBS = 98
		Matheny et al. (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	3	BPP = 1 MPBS = 100
Order	*Geastrales*	Hosaka et al. (2007, fig. 2)	LSU, mt-SSU, *atp6*, *rpb2*, *tef1*	21	BPP = 1 MPBS = 59
Order	*Gomphales*	Hosaka et al. (2007, fig. 2)	LSU, mt-SSU, *atp6*, *rpb2*, *tef1*	61	BPP = 1 MPBS = 63
Order	*Hysterangiales*	Hosaka et al. (2007, fig. 2)	LSU, mt-SSU, *atp6*, *rpb2*, *tef1*	99	BPP = 1 MPBS = 98
Order	*Phallales*	Hosaka et al. (2007, fig. 2)	LSU, mt-SSU, *atp6*, *rpb2*, *tef1*	41	BPP = 1 MPBS = 84
	Agaricomycetes incertae sedis (*not placed in any subclass*):				
Order	*Auriculariales*	Matheny et al. (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	3	BPP = 1 MPBS = 100
		Weiß & Oberwinkler (2001, fig. 6)	LSU	43	NJBS < 60
Order	*Cantharellales*	Matheny et al. (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	11	BPP = 1 MPBS = 69
		Moncalvo et al. (2007, fig. 1)	LSU, SSU, mtSSU, *rpb2*	29	BPP < 0.50 MPBS < 50
		Binder et al. (2005, fig. 4)	LSU, SSU, mt-LSU, mt-SSU	31	MPBS < 50
Order	*Corticiales*	Larsson et al. (2004, fig. 1)	LSU	7	MPBS = 96

Table 3 (*continued*)

Rank	Taxon	Reference	Data	OTUs	Support
		Binder *et al.* (2005, fig. 4)	LSU, SSU, mt-LSU, mt-SSU	8	MPBS = 81
Order	*Gloeophyllales*	Thorn *et al.* (2000, fig. 5)	LSU	5	MPBS = 71
		Binder *et al.* (2005, fig. 4)	LSU, SSU, mt-LSU, mt-SSU	6	MPBS = 54
	Hymenochaetales	Matheny *et al.* (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	7	BPP = 1 MPBS = 63
		Larsson *et al.* (2007, fig. 3)	LSU, 5.8S	174	BPP = 1
		Wagner & Fischer (2002, fig. 2)	LSU	104	NJBS = 100
Order	*Polyporales*	Matheny *et al.* (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	16	BPP = 1 MPBS = 85
		Binder *et al.* (2005, fig. 4)	LSU, SSU, mt-LSU, mt-SSU	122	MPBS < 50
Order	*Russulales*	Matheny *et al.* (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	8	BPP = 1 MPBS = 99
		Larsson & Larsson (2003, fig. 1)	LSU, 5.8S	127	MPBS = 96
		Miller *et al.* (2007, fig. 2)	LSU, ITS	143	MPBS = 100
Order	*Sebacinales*	Matheny *et al.* (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	2	BPP = 1 MPBS = 100
		Weiß & Oberwinkler (2001, fig. 6)	LSU	9	NJBS = 99
Order	*Thelephorales*	Matheny *et al.* (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	2	BPP = 1 MPBS = 100
		Binder *et al.* (2005, fig. 4)	LSU, SSU, mt-LSU, mt-SSU	13	MPBS = 97
		Larsson *et al.* (2004, fig. 1)	LSU, 5.8S	11	MPBS = 86
Order	*Trechisporales*	Matheny *et al.* (2007b, fig. 6)	LSU, SSU, 5.8S, *rpb2*, *tef1*	2	BPP = 1 MPBS = 100
		Binder *et al.* (2005, fig. 4)	LSU, SSU, mt-LSU, mt-SSU	20	MPBS = 69
		Larsson *et al.* (2004, fig. 1)	LSU, 5.8S	12	MPBS = 99
	Basidiomycota incertae sedis (*not placed in any subphylum*):				
Class/Order	*Wallemiomycetes*, *Wallemiales*	Matheny *et al.* (2007a, fig. 4)	LSU, SSU, 5.8S, *rpb1*, *rpb2*, *tef1*	3	BPP > 0.95 MPBS > 70
		Matheny *et al.* (2007a, fig. 5)	LSU, SSU, 5.8S	3	BPP = 1 MPBS > 70
Class/Order	*Entorrhizomycetes*, *Entorrhizales*	Matheny *et al.* (2007a, fig. 5)	LSU, SSU, 5.8S	3	BPP = 1 MPBS > 70
		Bauer *et al.* (2001, figs. 33–34)	LSU	2	MPBS = 100 NJBS = 100

See Table 1 for explanation.

Class: **Microbotryomycetes** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 47 (2006).

Equivalent to *Microbotryomycetidae* (Swann *et al.* 2001; Weiß *et al.* 2004a). The backbone of the *Microbotryomycetes* remains poorly resolved, and several genera of *Microbotryomycetes* are not placed in any order, including *Colacogloea* Oberw. & R. Bauer 1991, *Atractocolax* R. Kirschner, R. Bauer & Oberw. 1999, *Krieglsteinera* Pouzar 1987, *Camptobasidium* Marvanová & Suberkr. 1990, *Kriegeria* Bres. 1891 and certain species of the polyphyletic genera *Sporobolomyces* Kluyver & C. B. Niel 1924 *pro parte*, *Rhodotorula* F. C. Harrison 1927 *pro parte*, and *Leucosporidium* Fell, Statzell, I. L. Hunter & Phaff 1970, and others (Aime *et al.* 2007; Bauer *et al.* 2006; Sampaio *et al.* 2003; Weiß *et al.* 2004a).

Order: **Heterogastridiales** Oberw., R. Bauer & Bandoni R. J., *Mycologia* **82**: 57 (1990).

Exemplar genus: *Heterogastridium* Oberw. & R. Bauer 1990.

Bauer *et al.* (2006) placed *Colacogloea*, *Atractocolax* and *Krieglsteinera* in the *Heterogastridiales*. However, analyses of Bauer *et al.* (2006) and Aime *et al.* (2007) suggest that *Heterogastridium* and *Colacogloea* do not form a clade, while *Atractocolax* and *Krieglsteinera* have yet to be sampled in molecular phylogenetic studies.

Order: **Microbotryales** R. Bauer & Oberw., *in* Bauer *et al.*, *Can. J. Bot.* **75**: 1309 (1997).

Exemplar genera: *Microbotryum* Lév. 1847, *Ustilentyloma* Savile 1964.

Order: **Leucosporidiales** J. P. Samp., M. Weiß & R. Bauer, *in* Sampaio *et al.*, *Mycol. Progr.* **2**: 61 (2003).

Exemplar genera: *Leucosporidiella* J. P. Samp. 2003, *Leucosporidium* Fell, Statzell, I. L. Hunter & Phaff 1970, *Mastigobasidium* Golubev 1999.

Order: **Sporidiobolales** J. P. Samp., M. Weiß & R. Bauer, *in* Sampaio *et al.*, *Mycol. Progr.* **2**: 66 (2003).

Exemplar genera: *Sporidiobolus* Nyland 1949, *Sporobolomyces* Kluyver & C. B. Niel 1924, *Rhodosporidium* I. Banno 1967, *Rhodotorula* F. C. Harrison 1927 *pro parte*.

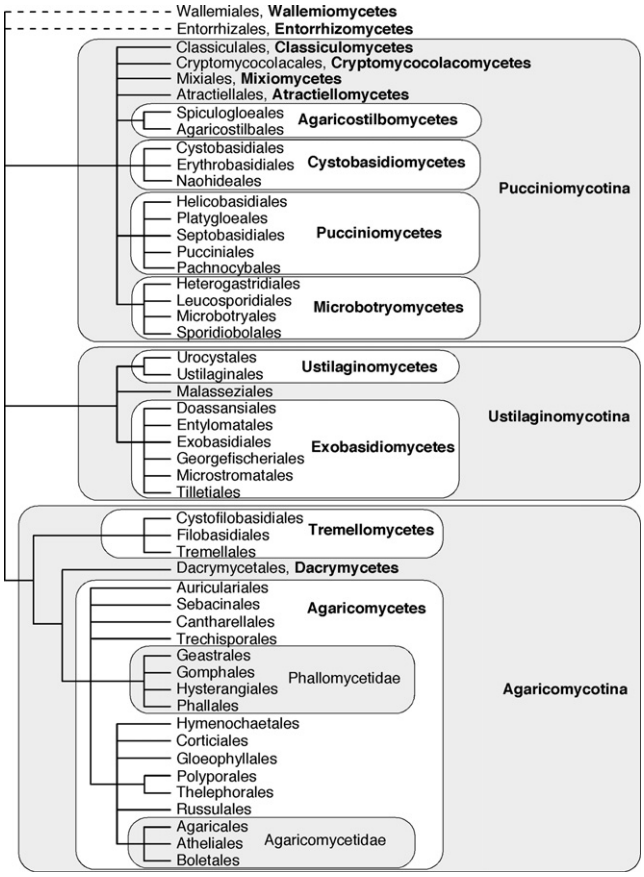


Fig 3 – Phylogeny and classification of *Fungi*. *Basidiomycota*. See Table 3 for support values for clades. Dashed lines indicate taxa that are of uncertain placement.

Class: **Atractiellomycetes** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 45 (2006).

Order: **Atractiellales** Oberw. & Bandoni, *Can. J. Bot.* **60**: 1740 (1982).

Emend. Oberw. & Bauer, *Sydowia* 41: 239 (1989).

Exemplar genera: *Atractiella* Sacc. 1886, *Saccoblastia* A. Møller 1895, *Helicogloea* Pat. 1892, *Phleogena* Link 1833.

Class: **Classiculomycetes** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 46 (2006).

Order: **Classiculales** R. Bauer, Begerow, Oberw. & Marvanová, *Mycologia* **95**: 763 (2003).

Exemplar genera: *Classicula* R. Bauer, Begerow, Oberw. & Marvanová 2003, *Jaculispora* H. J. Huds. & Ingold 1960.

Class: **Mixiomycetes** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 47 (2006).

Order: **Mixiales** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 47 (2006).

Exemplar genus: *Mixia* C. L. Kramer 1959 [‘1958’].

Class: **Cryptomycocolacomycetes** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 46 (2006).

Order: **Cryptomycocolacales** Oberw. & R. Bauer, *Mycologia* **82**: 672 (1990).

Exemplar genera: *Cryptomycocolax* Oberw. & R. Bauer 1990, *Colacosiphon* R. Kirschner, R. Bauer & Oberw. 2001.

Subphylum: **Ustilaginomycotina** R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 45 (2006).

Equivalent to *Ustilaginomycetes* (Bauer *et al.* 1997, 2001; Swann & Taylor 1995).

The classification of *Ustilaginomycotina* employed here largely parallels that of Begerow *et al.* (2007), with the primary differences being that here the *Entorrhizomycetes* are classified as *incertae sedis* among *Basidiomycota* (rather than being a class within *Ustilaginomycotina*).

Class: **Ustilaginomycetes** R. Bauer, Oberw. & Vánky, *Can. J. Bot.* **75**: 1311 (1997).

Emend. Begerow, Stoll & Bauer, *Mycologia* **98**: 908 (2007) [‘2006’].

Equivalent to *Ustilaginomycetidae* Jülich as emmended by Bauer & Oberwinkler (Bauer *et al.* 1997, 2001; Weiß *et al.* 2004a).

Order: **Urocystales** R. Bauer & Oberw., *in* Bauer *et al.*, *Can. J. Bot.* **75**: 1311 (1997).

Exemplar genera: *Urocystis* Rabenh. ex Fuckel 1870, *Ustacystis* Zundel 1945, *Doassansiopsis* (Setch.) Dietel 1897.

Melanotaenium de Bary 1874 has also been placed in this order (Bauer *et al.* 2001; Weiß *et al.* 2004a), but analyses of Begerow *et al.* (2007) and Matheny *et al.* (2007b) have supported its transfer to *Ustilaginales*.

Order: **Ustilaginales** G. Winter, *Rabenh. Krypt.-Fl.* 2nd ed. **1**(1.1): 73 (1880), as ‘*Ustilagineae*’.

Emend. Bauer & Oberwinkler, *in* Bauer *et al.*, *Can. J. Bot.* **75**: 1311 (1997).

Exemplar genera: *Ustilago* (Pers.) Roussel 1806, *Cintractia* Cornu 1883.

Thecaphora Fingerh. 1836 has also been placed in this order (Bauer *et al.* 2001), but analyses of Begerow *et al.* (2007) and Matheny *et al.* (2007b) have suggested that it is not nested in *Ustilaginales*. *Thecaphora* may be the sister group of *Urocystales* (Matheny *et al.* 2007b).

Class: Exobasidiomycetes Begerow, Stoll & R. Bauer, *Mycologia* 98: 908 (2007) [‘2006’].

Equivalent to *Exobasidiomycetidae* Jülich 1981 *emend.* Bauer & Oberwinkler, except for exclusion of *Malasseziales* (Bauer *et al.* 1997, 2001; Weiß *et al.* 2004a).

Monophyly of the *Exobasidiomycetidae*, as delimited here, is supported with high Bayesian posterior probability in analyses of *rpb1*, *rpb2*, and *tef1*, and nuclear lsu, ssu, and 5.8S ribosomal genes (Matheny *et al.* 2007b), but it is weakly supported in analyses using *atp6*, β-tubulin, and nuc-lsu ribosomal RNA genes (Begerow *et al.* 2007). See comments regarding *Malasseziales*.

Order: **Doassansiales** R. Bauer & Oberw., *in* Bauer *et al.*, *Can. J. Bot.* **75**: 1312 (1997).

Exemplar genera: *Doassansia* Cornu 1883, *Rhamphospora* D. D. Cunn. 1888, *Nannfeldtiomyces* Vánky 1981.

Order: **Entylomatales** R. Bauer & Oberw., *in* Bauer *et al.*, *Can. J. Bot.* **75**: 1311 (1997).

Exemplar genera: *Entyloma* de Bary 1874, *Tilletiopsis* Derx 1948.

Begerow *et al.* (2007) erected the monotypic order *Ceraceosorales* Begerow, Stoll & R. Bauer for *Ceraceosorus bombacis* (B. K. Bakshi) B. K. Bakshi 1976, which was weakly supported as the sister group of *Tilletiopsis albescens* Gokhale 1972. The *Ceraceosorus-T. albescens* clade was placed as the sister group of *Entylomatales*, again with weak support. *Ceraceosorales* is not included in the present classification, pending more robust resolution of the relationships among *Ceraceosorus*, *Tilletiopsis*, and *Entyloma*.

Order: **Exobasidiales** Henn., *in* Engler & Prantl (eds), *Nat. Pflanzenfam.* **1**(1**): 103 (1897), as '*Exobasidiineae*'.

Emend. Bauer, Oberwinkler & Vánky, *Can. J. Bot.* **75**: 1312 (1997).

Exemplar genera: *Exobasidium* Woronin 1867, *Clinoconidium* Pat. 1898, *Dicellomyces* L. S. Olive 1945.

Order: **Georgefischeriales** R. Bauer, Begerow & Oberw., *in* Bauer *et al.*, *Can. J. Bot.* **75**: 1311 (1997).

Exemplar genera: *Georgefischeria* Thirum. & Naras. *emend.* Gandhe 1980, *Phragmotaenium* R. Bauer, Begerow, A. Nagler & Oberw. 2001, *Tilletiaria* Bandoni & B. N. Johri 1972, *Tilletiopsis* Derx 1948 *pro parte*.

Order: **Microstromatales** R. Bauer & Oberw., *in* Bauer *et al.*, *Can. J. Bot.* **75**: 1311 (1997).

Exemplar genera: *Microstroma* Niessl 1861, *Sympodiomycopsis* Sugiy., Tokuoka & Komag. 1991, *Volvocisporium* Begerow, R. Bauer & Oberw. 2001.

Order: **Tilletiales** Kreisel ex R. Bauer & Oberw., *in* Bauer *et al.*, *Can. J. Bot.* **75**: 1311 (1997).

Exemplar genera: *Tilletia* Tul. & C. Tul. 1847, *Conidiosporomyces* Vánky 1992, *Erratomyces* M. Piepenbr. & R. Bauer 1997.

Ustilaginomycotina ***incertae sedis*** (not placed in any class):

Order: **Malasseziales** R. T. Moore, *Bot. Mar.* **23**: 371 (1980).

Emend. Begerow, Bauer & Boekhout, *Mycol. Res.* **104**: 59 (2000).

Exemplar genus: *Malassezia* Baill. 1889.

Analyses of the protein-coding genes *rpb1*, *rpb2*, and *tef1*, alone or in combination with nuclear LSU, SSU, and 5.8S ribosomal genes, suggest that *Malasseziales* are included in the *Ustilaginomycetes*, but analyses of nuclear ribosomal genes alone or in combination with *atp6* and β-tubulin suggest that *Malasseziales* is in the *Exobasidiomycetes* (Bauer *et al.* 2001; Begerow *et al.* 2007; Matheny *et al.* 2007b; Weiß *et al.* 2004a).

Subphylum: **Agaricomycotina** Dowell *Prosyllabus* LXXVII (2001).

Homonym: *Agaricomycotina* R. Bauer, Begerow, J. P. Samp., M. Weiß & Oberw., *Mycol. Progr.* **5**: 45 (2006). Equivalent to *Hymenomycetes* (Swann & Taylor 1995) or *Basidiomycetes* (Kirk *et al.* 2001; Hibbett 2007).

Class: **Tremellomycetes** Dowell, *Prosyllabus*: LXXVII (2001).

Dimorphic fungi. Fruiting bodies gelatinous or absent, parenthesomes sacculate or absent, basidia septate or non-septate. The least inclusive clade containing *Tremellales*, *Filobasidiales* and *Cystofilobasidiales*.

Equivalent to *Tremellomycetidae sensu* Swann & Taylor (1995) and Weiß *et al.* (2004a). The name *Tremellomycetidae* was earlier published by Locquin (1984), but without a Latin diagnosis, and it is therefore invalid under the *Code*.

Order: **Cystofilobasidiales** Fell, Roeijmans & Boekhout, *Int. J. Syst. Bacteriol.* **49**: 911 (1999).

Exemplar genera: *Cystofilobasidium* Oberw. & Bandoni 1983, *Mrakia* Y. Yamada & Komag. 1987, *Itersonilia* Derx 1948.

Order: **Filobasidiales** Jülich, *Biblthca Mycol.* **85**: 324 (1981).

Exemplar genera: *Filobasidiella* Kwon-Chung 1976, *Cryptococcus* Vuill. 1901 (*pro parte*).

Order: **Tremellales** Fr., *Syst. Mycol.* **1**: 2 (1821), as '*Tremellinae*'.

As delimited here, the group includes *Trichosporonales* Boekhout & Fell 2001 (Fell *et al.* 2001) and *Christianseniales* F. Rath 1991 (Wells & Bandoni 2001). *Filobasidiales*, which Weiß *et al.* (2004a) included in *Tremellales s. lat.*, has been resolved as the sister group of *Tremellales* (Fell *et al.* 2001; Matheny *et al.* 2007b; Swann & Taylor 1995).

Exemplar genera: *Tremella* Pers. 1794, *Trichosporon* Behrend 1890, *Christiansenia* Hauerslev 1969.

Class: **Dacrymycetes** Dowell, *Prosyllabus*: LXXVII (2001)

Fruiting bodies gelatinous, basidia furcate (rarely unisporous), parenthesomes imperforate.

Containing the single order *Dacrymycetales* (Wells & Bandoni 2001).

Order: **Dacrymycetales** Henn., *in* Engler & Prantl (eds), *Nat. Pflanzenfam.* **1**(1**): 96 (1898), as '*Dacryomycetineae*'.

Exemplar genera: *Dacrymyces* Nees 1861, *Calocera* (Fr.) Fr. 1828, *Guepiniopsis* Pat. 1883.

Class: **Agaricomycetes** Dowell, *Prosyllabus*: LXXVII (2001)

Fruiting bodies hymenomycetous or gasteroid, basidia two- to eight-spored, parenthesomes perforate or imperforate. The least-inclusive clade containing *Auriculariales*, *Sebacinales*, *Cantharellales*, *Phallomycetidae* and *Agaricomycetidae*.

This group is approximately equivalent to *Homobasidiomycetes sensu* Hibbett & Thorn (2001) plus *Auriculariales* and *Sebacinales*.

Subclass: **Agaricomycetidae** Parmasto, *Windahlia* **16:** 16 (1986). Synonym: *Basidiosporeae* Bessey, Univ. Studies, Univ. Nebr. **7**: 306 (1907).

The least-inclusive clade containing *Agaricales*, *Boletales* and *Atheliales*.

The delimitation of *Agaricomycetidae* adopted here differs from that of Parmasto (1986), who described *Agaricomycetidae*

as a subclass of *Cantharellomycetes* Parm. 1986. For example, many of the resupinate forms in the *Agaricomycetidae* were placed by Parmasto in the *Corticiomycetes* Parm. 1986. The name *Agaricomycetidae* was also published by Locquin (1984), but without a Latin diagnosis and it is therefore invalid under the *Code*.

Order: **Agaricales** Underw., *Moulds, Mildews Mushrooms:* 97 (1899).

Equivalent to euagarics clade (Hibbett & Thorn 2001).

Exemplar genera: *Agaricus* L. 1753, *Coprinus* Pers. 1797, *Pleurotus* (Fr.) P. Kumm. 1871.

Order: **Atheliales** Jülich, *Biblthca Mycol.* **85**: 343 (1981).

Equivalent to athelioid clade (Binder *et al.* 2005; Larsson *et al.* 2004).

Exemplar genera: *Athelia* Pers. 1822, *Piloderma* Jülich 1969, *Tylospora* Donk 1960.

Order: **Boletales** E.-J. Gilbert, *Livres Mycol.* **3**: 83 (1931).

Equivalent to bolete clade (Binder & Hibbett 2006; Hibbett & Thorn 2001).

Exemplar genera: *Boletus* Fr. 1821, *Scleroderma* Pers. 1801, *Coniophora* DC. 1815, *Rhizopogon* Fr. & Nordholm 1817.

Subclass: **Phallomycetidae** K. Hosaka, Castellano & Spatafora, *Mycologia* **98**: 955 (2007) ['2006'].

Equivalent to *Phallales sensu* Kirk *et al.* (2001), and the gomphoid-phalloid clade (Hibbett & Thorn 2001; Hosaka *et al.* 2007).

Order: **Geastrales** K. Hosaka & Castellano, *Mycologia* **98:** 957 (2007) ['2006'].

Exemplar genera: *Geastrum* Pers. 1794, *Radiigera* Zeller 1944, *Sphaerobolus* Tode 1790.

Order: **Gomphales** Jülich, *Biblthca Mycol.* **85**: 348 (1981).

Exemplar genera: *Gomphus* (Fr.) Weinm. 1826, *Gautieria* Vittad. 1831, *Ramaria* Holmsk. 1790.

Order: **Hysterangiales** K. Hosaka & Castellano, *Mycologia* **98**: 956 (2007) ['2006'].

Exemplar genera: *Hysterangium* Vittad. 1831, *Phallogaster* Morgan 1893, *Gallacea* Lloyd 1905, *Austrogautieria* E. L. Stewart & Trappe 1985.

Order: **Phallales** E. Fisch., *in* Engler & Prantl (eds), *Nat. Pflanzenfam.* **1**(1**): 276 (1898).

Equivalent to *Phallomycetidae* Locq. (Locquin 1984), which was invalidly published, owing to the absence of a Latin diagnosis.

Exemplar genera: *Phallus* Junius ex L. 1753, *Clathrus* P. Micheli ex L. 1753, *Claustula* K. M. Curtis 1926.

Agaricomycetes *incertae sedis* (not placed in any subclass):

Order: **Auriculariales** J. Schröt., *in* Cohn (ed.), *Krypt.-Fl. Schlesien* **1**: 382 (1889).

Exemplar genera: *Auricularia* Bull. ex Juss. 1789, *Exidia* Fr. 1822, *Bourdotia* (Bres.) Trotter 1913.

Order: **Cantharellales** Gäum., *Vergl. Morph. Pilze*: 495 (1926).

Equivalent to the cantharelloid clade (Hibbett & Thorn 2001; Moncalvo *et al.* 2007). The *Cantharellales* as delimited here includes *Tulasnella*, which is distinguished by unusual basidia with inflated sterigmata, and has been classified in a separate order, *Tulasnellales* Rea 1922 (e.g. Weiß *et al.* 2004a). Extreme evolutionary rate heterogeneity in the nuclear ribosomal RNA genes of *Tulasnella*, *Cantharellus* and *Craterellus* is a source of error in phylogenetics of *Cantharellales*. Analyses of Matheny *et al.* (2006) suggest that *Tulasnella* is nested within the *Cantharellales*, but it could also be the sister group to *Cantharellales s.str.* (Moncalvo *et al.* 2007). If so, then it may be appropriate to segregate *Tulasnellales* from *Cantharellales s.str.*

Exemplar genera: *Cantharellus* Fr. 1821, *Botryobasidium* Donk 1931, *Craterellus* Pers. 1825, *Tulasnella* J. Schröt. 1888.

Order: **Corticiales** K. H. Larss., **ord. nov.**
MycoBank no.: MB 501299

Basidiomata resupinata, effuso-reflexa vel discoidea; hymenophora laevia; systema hypharum monomiticum; dendrohyphidia raro absentia; basidia saepe e probasidiis oriuntur. Cystidia presentia vel absentia. Sporae hyalinae, tenuitunicatae, albae vel aggregatae roseae.

Typus: Corticium Pers. 1794.

Basidiomycetes with effused or discoid (*Cytidia*) basidiomata, a smooth hymenophore, and a monomitic hyphal system with clamped, rarely simple-septate, hyphae. Dendrohyphidia common. Species with or without cystidia. A probasidial resting stage is present in many species. Spores smooth, in masses white to pink. Saprotrophic, parasitic, or lichenicolous.

Equivalent to *Vuilleminiales* Boidin *et al.* 1998 and the corticioid clade (Binder *et al.* 2005; Larsson *et al.* 2004). Boidin *et al.* (1998) explicitly included *Corticium* in their new order, as a member of the family *Vuilleminiaceae* Maire 1902. Jülich (1981) also placed *Corticium* in *Vuilleminiaceae* but referred them to *Aleurodiscales* Jülich 1981. *Corticium* is the type of *Corticiaceae* Herter 1910, a family name conserved against *Vuilleminiaceae*. The introduction of *Corticiales* as a new name for this order is, therefore, the preferred option.

Exemplar genera: *Corticium* Pers. 1794, *Vuilleminia* Maire 1902, *Punctularia* Pat. 1895.

Order: **Gloeophyllales** Thorn, **ord. nov.**
MycoBank no.: MB 501300

Basidiomata annua vel perennia, resupinata, effuso-reflexa, dimidiata vel pileata; hymenophora laevia, merulioidea, odontioidea vel poroidea. Systema hypharum monomiticum, dimiticum vel trimiticum. Hyphae generativae fibulatae vel efibulatae. Leptocystidia ex trama in hymenium projecta, hyalina vel brunnea, tenuitunicata vel crassitunicata. Basidiosporae laeves, hyalinae, tenuitunicatae, ellipsoideae vel cylindricae vel allantoideae, inamyloideae. Lignum decompositum brunneum vel album.

Typus: Gloeophyllum P. Karst. 1882.

Fruiting bodies perennial or annual and long-lived, with hymenium maturing and thickening over time. Stature resupinate, effused-reflexed or dimidiate, with smooth, wrinkled,

dentate, lamellate or regularly poroid hymenophore, or pileate-stipitate with lamellae. (Aborted, coralloid or flabelliform fruiting bodies may be formed under conditions of darkness or high carbon dioxide concentration). Leptocystidia or hyphoid hairs originating in the context and extending into or protruding from the hymenial layer (or lamellar margin in *Neolentinus*) are common; these often with thick brown walls and brownish incrustation. Context brown (but pallid in *Neolentinus*) and generally darkening in potassium hydroxide (the brownish incrustation in *Boreostereum* turning green in potassium hydroxide). Monomitic (if so, with sclerified generative hyphae), dimitic, or trimitic; generative hyphae with or without clamp connections. Basidiospores hyaline, ellipsoid to cylindrical or suballantoid, with thin, smooth walls, and neither amyloid, dextrinoid nor cyanophilous. Where this is known, basidiospores are binucleate and sexuality is heterothallic and bipolar (but tetrapolar in *V. berkeleyi*).

Causing brown rots (*Gloeophyllum*, *Neolentinus*, *Veluticeps*) or stringy white rot (*Boreostereum*, *Donkioporia*) of wood of gymnosperms, monocots and dicots. Occurrence on 'wood in service' (e.g. railway ties, paving blocks, wooden chests) seems to be common (in *Donkioporia*, *Gloeophyllum*, *Heliocybe* and *Neolentinus*); often on charred wood (*Boreostereum* and *Veluticeps*).

Equivalent to *Gloeophyllum* clade (Binder *et al.* 2005).

Exemplar genera: *Gloeophyllum* P. Karst. 1882, *Neolentinus* Redhead & Ginns 1985, *Veluticeps* (Cooke) Pat. 1894.

Order: **Hymenochaetales** Oberw., *in* Frey *et al.* (eds), *Beitr. Biol. niederen Pflanz.*: 89 (1977).

Equivalent to the hymenochaetoid clade (Hibbett & Thorn 2001; Larsson *et al.* 2007).

Exemplar genera: *Hymenochaete* Lév. 1846, *Phellinus* Quél. 1886, *Trichaptum* Murrill 1904.

Order: **Polyporales** Gäum., *Vergl. Morph. Pilze*: 503 (1926).

Equivalent to polyporoid clade (Hibbett & Thorn 2001).

Exemplar genera: *Polyporus* Fr. 1815, *Fomitopsis* P. Karst. 1881, *Phanerochaete* P. Karst. 1889.

Order: **Russulales** Kreisel ex P. M. Kirk, P. F. Cannon & J. C. David, *in* Kirk *et al.*, *Ainsworth & Bisby's Dict. Fungi* (9th edn): xi (2001).

Equivalent to the russuloid clade (Hibbett & Thorn 2001; Larsson & Larsson 2003; Miller *et al.* 2007).

Exemplar genera: *Russula* Pers. 1796, *Aleurodiscus* Rabenh. ex J. Schröt. 1888, *Bondarzewia* Singer 1940, *Hericium* Pers. 1794, *Peniophora* Cooke 1879, *Stereum* Pers. 1794.

Order: **Sebacinales** M. Weiß, Selosse, Rexer, A. Urb. & Oberw., *Mycol. Res.* **108**: 1007 (2004b).

Exemplar genera: *Sebacina* Tul. 1871, *Tremellodendron* G. F. Atk. 1902, *Piriformospora* Sav. Verma, Aj. Varma, Rexer, G. Kost & P. Franken 1998.

Order: **Thelephorales** Corner ex Oberw., *Sydowia* **78**: 361 (1976).

Equivalent to the thelephoroid clade (Hibbett & Thorn 2001).

Exemplar genera: *Thelephora* Ehrh. ex Willd. 1787, *Bankera* Coker & Beers ex Pouzar 1955, *Polyozellus* Murrill 1910.

Order: **Trechisporales** K. H. Larss., **ord. nov.**
MycoBank no.: MB 501301

Basidiomata resupinata, stipitata vel clavarioidea. Hymenophora laevia, grandinioidea, hydnoidea vel poroidea. Systema hypharum monomiticum vel dimiticum. Hyphae fibulatae, septa hypharum interdum inflata (ampullata). Cystidia praesentia vel absentia. Basidia 4-6 sterigmata formantia. Sporae laeves vel ornatae. Species lignicolae vel terricolae.

Typus: *Trechispora* P. Karst. 1890.

Basidiomycetes with effused, stipitate or clavarioid basidiomata. Hymenophore smooth, grandinioid, hydnoid or poroid. Hyphal system monomitic, hyphae clamped, subicular hyphae with or without ampullate septa. Cystidia present in some species, mostly lacking. Basidia with four to six sterigmata. Spores smooth or ornamented. On wood or soil.

Equivalent to *Hydnodontales* Jülich 1981 and trechisporoid clade (Binder *et al.* 2005; Larsson *et al.* 2004). *Hydnodon* Banker 1913 was recently placed in synonomy under *Trechispora* (Ryvarden 2002) and this synonomy is supported by molecular data (K.H. Larsson, unpubl.). The introduction of a new name for the group, a name that connects to the clade name already established and that is based on the most species-rich genus is, therefore, justified.

Exemplar genera: *Trechispora* P. Karst. 1890, *Sistotremastrum* J. Erikss. 1958, *Porpomyces* Jülich 1982.

Basidiomycota *incertae sedis* (not placed in any subphylum):

Class: **Wallemiomycetes** Zalar, de Hoog & Schroers, *Antonie van Leeuwenhoek* **87**: 322 (2005).

Analyses of *rpb1*, *rpb2*, *tef1*, and nuc-lsu, nuc-ssu, and 5.8S ribosomal RNA genes suggest that the *Wallemiomycetes* is the sister group of the rest of the *Basidiomycota* (possibly along with *Entorrhizomycetes*, see below), but subsets of this dataset produce alternative placements (Matheny *et al.* 2007b; Zalar *et al.* 2005).

Order: **Wallemiales** Zalar, de Hoog & Schroers, *Antonie van Leeuwenhoek* **87**: 322 (2005).

Exemplar genus: *Wallemia* Johan-Olsen 1887.

Class: **Entorrhizomycetes** Begerow, Stoll & R. Bauer, *Mycologia* **98**: 908 (2007) ['2006'].

Equivalent to *Entorrhizomycetidae* R. Bauer & Oberw. (Bauer *et al.* 1997). So far, only ribosomal RNA genes have been sequenced in *Entorrhizomycetes*. Analyses with broad sampling across all groups of *Basidiomycota* and including *Ascomycota* and *Glomeromycota* as outgroups suggest that *Entorrhizomycetes* is not nested within any subphylum, and may be the sister group of the rest of the *Basidiomycota* (Matheny *et al.* 2007a; also see Begerow *et al.* 1997).

Order: **Entorrhizales** R. Bauer & Oberw., *in* Bauer *et al.*, , *Can. J. Bot.* **75**: 1311 (1997).

Exemplar genus: *Entorrhiza* C. A. Weber 1884.

Acknowledgments

Preparation of this classification was facilitated by the Assembling the Fungal Tree of Life (AFTOL) project and the Deep Hypha Research Coordination Network, which were supported by the US National Science Foundation grants DEB-0228657, DEB-0228668, DEB-0228671, DEB-0228725 and NSF-0090301.

Note added in proof

After this article went to press, the authors became aware of the following publication, which includes alternative citations for many of the names included here: Doweld A, 2001. *Prosyllabus tracheophytorum: Tentamen systematis plantarum vascularium (Tracheophyta)*. Geos, Moscow.

REFERENCES *

Adl SM, Simpson AGB, Farmer MA, Andersen RA, Anderson OR, Barta JR, Bowser SS, Brugerolle G, Fensome RA, Fredericq S, James TY, Karpov S, Kugrens P, Krug J, Lane CE, Lewis LA, Lodge J, Lynn DH, Mann DG, McCourt RM, Mendoza L, Moestrup Ø, Mozley-Standridge SE, Nerad TA, Shearer CA, Smirnov AV, Spiegel FW, Taylor MFJR, 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology* **52**: 399–451.

Aime MC, Matheny PB, Henk DA, Frieders EM, Nilsson RH, Piepenbring M, McLaughlin DJ, Szabo LJ, Begerow D, Sampaio JP, Bauer R, Weiß M, Oberwinkler F, Hibbett DS, 2007 ['2006']. . An overview of the higher-level classification of *Pucciniomycotina* based on combined analyses of nuclear large and small subunit rDNA sequences. *Mycologia* **98**: 896–905.

Alexopoulos CJ, Mims CW, Blackwell M, 1996. *Introductory Mycology*, fourth ed. John Wiley, New York.

von Arx JA, 1967. *Pilzkunde*. J. Cramer, Lehre.

Balbiani G, 1882. Sur les microsporides ou psorospermies des Articulés. *Comptes rendus de l'Académie des Sciences, Paris* **95**: 1168–1171.

Baldauf SL, Roger AJ, Wenk-Siefert I, Doolittle WF, 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* **290**: 972–977.

Barr DJS, 1980. An outline for the reclassification of the *Chytridiales*, and for a new order, the *Spizellomycetales*. *Canadian Journal of Botany* **58**: 2380–2394.

Barr ME, 1983. The ascomycete connection. *Mycologia* **75**: 1–13.

Barr ME, 1987a. New taxa and combinations in the *Loculoascomycetes*. *Mycotaxon* **29**: 501–505.

Barr ME, 1987b. *Prodromus to Class Loculoascomycetes*. University of Massachusetts, Amherst.

Barron GL, 1975. Nematophagus fungi: *Helicocephalum*. *Transactions of the British Mycological Society* **65**: 309–310.

Bauer R, Begerow D, Oberwinkler F, Piepenbring M, Berbee ML, 2001. *Ustilaginomycetes*. In: McLaughlin DJ, McLaughlin EJ, Lemke P (eds), *The Mycota. Vol. VIII: Part B. Systematics and Evolution*. Springer-Verlag, Berlin, pp. 57–84.

Bauer R, Begerow D, Sampaio JP, Weiß M, Oberwinkler F, 2006. The simple-septate basidiomycetes: a synopsis. *Mycological Progress* **5**: 41–66.

Bauer R, Oberwinkler F, Vánky K, 1997. Ultrastructural markers and systematics in smut fungi and allied taxa. *Canadian Journal of Botany* **75**: 1273–1314.

Begerow D, Bauer R, Boekhout T, 2000. Phylogenetic placements of ustilaginomycetous anamorphs as deduced from nuclear LSU rDNA sequences. *Mycological Research* **104**: 53–60.

Begerow D, Bauer R, Oberwinkler F, 1997. Phylogenetic studies on nuclear large subunit ribosomal DNA sequences of smut fungi and related taxa. *Canadian Journal of Botany* **75**: 2045–2056.

Begerow D, Stoll M, Bauer R, 2007 ['2006']. A phylogenetic hypothesis of *Ustilaginomycotina* based on multiple gene analyses and morphological data. *Mycologia* **98**: 906–916.

Benjamin RK, 1979. Zygomycetes and their spores. In: Kendrick B (ed), *The Whole Fungus: the Sexual–Asexual Synthesis*. National Museum of Natural Sciences, Ottawa, pp. 573–616.

Benny GL, Kimbrough JW, 1980. A synopsis of the orders and families of *Plectomycetes* with keys to genera. *Mycotaxon* **12**: 1–91.

Benny GL, White MM, 2001. The classification and phylogeny of *Trichomycetes* and *Zygomycetes*. In: Misra JK, Horn BW (eds), *Trichomycetes and Other Fungal Groups*. Science Publishers, Enfield, NH, pp. 39–53.

Berres ME, Szabo LJ, McLaughlin DJ, 1995. Phylogenetic relationships in auriculariaceous basidiomycetes based on 25S ribosomal DNA sequences. *Mycologia* **87**: 821–840.

Binder M, Hibbett DS, Larsson K-H, Larsson E, Langer E, Langer G, 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (*Homobasidiomycetes*). *Systematics and Biodiversity* **3**: 1–45.

Binder M, Hibbett DS, 2007 ['2006']. Molecular systematics and biological diversification of *Boletales*. *Mycologia* **98**: 971–983.

Blackwell M, Hibbett DS, Taylor JW, Spatafora JW, 2007 ['2006']. Research coordination networks: a phylogeny for kingdom *Fungi*. *Mycologia* **98**: 829–837.

Boidin J, Mugnier J, Canales R, 1998. Taxonomie moléculaire des *Aphyllophorales*. *Mycotaxon* **66**: 445–492.

Bold HC, 1957. *The Morphology of Plants*. Harper Row, New York.

Bullerwell CE, Forget L, Lang BF, 2003. Evolution of monoblepharidalean fungi based on complete mitochondrial genome sequences. *Nucleic Acids Research* **31**: 1614–1623.

Campbell J, Anderson JL, Shearer CA, 2003. Systematics of *Halosarpheia* based on morphological and molecular data. *Mycologia* **95**: 530–552.

Campbell J, Volkmann-Kohlmeyer B, Gräfenhan T, Spatafora JW, Kohlmeyer J, 2005. A re-evaluation of *Lulworthiales*: relationships based on 18S and 28S rDNA. *Mycological Research* **109**: 556–568.

Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN, 2002. A preliminary overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.

Castlebury LA, Rossman AY, Sung G-H, Hyten AS, Spatafora JW, 2004. Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. *Mycological Research* **108**: 864–872.

Cavalier-Smith T, 1981. Eukaryote kingdoms: seven or nine? *BioSystems* **14**: 461–481.

Cavalier-Smith T, 1998. A revised six-kingdom system of Life. *Biological Reviews* **73**: 203–266.

Cejp K, 1957. *Houby; celostátní vysokoškolská učebnice*. **Vol. 1**. Czechoslovakian Academy of Sciences Press, Prague.

Clements FE, Shear CL, 1931. *The Genera of Fungi*, second ed. H.W. Wilson, New York.

Cohn F, 1879. Ueber ein Thallophytensystem. *Jahresbericht der Schlesischen Gesellschaft für vaterländische Cultur, Breslau* **57**: 279.

Cracraft J, Donoghue MJ (eds), 2004. *Assembling the Tree of Life*. Oxford University Press, Oxford.

Currah RS, 1985. Taxonomy of the *Onygenales*: *Arthrodermataceae*, *Gymnoacaceae*, *Myxotrichaceae* and *Onygenaceae*. *Mycotaxon* **24**: 1–216.

* This list of references, in addition to including papers cited in the text, also contains the full bibliographic details of some papers otherwise cited only as places of publications of names where those works may otherwise be difficult to locate.

David JC, 2002. A preliminary catalogue of the names of fungi above the rank of order. *Constancea* **83**: 1–30.
Del Prado R, Schmitt I, Kautz S, Palice Z, Luecking R, Lumbsch HT, 2006. Molecular data place *Trypetheliaceae* in *Dothideomycetes*. *Mycological Research* **110**: 511–520.
Donk MA, 1964. A conspectus of the families of *Aphyllophorales*. *Persoonia* **3**: 199–324.
Ekman S, Tønsberg T, 2002. Most species of *Lepraria* and *Leproloma* form a monophyletic group closely related to *Stereocaulon*. *Mycological Research* **106**: 1262–1276.
Engler A, 1898. *Syllabus der Pflanzenfamilien: Eine Übersicht über das gesammte Pflanzensystem*, second ed. Gebrüder Borntraeger Verlag, Berlin, 46.
Eriksson O, 1982. Outline of the ascomycetes. *Mycotaxon* **15**: 203–248.
Eriksson OE, 1986. *Lahmia* Körber (=*Parkerella* A. Funk) a misinterpreted genus with isolated position. *Mycotaxon* **27**: 347–360.
Eriksson OE, 1992. *Huangshania verrucosa* gen. et sp. nov. (*Triblidiaceae*, *Triblidiales* ordo nov.), a discomycete on *Pinus* from China. *Systema Ascomycetum* **11**: 1–10.
Eriksson OE, 1994. *Pneumocystis carinii*, a parasite in lungs of mammals, referred to a new family and order (*Pneumocystidaceae*, *Pneumocystidales*, *Ascomycota*). *Systema Ascomycetum* **13**: 165–180.
Eriksson OE, Baral H-O, Currah RS, Hansen K, Kurtzman CP, Rambold G, Læssøe T, 2003. Notes on ascomycete systematics. Nos 3580-3623. *Myconet* **9**: 91–103.
Eriksson OE, Svedskog A, Landvik S, 1993. Molecular evidence for the evolutionary hiatus between *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Systema Ascomycetum* **11**: 119–162.
Eriksson OE, Winka K, 1997. Supraordinal taxa of *Ascomycota*. *Myconet* **1**: 1–16.
Fell JW, Boekhout T, Fonseca A, Sampaio JP, 2001. Basidiomycetous yeasts. In: McLaughlin DJ, McLaughlin EJ, Lemke P (eds), *The Mycota. Vol. VII. Part B., Systematics and Evolution*. Springer-Verlag, Berlin, pp. 3–35.
Fell JW, Roeijmans H, Boekhout T, 1999. a new order of basidiomycetous yeasts. International Journal of Systematic Bacteriology. *Cystofilobasidiales* **49**: 907–913.
Fell JW, Scorzetti G, 2004. Reassignment of the basidiomycetous yeasts *Trichosporon pullulans* to *Guehomyces pullulans* gen. nov., comb. nov. and *Hyalodendron lignicola* to *Trichosporon lignicola* comb. nov. *International Journal of Systematic and Evolutionary Microbiology* **54**: 995–998.
Fischer A, 1892. Phycomycetes. *Rabenhorst's Kryptogamen-Flora von Deutschland*, *Österreich und der Schweiz* **1**: 1–128.
Fischer E, 1898. Phallineae. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere der Nutzpflanzen,* **Vol. 1**. Wilhelm Engelmann, Leipzig, pp. 276–295.
Fries EM, 1821. *Systema Mycologicum,* **Vol. 1**. Berling, Lund.
Fries EM, 1832. *Systema Mycologicum,* **Vol. 3**. Ernest Mauriti, Greifswald.
Galagan JE, Henn MR, Ma L-J, Cuomo CA, Birren B, 2005. Genomics of the fungal kingdom: insights into eukaryotic biology. *Genome Research* **15**: 1620–1631.
Gamundí IJ, 1971. Las *Cyttariales* sudamericanas. *Darwiniana* **16**: 461–510.
Gäumann E, 1926. *Vergleichende Morphologie der Pilze*. Gustav Fischer, Jena.
Gäumann E, Dodge CW, 1928. *Comparative Morphology of the Fungi*. McGraw-Hill, London.
Geiser DM, Gueidan C, Miądlikowska J, Lutzoni F, Kauff F, Hofstetter V, Fraker E, Schoch CL, Tibell L, Untereiner WA, Aptroot A, 2007 ['2006']. *Eurotiomycetes: Eurotiomycetidae* and *Chaetothyriomycetidae*. *Mycologia* **98**: 1054–1065.
Germot A, Philipe H, Le Guyader H, 1997. Evidence for loss of mitochondria in microsporidia from a mitochondrial HSP70 in *Nosema locustae*. *Molecular and Biochemical Parasitology* **87**: 159–168.
Gibson JL, Kimbrough JW, Benny GL, 1986. Ultrastructural observations on *Endogonaceae* (*Zygomycetes*). II. *Glaziellales* ord. nov. and *Glaziellaceae* fam. nov.: new taxa based upon light and electron microscopic observations of *Glaziella aurantiaca*. *Mycologia* **78**: 941–954.
Gilbert EJ, 1931. *Les Bolets. [Les Livres du Mycologue, Vol. 3]*. E. Le François, Paris.
Gill EE, Fast NM, 2006. Assessing the microsporidia–fungi relationship: combined phylogenetic analysis of eight genes. *Gene* **375**: 103–109.
Grube M, Baloch E, Lumbsch HT, 2004. The phylogeny of *Porinaceae* (*Ostropomycetidae*) suggests a neotenic origin of perithecia in *Lecanoromycetes*. *Mycological Research* **108**: 1111–1118.
Gwynne-Vaughan HCI, 1922. *Fungi: Ascomycetes, Ustilaginales, Uredinales*. Cambridge University Press, Cambridge.
Hausner G, Reid J, 2004. The nuclear small subunit ribosomal genes of *Sphaeronaemella helvellae*, *Sphaeronaemella fimicola*, *Gabarnaudia betae*, and *Cornuvesica falcata*: phylogenetic implications. *Canadian Journal of Botany* **82**: 752–762.
Hawksworth DL, Kirk PM, Sutton BC, Pegler DN, 1995. *Ainsworth & Bisby's Dictionary of the Fungi*, eighth ed. CAB International, Wallingford.
Henk DA, Weir A, Blackwell M, 2003. *Laboulbeniopsis*, an ectoparasite of termites newly recognized as a member of the *Laboulbeniomycetes*. *Mycologia* **95**: 561–564.
Hennings P, 1897. *Exobasidiineae*. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien, Vol. 1*. Wilhelm Engelmann Verlag, Leipzig, pp. 103–105.
Hennings P, 1898. *Dacryomycetineae*. In: Engler A, Prantl K (eds), *Die Natürlichen Pflanzenfamilien,* **Vol. 1**. Wilhelm Engelmann Verlag, Leipzig, pp. 96–102.
Henssen A, Jahms HM, 1973 [1974]. *Lichenes: Eine Einfürhung in die Flechtenkunde*. George Thieme, Stuttgart.
Hibbett DS, 2007 ['2006']. A phylogenetic overview of the *Agaricomycotina*. *Mycologia* **98**: 917–925.
Hibbett DS, Donoghue MJ, 1998. Integrating phylogenetic analysis and classification in fungi. *Mycologia* **90**: 347–356.
Hibbett DS, Thorn RG, 2001. *Homobasidiomycetes*. In: McLaughlin DJ, McLaughlin EJ, Lemke P (eds), *The Mycota*. **Vol. VII**. *Part B., Systematics and Evolution*. Springer-Verlag, Berlin, pp. 121–168.
Hirt RP, Healy B, Vossbrinck CR, Canning EU, Embley TM, 1997. A mitochondrial Hsp70 orthologue in *Vairimorpha necatrix*: molecular evidence that *Microsporidia* once contained mitochondria. *Current Biology* **7**: 995–998.
Hofstetter V, Miądlikowska J, Kauff F, Lutzoni F, 2007. Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: a case study of the *Lecanoromycetes* (*Ascomycota*). *Molecular Phylogenetics and Evolution,* in press.
de Hoog GS, Göttlich E, Platas G, Genilloud O, Leotta G, Brummelen J, 2005. Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. *Studies in Mycology* **51**: 33–76.
Hosaka K, Bates ST, Beever RT, Castellano MA, Colgan III W, Domínguez LS, Nouhra ER, Geml J, Giachini AJ, Kenney SR, Simpson NB, Spatafora JW, Trappe JM, 2007 ['2006']. Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass *Phallomycetidae* and two new orders. *Mycologia* **98**: 949–959.
Huhndorf SM, Miller AN, Fernández FA, 2004a. Molecular systematics of the Coronophorales and new species of

Bertia, *Lasiobertia* and *Nitschkia*. *Mycological Research* **108**: 1384–1398.

Huhndorf SM, Miller AN, Fernández FA, 2004b. Molecular systematics of the *Sordariales*: the order and the family *Lasiosphaeriaceae* redefined. *Mycologia* **96**: 368–387.

Inderbitzin P, Landvik S, Abdel-Wahab MA, Berbee ML, 2001. *Aliquandostipitaceae*, a new family for two new tropical ascomycetes with unusually wide hyphae and dimorphic ascomata. *American Journal of Botany* **88**: 52–61.

Inderbitzin P, Lim SR, Volkmann-Kohlmeyer B, Kohlmeyer J, Berbee ML, 2004. The phylogenetic position of *Spathulospora* based on DNA sequences from dried herbarium material. *Mycological Research* **108**: 737–748.

International Commission on Zoological Nomenclature, 1999. *International Code of Zoological Nomenclature*. International Trust for Zoological Nomenclature, London.

Jahn TL, Jahn FF, 1949. *How to Know the Protozoa*. Wm C. Brown, Dubuque.

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox C, Celio G, Gueidan C, Fraker E, Miądlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold EA, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüßler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton J, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin D, Spatafora J, Vilgalys R, 2006. Reconstructing the early evolution of the fungi using a six gene phylogeny. *Nature* **443**: 818–822.

James TY, Letcher PM, Longcore JE, Mozley-Standridge SE, Porter D, Powell MJ, Griffith GW, Vilgalys R, 2007 ['2006']. A molecular phylogeny of the flagellated fungi (*Chytridiomycota*) and a proposal for a new phylum (*Blastocladiomycota*). *Mycologia* **98**: 860–871.

James TY, Porter D, Leander CA, Vilgalys R, Longcore JE, 2000. Molecular phylogenetics of the *Chytridiomycota* supports the utility of ultrastructural data in chytrid systematics. *Canadian Journal of Botany* **78**: 336–350.

Jülich W, 1981. Higher taxa of basidiomycetes. *Bibliotheca Mycologica* **85**: 1–485.

Kauff F, Lutzoni F, 2002. Phylogeny of the *Gyalectales* and *Ostropales* (*Ascomycota*, *Fungi*): among and within order relationships based on nuclear ribosomal RNA small and large subunits. *Molecular Phylogenetics and Evolution* **25**: 138–156.

Keeling PJ, 2003. Congruent evidence for alpha-tubulin and beta-tubulin gene phylogenies for a zygomycete origin of *Microsporidia*. *Fungal Genetics and Biology* **38**: 298–309.

Keeling PJ, Luker MA, Palmer JD, 2000. Evidence from beta-tubulin phylogeny that *Microsporidia* evolved from within the fungi. *Molecular Biology and Evolution* **17**: 23–31.

Kendrick B (ed), 1979. *The Whole Fungus: the Sexual–asexual Synthesis, Vol. 2*. National Museum of Natural Sciences, Ottawa.

Kendrick B, 1985. *The Fifth Kingdom*. Mycologue Publications, Waterloo.

Kirk PM, Cannon PF, David JC, Stalpers JA (eds), 2001. *Ainsworth & Bisby's Dictionary of the Fungi*, ninth ed. CABI Publishing, Wallingford.

Kohlmeyer J, Spatafora JW, Volkmann-Kohlmeyer B, 2000. *Lulworthiales*, a new order of marine *Ascomycota*. *Mycologia* **92**: 453–458.

Kruys A, Eriksson OE, Wedin M, 2006. Phylogenetic relationships of coprophilous *Pleosporales* (*Dothideomycetes*, *Ascomycota*), and the classification of some bitunicate taxa of unknown position. *Mycological Research* **110**: 527–536.

Kuramae EE, Robert V, Snel B, Weiß M, Boekhout T, 2006. Phylogenomics reveal a robust fungal tree of life. *FEMS Yeast Research* **6**: 1213–1220.

Kudrjazew W, 1960. *Die Systematik der Hefen*. Akademie Verlag, Berlin.

Kurtzman CP, Sugiyama J, 2001. Ascomycetous yeasts and yeastlike taxa. In: McLaughlin DJ, McLaughlin EJ, Lemke P (eds), *The Mycota*. Vol. VII. Part A. *Systematics and Evolution*. Springer-Verlag, Berlin, pp. 179–200.

Landvik S, Eriksson OE, 1994. Relationship of the genus *Glaziella* (*Ascomycota*) inferred from 18S rDNA sequences. *Systema Ascomycetum* **13**: 13–23.

Landvik S, Eriksson OE, Berbee ML, 2001. *Neolecta* — a fungal dinosaur? Evidence from β-tubulin amino acid sequences. *Mycologia* **93**: 1151–1163.

Landvik S, Eriksson OE, Gargas A, Gustafsson P, 1993. Relationships of the genus *Neolecta* (*Neolectales* ordo nov., *Ascomycotina*) inferred from 18S rDNA sequences. *Systema Ascomycetum* **11**: 107–118.

Larsson E, Larsson K-H, 2003. Phylogenetic relationships of russuloid basidiomycetes with emphasis on aphyllophoralean taxa. *Mycologia* **95**: 1037–1065.

Larsson JIR, 2000. The hyperparasitic microsporidium *Amphiacantha longa* Caullery et Mesnil, 1914 (*Microspora: Metchnikovellidae*) — description of the cytology, redescription of the species, emended diagnosis of the genus *Amphiacantha* and establishment of the new family *Amphiacanthidae*. *Folia Parasitologica* **47**: 241–256.

Larsson K-H, Larsson E, Kõljalg U, 2004. High phylogenetic diversity among corticioid homobasidiomycetes. *Mycological Research* **108**: 983–1002.

Larsson K-H, Parmasto E, Fischer M, Langer E, Nakasone KK, Redhead SA, 2007 ['2006']. *Hymenochaetales*: a molecular phylogeny for the hymenochaetoid clade. *Mycologia* **98**: 926–936.

Letcher PM, Powell MJ, Churchill PF, Chambers JG, 2006. Ultrastructural and molecular phylogenetic delineation of a new order, the *Rhizophydiales* (*Chytridiomycota*). *Mycological Research* **110**: 898–915.

Li JL, Heath IB, Packer L, 1993. The phylogenetic relationships of the anaerobic chytridiomycetous gut fungi (*Neocallimasticaceae*) and the *Chytridiomycota*. II. Cladistic analysis of structural data and description of *Neocallimasticales* ord. nov. *Canadian Journal of Botany* **71**: 393–407.

Lichtwardt RW, Manier JF, 1978. Validation of the *Harpellales* and *Asellariales*. *Mycotaxon* **7**: 441–442.

Lindau G, 1896. *Hysteriineae*. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien, Vol. 1*. Wilhelm Engelmann Verlag, Leipzig, pp. 265–278.

Lindau G, 1897a. *Dothideales*. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien, Vol. 1*. Wilhelm Engelmann Verlag, Leipzig, pp. 373–383.

Lindau G, 1897b. *Hypocreales*. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien, Vol. 1(1)*. *Leipzig,*. Wilhelm Engelmann Verlag, pp. 343–372.

Lindau G, 1897c. *Laboulbeniineae*. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien, Vol. 1(1)*. *Leipzig,*. Wilhelm Engelmann Verlag, pp. 491–505.

Lindemuth R, Wirtz N, Lumbsch HT, 2001. Phylogenetic analysis of nuclear and mitochondrial rDNA sequences supports the view that loculoascomycetes (*Ascomycota*) are not monophyletic. *Mycological Research* **105**: 1176–1181.

Liu YJ, Hall BD, 2004. Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. *Proceedings of the National Academy of Sciences, USA* **101**: 4507–4512.

Liu YJ, Hodson MC, Hall BD, 2006. Loss of the flagellum happened only once in the fungal lineage: phylogenetic structure of kingdom *Fungi* inferred from RNA polymerase II subunit genes. *BMC Evolutionary Biology* **6**: 74.www.biomedcentral.com/1471-2148/6/74.

Locquin M, 1984. *Mycologie Générale et Structurale*. Masson, Paris.

Lücking R, Stuart B, Lumbsch HT, 2004. Phylogenetic relationships of *Gomphillaceae* and *Asterothyriaceae*: evidence from a combined Bayesian analysis of nuclear and mitochondrial sequences. *Mycologia* **96**: 283–294.

Lumbsch T, Palice Z, Wiklund E, Ekman S, Wedin M, 2004. Supraordinal phylogenetic relationships of *Lecanoromycetes* based on Bayesian analysis of combined nuclear and mitochondrial sequences. *Molecular Phylogenetics and Evolution* **31**: 822–832.

Lumbsch HT, Schmitt I, Lindemuth R, Miller A, Mangold A, Fernandez F, Huhndorf S, 2005. Performance of four ribosomal DNA regions to infer higher-level phylogenetic relationships of inoperculate euascomycetes (*Leotiomyceta*). *Molecular Phylogenetics and Evolution* **34**: 512–524.

Lumbsch HT, Schmitt I, Lücking R, Wiklund RE, Wedin M, 2007. The phylogenetic placement of *Ostropales* within *Lecanoromycetes* (*Ascomycota*) revisited. *Mycological Research* **111**.

Lumbsch HT, Wirtz N, Lindemuth R, Schmitt I, 2002. Higher level phylogenetic relationships of euascomycetes (*Pezizomycotina*) inferred from a combined analysis of nuclear and mitochondrial sequence data. *Mycological Progress* **1**: 57–70.

Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett DS, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miądlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung G-H, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim Y-W, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R, 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* **91**: 1446–1480.

Manier J-F, 1973. Quelques aspects ultrastructuraux du trichomycète asellariale, *Asellaria ligae* Tuzet et Manier, 1950 ex Manier, 1958. *Comptes Rendu hebdomadaires des séances de l'Académie des Sciences de Paris, séries D* **276**: 3429–3431.

Margulis L, Corliss JO, Melkonian M, Chapman DJ, 1990. *Handbook of Protoctista: the structure, cultivation, habitats and life histories of the eukaryotic micro-organisms and their descendants*. Jones & Bartlett, Boston.

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo J-M, Ge Z-W, Yang Z-L, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS, 2007 [''2006'']a. Major clades of *Agaricales*: a multi-locus phylogenetic overview. *Mycologia* **98**: 984–997.

Matheny PB, Gossman JA, Zalar P, Arun Kumar TK, Hibbett DS, 2007a. Resolving the phylogenetic position of the *Wallemiomycetes*: an enigmatic major lineage of *Basidiomycota*. *Canadian Journal of Botany* **84**: 1794–1805.

Matheny PB, Wang Z, Binder M, Curtis JM, Lim YW, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer GE, McLaughlin DJ, Wilson AW, Frøslev T, Ge ZW, Kerrigan RW, Slot JC, Vellinga EC, Liang ZL, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vaura J, Hibbett DS, 2006. Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (*Basidiomycota*, *Fungi*). *Molecular Phylogenetics and Evolution* in press.

McLaughlin DJ, McLaughlin EG, Lemke PA (eds), 2001a. *The Mycota*. **Vol. VII**. Part A. *Systematics and Evolution*. Springer-Verlag, Berlin.

McLaughlin DJ, McLaughlin EG, Lemke PA (eds), 2001b. *The Mycota*. **Vol. VII**. Part B. *Systematics and Evolution*. Springer-Verlag, Berlin.

McNeill JF, Barrie F, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema J, Turland NJ (eds), 2006. *International Code of Botanical Nomenclature (Vienna Code) [Regnum Vegetabile* **Vol. 146**.*]*. A.R.G. Ganter Verlag, Ruggell.

Miądlikowska J, Kauff F, Hofstetter V, Fraker E, Reeb V, Grube M, Hafellner J, Kukwa M, Lücking R, Hestmark G, Otalora MG, Rauhut A, Büdel B, Scheidegger C, Timdal E, Stenroos S, Brodo I, Perlmutter GB, Ertz D, Diederich P, Lendemer JC, May P, Schoch CL, Arnold AE, Hodkinson BP, Gueidan C, Tripp E, Yahr R, Robertson C, Lutzoni F, 2007 ['2006']. New insights into classification and evolution of the *Lecanoromycetes* (*Pezizomycotina*, *Ascomycota*) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia* **98**: 1089–1102.

Miądlikowska J, Lutzoni F, 2004. Phylogenetic classification of peltigeralean fungi (*Peltigerales*, *Ascomycota*) based on ribosomal RNA small and large subunits. *American Journal of Botany* **91**: 449–464.

Miller AN, Huhndorf SM, 2004. A natural classification of *Lasiosphaeria* based on nuclear LSU rDNA sequences. *Mycological Research* **108**: 26–34.

Miller AN, Huhndorf SM, 2005. Multi-gene phylogenies indicate ascomal wall morphology is a better predictor of phylogenetic relationships than ascospore morphology in the *Sordariales* (*Ascomycota*, *Fungi*). *Molecular Phylogenetics and Evolution* **35**: 60–75.

Miller SL, Larsson E, Larsson K-H, Verbeken A, Nuytinck J, 2007 ['2006']. Perspectives in the new *Russulales*. *Mycologia* **98**: 960–970.

Moncalvo J-M, Nilsson RH, Koster B, Dunham SM, Bernauer T, Matheny PB, McLenon T, Margaritescu S, Weiß M, Garnica S, Danell E, Langer G, Langer E, Larsson E, Larsson K-H, Vilgalys R, 2007 ['2006']. The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia* **98**: 937–948.

Moncalvo J-M, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémençon H, Miller Jr OK, 2002. One hundred seventeen clades of euagarics. *Molecular Phylogenetics and Evolution* **23**: 357–400.

Moore RT, 1980. Taxonomic proposals for the classification of marine yeasts and other yeast-like fungi including the smuts. *Botanica Marina* **23**: 361–373.

Moore RT, 1990. Order *Platygloeales* ord. nov. *Mycotaxon* **39**: 245–248.

Moreau F, 1954. *Les Champignons. Physiologie, morphologie, développement et systématique. Vol. 2*. Lechevalier, Paris.

Morton JB, Benny GL, 1990. Revised classification of arbuscular mycorrhizal fungi (*Zygomycetes*): a new order, *Glomales*, two new suborders, *Glomineae* and *Gigasporineae*, and two new families, *Acaulosporaceae* and *Gigasporaceae*, with an emendation of *Glomaceae*. *Mycotaxon* **37**: 471–491.

Moss ST, 1975. Septal structure in the *Trichomycetes* with special reference to *Astreptonema gammari* (*Eccrinales*). *Transactions of the British Mycological Society* **65**: 115–127.

Nagahama T, Hamamoto M, Nakase T, Shimamura S, Horikoshi K, 2006. Phylogenetic relationship within the *Erythrobasidium* clade: molecular phylogenies, secondary structure, and intron positions inferred from partial sequences of ribosomal RNA and elongation factor-1α genes. *Journal of General and Applied Microbiology* **52**: 37–45.

Nagahama T, Sato H, Shimazu M, Sugiyama J, 1995. Phylogenetic divergence of the entomophthoralean fungi: evidence from nuclear 18S ribosomal RNA gene sequences. *Mycologia* **87**: 203–209.

Nannfeldt JA, 1932. Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. *Nova Acta Regiae Societatis Scientarum Upsaliensis, ser. 4,* **8** (2): 1–368.
Nishida H, Sugiyama J, 1994. *Archiascomycetes*: detection of a major new lineage within the *Ascomycota*. *Mycoscience* **35**: 361–366.
Oberwinkler F, 1976. Eine agaricoide Gattung der *Thelephorales*. *Sydowia* **78**: 359–361.
Oberwinkler F, 1977. Das neue System der Basidiomyceten. In: Frey W, Hurka H, Oberwinkler F (eds), *Beiträge zur Biologie der niederen Pflanzen*. Gustav Fischer Verlag, Stuttgart, pp. 59–105.
Oberwinkler F, Bandoni RJ, 1982. A taxonomic survey of the gasteroid, auricularioid *Heterobasidiomycetes*. *Canadian Journal of Botany* **60**: 1726–1750.
Oberwinkler F, Bauer R, 1989. The systematics of gasteroid, auricularioid *Heterobasidiomycetes*. *Sydowia* **41**: 224–256.
Oberwinkler F, Bauer R, 1990. *Cryptomycocolax*: a new mycoparasitic heterobasidiomycete. *Mycologia* **82**: 671–692.
O'Donnell K, Cigelnik E, Benny GL, 1998. Phylogenetic relationships among the *Harpellales* and *Kickxellales*. *Mycologia* **90**: 286–297.
Pang KL, Abdel-Wahab MA, Sivichai S, El-Sharouney HM, Jones EBG, 2002. *Jahnulales* (*Dothideomycetes*, *Ascomycota*): a new order of lignicolous freshwater ascomycetes. *Mycological Research* **106**: 1031–1042.
Parmasto E, 1986. On the origin of the hymenomycetes (what are corticioid fungi?). *Windahlia* **16**: 3–20.
Peyretaillade E, Broussolle V, Peyret P, Méténier G, Gouy M, Vivarès CP, 1998. *Microsporidia*, amitochondrial protists, possess a 70-kDa heat shock protein gene of mitochondrial evolutionary origin. *Molecular Biology and Evolution* **15**: 683–689.
Réblová M, 2006. Molecular systematics of *Ceratostomella sensu lato* and morphologically similar fungi. *Mycologia* **98**: 68–93.
Réblová M, Mostert L, Gams W, Crous PW, 2004. New genera in the Calosphaeriales: *Togniniella* and its anamorph *Phaeocrella*, and *Calosphaeriophora* as anamorph of *Calosphaeria*. *Studies in Mycology* **50**: 533–550.
Réblová M, Seifert KA, 2004. *Cryptadelphia* (*Trichosphaeriales*), a new genus for holomorphs with *Brachysporium* anamorphs and clarification of the taxonomic status of *Wallrothiella*. *Mycologia* **96**: 343–367.
Reeb V, Lutzoni F, Roux C, 2004. Multilocus phylogenetic circumscription of the lichen-forming fungi family *Acarosporaceae* and its position within the *Ascomycota*. *Molecular Phylogenetics and Evolution* **32**: 1036–1060.
Robbertse B, Reeves J, Schoch C, Spatafora JW, 2006. A phylogenomic analysis of the *Ascomycota*. *Fungal Genetics and Biology* **43**: 715–725.
Rossman AY, Aime MC, Farr DF, Castelbury LA, Peterson KR, Leahy R, 2004. The coelomycetous genera *Chaetomella* and *Pilidium* represent a newly discovered lineage of inoperculate discomycetes. *Mycological Progress* **3**: 275–290.
Ryvarden L, 2002. A note on the genus *Hydnodon* Banker. *Synopsis Fungorum* **15**: 31–33.
Saenz GS, Taylor JW, 1999. Phylogenetic relationships of *Meliola* and *Meliolina* inferred from nuclear small subunit rRNA sequences. *Mycological Research* **103**: 1049–1056.
Saikawa M, Sugiura K, Sato H, 1997. Electron microscopy of two trichomycetous fungi attached to the hindgut lining of pillbugs. *Canadian Journal of Botany* **75**: 1479–1484.
Sampaio JP, Gadanho M, Bauer R, Weiß M, 2003. Taxonomic studies in the *Microbotryomycetidae*: *Leucosporidium golubevii* sp. nov., *Leucosporidiella* gen. nov. and the new orders *Leucosporidiales* and *Sporidiobolales*. *Mycological Progress* **2**: 53–68.
Sampaio JP, 2004. Diversity, phylogeny and classification of basidiomycetous yeasts. In: Agerer R, Piepenbring M, Blanz P (eds), *Frontiers in Basidiomycote Mycology*. IHW Verlag, Eching, pp. 49–80.
Schaffner JH, 1909. The classification of plants, IV. *Ohio Naturalist* **9**: 446–455.
Schmitt I, Mueller G, Lumbsch HT, 2005. Ascoma morphology is homoplaseous and phylogenetically misleading in some pyrenocarpous lichens. *Mycologia* **97**: 362–374.
Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW, 2007 ['2006']. A multigene phylogeny of the *Dothideomycetes* using four nuclear loci. *Mycologia* **98**: 1042–1053.
Schröter J, 1889. Pilze. In: Cohn F (ed), *Kryptogamen-Flora von Schlesien, Vol. 3*. J. U. Kern's Verlag, Breslau, pp. 382–386.
Schröter J, 1892. Phycomycetes. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien, Vol. 1*. Wilhelm Engelmann Verlag, Leipzig, pp. 63–87.
Schröter J, 1893. *Monoblepharidineae*. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien, Vol. 1*. Wilhelm Engelmann Verlag, Leipzig, pp. 106–107.
Schröter J, 1894. *Pezizineae*. In: Engler A, Prantl K (eds), *Die natürlichen Pflanzenfamilien, Vol. 1*. Wilhelm Engelmann Verlag, Leipzig, pp. 173–243.
Schüßler A, Schwarzott D, Walker C, 2001. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* **105**: 1413–1421.
Seif E, Leigh L, Roewer I, Forget L, Lang BF, 2005. Comparative mitochondrial genomics in zygomycetes: bacteria-like RNase P RNAs, mobile elements and a close source of the group I intron invasion in angiosperms. *Nucleic Acids Research* **33**: 734–744.
Serbinow JL, 1907. Organisazija I rasitie nekotorich gribow *Chytridineae* Schröt. *Scripa Botanica Horti Universitatis Imperialis Petropolitana* **24**: 1–147.
Shenoy BD, Jeewon R, Wu WP, Bhat DJ, Hyde KD, 2006. Ribosomal and RPB2 DNA sequence analyses suggest that *Sporidesmium* and morphologically similar genera are polyphyletic. *Mycological Research* **110**: 916–928.
Sparrow FK, 1943. *Aquatic Phycomycetes exclusive of the Saprolegniaceae and Pythium*. University of Michigan Press, Ann Arbor.
Sparrow FK, 1958. Interrelationships and phylogeny of the aquatic *Phycomycetes*. *Mycologia* **50**: 797–813.
Spatafora JW, Johnson D, Sung G-H, Hosaka K, O'Rourke B, Serdani M, Spotts R, Lutzoni F, Hofstetter V, Fraker E, Gueidan C, Miądlikowska J, Reeb V, Lumbsch T, Lücking R, Schmitt I, Aptroot A, Roux C, Miller A, Geiser D, Hafellner J, Hestmark G, Arnold AE, Büdel B, Rauhut A, Hewitt D, Untereiner W, Cole MS, Scheidegger C, Schultz M, Sipman H, Schoch CL, 2007 ['2006']. A five-gene phylogenetic analysis of the *Pezizomycotina*. *Mycologia* **98**: 1020–1030.
Sprague V, Becnel JJ, 1998. Note on the name-author-date combination for the taxon *Microsporidies* Balbiani, 1882, when ranked as a phylum. *Journal of Invertebrate Pathology* **71**: 91–94.
Starbäck K, 1899. Ascomyceten der Ersten Regnellschen Expedition. *Bihang till Kungliga Svenska Vetenskaps-Akademiens Handlingar, ser. 3* **25**: 1–68.
Steenkamp ET, Wright J, Baldauf SL, 2006. The protistan origins of animals and fungi. *Molecular Biology and Evolution* **23**: 93–106.
Sugiyama J, Hosaka K, Suh S-O, 2007 ['2006']. Early diverging *Ascomycota*: phylogenetic divergence and related evolutionary enigmas. *Mycologia* **98**: 998–1007.
Suh S-O, Blackwell M, Kurtzman CP, Lachance M-A, 2007 ['2006']. Phylogenetics of *Saccharomycetales*, the ascomycete yeasts. *Mycologia* **98**: 1008–1019.
Swann EC, Frieders EM, McLaughlin DJ, 2001. *Urediniomycetes*. In: McLaughlin DJ, McLaughlin EJ, Lemke P (eds), *The Mycota*. **Vol. VII**. Part B, *Systematics and Evolution*. Springer-Verlag, Berlin, pp. 37–56.
Swann EC, Taylor JW, 1995. Phylogenetic perspectives on basidiomycete systematics: evidence from the 18S rRNA gene. *Canadian Journal of Botany* **73**: S862–S868.
Takamatsu S, 2004. Phylogeny and evolution of the powdery mildew fungi (*Erysiphales*, *Ascomycota*) inferred from nuclear ribosomal DNA sequences. *Mycoscience* **45**: 147–157.

Tanabe Y, O'Donnell K, Saikawa M, Sugiyama J, 2000. Molecular phylogeny of parasitic *Zygomycota* (*Dimargaritales*, *Zoopagales*) based on nuclear small subunit ribosomal DNA sequences. *Molecular Phylogenetics and Evolution* **16**: 253–262.

Tanabe Y, Saikawa M, Watanabe MM, Sugiyama J, 2004. Molecular phylogeny of *Zygomycota* based on EF-1 and RPB1 sequences: limitations and utility of alternative markers to rDNA. *Molecular Phylogenetics and Evolution* **30**: 438–449.

Tanabe Y, Watanabe MM, Sugiyama J, 2005. Evolutionary relationships among basal fungi (*Chytridiomycota* and *Zygomycota*): Insights from molecular phylogenetics. *Journal of General and Applied Microbiology* **51**: 267–276.

Tang AMC, Jeewon R, Hyde KD, 2007. Phylogenetic utility of protein (RPB2, B-tubulin) and ribosomal (LSU, SSU) gene sequences in the systematics of *Sordariomycetes* (*Ascomycota*, *Fungi*). *Antonie van Leeuwenhoek* in press.

Taylor JW, Spatafora J, O'Donnell K, Lutzoni F, James T, Hibbett DS, Geiser D, Bruns TD, Blackwell M, 2004. The fungi. In: Cracraft J, Donoghue MJ (eds), *Assembling the Tree of Life*. Oxford University Press, Oxford, pp. 171–196.

Tehler A, 1988. A cladistic outline of the *Eumycota*. *Cladistics* **4**: 227–277.

Tehler A, Little DP, Farris JS, 2003. The full-length phylogenetic tree from 1551 ribosomal sequences of chitinous fungi, *Fungi*. *Mycological Research* **107**: 901–916.

Thorn RG, Moncalvo J-M, Reddy CA, Vilgalys R, 2000. Phylogenetic analyses and the distribution of nematophagy support a monophyletic *Pleurotaceae* within the polyphyletic pleurotoid-lentinoid fungi. *Mycologia* **92**: 241–252.

Tibell L, Vinuesa M, 2005. *Chaenothecopsis* in a molecular phylogeny based on nuclear rDNA ITS and LSU sequences. *Taxon* **54**: 427–442.

Tibell L, Wedin M, 2000. *Mycocaliciales*, a new order for nonlichenized calicioid fungi. *Mycologia* **92**: 577–581.

Underwood LM, 1899. *Moulds, mildews and mushrooms: a guide to the systematic study of the Fungi and Mycetozoa and their literature*. Henry Holt, New York.

Vijaykrishna D, Mostert L, Jeewon R, Gams W, Hyde KD, Crous PW, 2004. *Pleurostomophora*, an anamorph of *Pleurostoma* (*Calosphaeriales*), a new anamorph genus morphologically similar to *Phialophora*. *Studies in Mycology* **50**: 387–395.

Vossbrinck CR, Debrunner-Vossbrinck BA, 2005. Molecular phylogeny of the *Microsporidia*: ecological, ultrastructural and taxonomic considerations. *Folia Parasitologica* **52**: 131–142.

Wagner T, Fischer M, 2002. Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. *Mycologia* **94**: 998–1016.

Walker C, Schüßler A, 2004. Nomenclatural clarifications and new taxa in the *Glomeromycota*. *Mycological Research* **108**: 981–982.

Wang Z, Binder M, Schoch C, Johnston PR, Spatafora JW, Hibbett DS, 2006. Evolution of helotialean fungi (*Leotiomycetes*, *Pezizomycotina*): a nuclear rDNA phylogeny. *Molecular Phylogenetics and Evolution* **41**: 295–312.

Wang Z, Johnston PR, Takamatsu S, Spatafora JW, Hibbett DS, 2007 ['2006']. Phylogenetic classification of the *Leotiomycetes* based on rDNA data. *Mycologia* **98**: 1066–1076.

Wedin M, Wiklund E, Crewe A, Döring H, Ekman S, Nyberg Å Schmitt I, Lumbsch HT, 2005. Phylogenetic relationships of *Lecanoromycetes* (*Ascomycota*) as revealed by analyses of mtSSU and nLSU rDNA sequence data. *Mycological Research* **109**: 159–172.

Weir A, Blackwell M, 2001. Molecular data support the *Laboulbeniales* as a separate class of *Ascomycota*, *Laboulbeniomycetes*. *Mycological Research* **105**: 1182–1190.

Weiß M, Bauer R, Begerow D, 2004a. Spotlights on heterobasidiomycetes. In: Agerer R, Piepenbring M, Blanz P (eds), *Frontiers in Basidiomycote Mycology*. IHW Verlag, Eching, pp. 7–48.

Weiß M, Oberwinkler F, 2001. Phylogenetic relationships in *Auriculariales* and related groups—hypotheses derived from nuclear ribosomal DNA sequences. *Mycological Research* **105**: 403–415.

Weiß M, Selosse M-A, Rexer K-H, Urban A, Oberwinkler O, 2004b. *Sebacinales*: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycological Research* **108**: 1003–1010.

Wells K, Bandoni RJ, 2001. *Heterobasidiomycetes*. In: McLaughlin DJ, McLaughlin EJ, Lemke P (eds), *The Mycota*. **Vol. VII**. Part B, *Systematics and Evolution*. Springer-Verlag, Berlin, pp. 85–120.

White TJ, Bruns TD, Lee S, Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky J, White TJ (eds), *PCR Protocols: a Guide to Methods and Applications*. Academic Press, San Diego, pp. 315–322.

White MM, James TY, O'Donnell K, Cafaro MJ, Tanabe Y, Sugiyama J, 2007 ['2006']. Phylogeny of the *Zygomycota* based on nuclear ribosomal sequence data. *Mycologia* **98**: 872–884.

Whittaker RH, 1959. On the broad classification of organisms. *Quarterly Review of Biology* **34**: 210–226.

Wiklund E, Wedin M, 2003. The phylogenetic relationships of the cyanobacterial lichens in the *Lecanorales* suborder *Peltigerineae*. *Cladistics* **19**: 419–431.

Wingfield B, Viljoen CD, Wingfield MJ, 1999. Phylogenetic relationships of ophiostomatoid fungi associated with *Protea infrusctescences* in South Africa. *Mycological Research* **103**: 1616–1620.

Winka K, 2000. Phylogenetic relationships within the Ascomycota based on 18S rDNA sequences. PhD thesis, Umeå University, Umeå.

Winter G, 1880. *Schizomyceten, Saccharomyceten und Basidiomyceten*. In: *Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz, Vol. 1*. E. Kummer, Leipzig, pp. 1–924.

Woronichin NN, 1925. Über die *Capnodiales*. *Annales Mycologici* **23**: 174–178.

Zalar P, de Hoog GS, Schroers H-J, Frank JM, Gunde-Cimerman N, 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (*Wallemiomycetes* and *Wallemiales*, cl. et ord. nov.). *Antonie van Leeuwenhoek* **87**: 311–328.

Zhang N, Castlebury LA, Miller AN, Huhndorf S, Schoch CL, Seifert K, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung G-H, 2007 ['2006']. *Sordariomycetes* systematics: an overview of the systematics of the *Sordariomycetes* based on a four-gene phylogeny. *Mycologia* **98**: 1077–1088.

PERSPECTIVES

ESSAY

Fungal systematics: is a new age of enlightenment at hand?

David S. Hibbett and John W. Taylor

Abstract | Fungal taxonomists pursue a seemingly impossible quest: to discover and give names to all of the world's mushrooms, moulds and yeasts. Taxonomists have a reputation for being traditionalists, but as we outline here, the community has recently embraced the modernization of its nomenclatural rules by discarding the requirement for Latin descriptions, endorsing electronic publication and ending the dual system of nomenclature, which used different names for the sexual and asexual phases of pleomorphic species. The next, and more difficult, step will be to develop community standards for sequence-based classification.

Taxonomists create the language of biodiversity, enabling communication about different organisms among basic and applied scientists, educators, students and the general public. This essential work is particularly challenging in hyperdiverse and morphologically cryptic groups, such as the kingdom Fungi. Roughly 100,000 species of fungi are accepted in the current taxonomy[1], but more than 400,000 fungal species names — including numerous synonyms — are recorded in the literature, and it is likely that millions of new species[2] still await description. Thus, the challenge for modern fungal taxonomy is to weed out redundant published names while accelerating the naming of newly discovered species. To regulate the naming of fungi, mycologists adhere to the *International Code of Botanical Nomenclature*[3]. The code provides stability to a potentially chaotic discipline, but it is updated only once every 7 years and only at meetings of the Nomenclature Section during the International Botanical Congress (IBC), which makes the code slow to adapt to modern practices in systematics. The fungal elements of the code that have been criticized as archaic include the dual system of nomenclature[4], which creates different names for the anamorphs (asexual forms) and teleomorphs (sexual forms) of the same species (FIG. 1), and the requirement for physical type specimens, which complicates efforts to classify taxa that are discovered through metagenomics[5].

In the lead-up to the last IBC in July 2011, a vocal and well-organized group of mycologists launched a 'One fungus, one name' campaign aimed at ending the system of dual nomenclature. The movement culminated in the publication of 'The Amsterdam declaration on fungal nomenclature' (by 88 co-authors from 26 countries)[4], which suggested that if dual nomenclature were retained in the botanical code, it might be necessary to create a separate MycoCode for the kingdom Fungi[6]. Independently, some mycologists had already begun to publish new fungal names that ignored reproductive morphology, putting sexual and asexual species in the same genus and thus deliberately disregarding the code[7–9]. Facing nomenclatural disobedience and the threat of secession, the Nomenclature Section of the 2011 IBC voted to abolish the dual system of fungal nomenclature[10,11]. At the same time, and in response to pressure from other activists, the Nomenclature Section also voted to eliminate Latin descriptions (English will now suffice), to allow the publication of new names in online-only journals (previously, print was required) and to require registration of new fungal names in a publically accessible database such as Index Fungorum or MycoBank[10]. Finally, the code itself was renamed the *International Code of Nomenclature for Algae, Fungi, and Plants* (ICN). To many scientists, these may seem like overdue, common-sense measures, but to some fungal taxonomists, the changes were seismic[11].

In the long run, a unitary nomenclature system for pleomorphic fungi, along with the other changes, will promote effective communication. In the short term, however, the abandonment of dual nomenclature will require mycologists to work together to resolve the correct names for large numbers of fungi, including many economically important pathogens and industrial organisms. Here, we consider the opportunities and challenges posed by the repeal of dual nomenclature and the parallels and contrasts between nomenclatural practices for fungi and prokaryotes. We also explore the options for fungal taxonomy based on environmental sequences and ask whether sequence-based taxonomy can be reconciled with the ICN.

One name, one fungus

The dual nomenclature system for pleomorphic fungal species arose in the nineteenth century, influenced by the use of sexual morphology in the Linnaean classification of plants[12,13]. Despite the fact that is illogical to assign multiple names to one species, the dual nomenclature system persisted, in part because the morphology of sexual reproductive structures was assumed to be superior to that of asexual forms for inferring the evolutionary relationships of fungi[14]. However, sexual characteristics lost their pre-eminence for classifying fungi in the late 1980s, when PCR made DNA variation accessible to systematic mycologists. More than 20 years later, the dual nomenclature system was finally abolished.

As is always the case, the hard work begins after the revolution. For mycologists, this means choosing names for thousands of pleomorphic fungal species. Some choices will be difficult. For example, the anamorphic genus *Penicillium* (with teleomorphic genera *Eupenicillium* and *Talaromyces*) contains fungi as important as *Penicillium rubens* (the original source of penicillin), *Penicillium marneffei* (the causative agent of an AIDS-defining disease in Thailand),

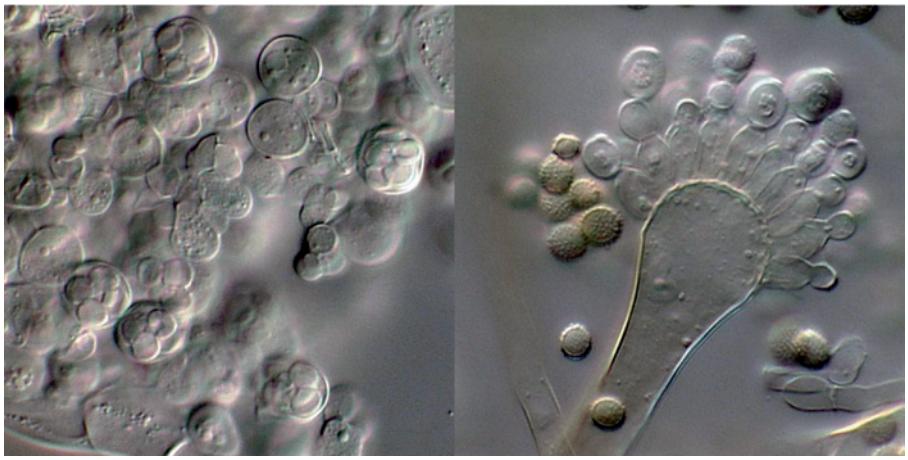


Figure 1 | **Two names, one fungus.** *Eurotium herbariorum* is a pleomorphic fungus that has a sexual phase, reproducing by ascospores (the teleomorphic form; left), as well as a conidium-producing asexual phase (the anamorphic form; right) that has been named *Aspergillus glaucus*. Images courtesy of Paul F. Cannon, Royal Botanical Gardens, Kew, London, UK, and the Centre for Agricultural Bioscience International (CABI).

and *Penicillium camemberti* and *Penicillium roqueforti* (used to make Camembert and Brie, and Roquefort cheeses, respectively). However, *Penicillium* spp., as traditionally delimited, are paraphyletic as well as pleomorphic, so these well-known species cannot all remain in this historic genus[15].

Under the revised code, any of the existing valid names for a species can be selected as its correct name, with preference given to the oldest name. However, this libertarian view is tempered by two additional revisions, both involving review by the General Committee (GC) of the ICN, which is empowered to vote on proposals to conserve or reject names of fungal taxa, as well as to modify the ICN itself[3]. First, in situations in which both the anamorph and teleomorph names for the same taxon are widely used — for example, *Fusarium* (anamorph) and *Gibberella* (teleomorph) at the genus level — the teleomorph name can be chosen without approval of the GC, but selection of the anamorph name, even if it is the older name, requires approval. Apparently, it is hard for systematists to abandon the primacy of sexual characteristics. Second, the GC has the authority to approve lists of names, which presumably will be generated by committees of mycologists with expertise in particular taxonomic groups. However, mycologists have retained the right to appeal any decision about names through the established process of conservation of names.

No one has had a chance to choose a name for a pleomorphic fungal species under the new code, which only came into effect on 1 January 2013, but the nomenclatural changes mentioned above illustrate what might lie ahead. Another example is the work of Gräfenhan *et al.*[9] on the taxonomy of the anamorphic genus *Fusarium*, one of the largest genera of fungi, containing nearly 1,500 species, subspecies, varieties and formae speciales. *Fusarium* spp. include important plant and animal pathogens and mycotoxin producers and have been linked to as many as seven teleomorph genera. On the basis of sequence analyses for RNA polymerase II and ATP citrate lyase genes, Gräfenhan *et al.*[9] identified 15 clades with *Fusarium*-like asexual forms and gave six of them names based on anamorphs, although five of these six have known teleomorphic forms. The reclassification of *Fusarium* by Gräfenhan *et al.* is based on robust phylogenies and would be nomenclaturally valid under the forthcoming ICN. Nevertheless, name changes in the genus *Fusarium sensu lato* might confuse and inconvenience user communities and regulatory bodies in agriculture and medicine, and it remains to be seen how these constituencies will react to the new taxonomy.

The complex nomenclatural history of many groups of pleomorphic fungi, coupled with phylogenetic uncertainty and the sometimes passionate opinions of stakeholders, presents a very challenging taxonomic problem. The code provides guidance, but many decisions about names cannot be reduced to 'legal' algorithms. As a follow-up to the 'One fungus, one name' movement that led to the repeal of dual nomenclature, a 'One fungus, which name?' conference was held in Amsterdam in April 2012 (REF. 16). This time, the goal was to begin working through the myriad options for the classification of pleomorphic fungi in light of the new rules. Similar meetings and workshops on the taxonomy of the genus *Fusarium*, the order Hypocreales and other groups were held in association with meetings of the Mycological Society of Japan (May 2012), the Mycological Society of America (July 2012) and the Mycological Society of China (August, 2012).

Classification of environmental sequences

Now that dual nomenclature has been abolished, the next major challenge for fungal taxonomy is to develop strategies for classifying environmental sequences (FIG. 2). Nobody knows how many unnamed species have already been detected through metagenomic studies (and this fact alone indicates the need for a centralized database of species that are based on environmental sequences), but as early as 2007 the number of clusters of closely related rRNA genes being discovered with Sanger chemistry approached the number of species being described from specimens[5], and the rate of molecular species discovery has surely increased with the application of next-generation sequencing in metagenomics.

Environmental studies have revealed not only individual species, but also major clades of fungi, such as the class Archaeorhizomycetes[17], containing a diverse group of soil-inhabiting fungi from the phylum Ascomycota. Sequences of Archaeorhizomycetes members have been reported in more than 50 independent studies, and they can be grouped into more than 100 species-level entities[17]. Nevertheless, only one species, *Archaeorhizomyces finlayi*, has been formally described, based on a culture that was obtained from conifer roots. A similar example is provided by the phylum Rozellomycota[18] (also known as Cryptomycota[19]), a large clade of aquatic and soil-inhabiting fungi that is known almost entirely from environmental sequences. The phylum Rozellomycota has been shown to contain the previously described chytrid genus *Rozella*[19], but most of the diversity of this phylum is in groups that are known only from environmental sequences and have not been named. These examples, and many others from fungal molecular ecology, illustrate the profound disconnect that now exists between formal taxonomy and species discovery through environmental sequences. Barriers to the naming of such species include a perceived conflict with the code, and errors and

Figure 2 | **Unnamed diversity.** A demonstration of the problem posed by unnamed fungi that are known only from environmental DNA sequences. When a new environmental sequence (the bottom-most operational taxonomic unit, gi|22497358; blue box) was used in a BLAST search of the GenBank database and the result displayed using the BLAST distance tree tool, only two of the 35 most closely related sequences were from cultured organisms (green boxes), and only one was named (*Fibulobasidium murrhardtense*). Without names, the information content of this tree leaves much to be desired. ITS, internal transcribed spacer; PS, partial sequence.

incomplete taxon sampling in reference sequence databases.

The perceived incompatibility of the code with sequence-based taxonomy is a consequence of the requirement for type specimens. However, the code places no restrictions on the form of type specimens, which need not be complete or representative; all that is required of a type specimen is that it should be a physical specimen. In principle, an aliquot of DNA extracted from an environmental sample, or a portion of the substrate from which the DNA was isolated, can serve as a legitimate type specimen. To prove this point, Kirk *et al.*[20] recently described a new species of rumen chytrid, *Piromyces cryptodigmaticus*, based on sequence data, and typified it with a sample from the fermenter from which the DNA was extracted. The new taxon name was validly published, even though the fungus was never directly observed. In the future, if purely sequence-based taxonomy is incorporated into the code, it may be possible to forego the deposition of physical type materials altogether. In the meantime, the publication of *P. cryptodigmaticus* provides a model for environmental molecular biologists who would like to formalize their discoveries through code-compliant taxonomic names.

Errors and incomplete taxonomic sampling in sequence databases, such as GenBank, present a psychological barrier to naming environmental sequences; if an environmental sequence has no match in GenBank, it could still represent a described but unsequenced species. Faced with such uncertainty, fungal taxonomists might be reluctant to describe new species based on environmental sequences. They should not be; current estimates of the actual diversity in the kingdom Fungi range from as few as 500,000 species to millions of species[2], suggesting that most unmatched environmental sequences probably do represent new species[5]. Even if some environmental species prove to be redundant, taxonomists are accustomed to resolving synonymy based on the principle of priority. Finally, the solution to the GenBank problem is conceptually straightforward — that is, generate well-documented reference sequences[21] — and is already being pursued through the fungal bar-coding initiative[22] and the creation of custom-curated databases of well-documented reference sequences, such as the RefSeq collection within GenBank, and the UNITE database for mycorrhizal fungi[23].

Lessons from prokaryotic taxonomy

Many of the taxonomic challenges faced by mycologists parallel those faced by researchers studying prokaryotes, but the nomenclatural practices adopted by the two groups are often divergent. For example, the expanded power of the GC to rule on the legitimacy of choices among existing names under the forthcoming ICN might worry some mycologists, who could fear a loss of taxonomic freedom, but the new system for fungi might seem familiar to prokaryote taxonomists, who have long used a Judicial Commission to accept or reject newly proposed names[24,25]. Another key difference between the nomenclatural codes for prokaryotes[26] and fungi[3] is that

the prokaryotic code specifies the technical means to recognize new species, and all new species are recorded in the *International Journal of Systematic and Evolutionary Microbiology*, whereas the ICN specifies no particular technique for the recognition of fungal species, which can be published in diverse venues. Under the ICN, acceptance of fungal species is left to the mycology community; new names are picked up by other mycologists and appear in the literature, or they are simply ignored. The highly regulated system for prokaryotes promotes uniformity in the species recognition criteria and preserves the stability of names, but it can also limit the rate of species description. By contrast, the laissez-faire system for fungi results in non-uniform species recognition criteria (for example, many new species descriptions lack supporting molecular data[5]), extensive synonymy, an ongoing challenge in compiling new names (although the new requirement for name registration will solve this problem) and frequent changes in species-level classifications. At the same time, the fungal system promotes rapid taxonomic updates to reflect new discoveries and advances in phylogenetic reconstruction.

Changes in fungal species classifications often occur when evidence for genetic diversity is discovered within morphological taxa. For example, it might have surprised readers to learn that Alexander Fleming's *Penicillium* species, *Penicillium chrysogenum*, is now known as *P. rubens*[27], but the change was necessitated when phylogenetic and population genetics data showed that the *P. chrysogenum* of old harboured several genetically isolated species[28]. Older mycologists may grumble about having to learn a new name, but the new classification reflects the current state of knowledge, and new students will not be bothered by the change. By contrast, the archaeon *Sulfolobus islandicus* was shown to comprise several genetically isolated species according to population genetics techniques, which showed genetic isolation by distance[29] and also evidence of ecological speciation[30], but these species were left unnamed, in part because the now passé technique of DNA–DNA hybridization would have been required for formal species descriptions[24]. Admittedly, there are huge challenges in determining species limits in bacteria and archaea, particularly in the face of extensive horizontal gene transfer[31]. Nonetheless, the differences in nomenclatural practices for bacteria and archaea versus fungi may be part of the reason why the number of new species described per year is about twice as many for fungi as it is for prokaryotes[5,32]. The ICN will increase the centralization of taxonomic authority for fungi, although the basic criteria for fungal species recognition will remain unrestricted. It is important that as the new rules of the ICN are implemented, the GC acts with restraint and does nothing to impede progress in fungal species description.

Mycologists can also learn from the experience of bacterial and archaeal researchers with regard to the classification of environmental sequences. The requirement for a living type culture for describing bacterial or archaeal species[26] is comparable to the requirement for a physical type specimen for naming fungal species. To enable the naming of bacteria that lack cultures but are known by "more than a mere sequence" (REF. 33), Murray and Schleifer[34] suggested that the prefix *Candidatus* be used, indicating that the name is provisional. This recommendation has been appended to the bacterial code[25], but fewer than 400 bacteria and archaea have been described as *Candidatus* species[35]. If mycologists wish to adopt a new category similar to *Candidatus* to accommodate the huge numbers of species discovered through environmental sequences, as has been suggested[5], they will need to find ways to facilitate high-throughput taxonomy, almost certainly involving automated work flows.

The future of fungal taxonomy

Twenty-five years after the first description of PCR, species-level fungal taxonomy is finally catching up with the molecular revolution. Change has come slowly and has been prompted by the actions of radicals, who flouted and subverted the code by naming taxa based on anamorphs[7,8] or environmental sequences[20]. Such individual acts of rebellion illuminate the way forward, but ultimately fungal taxonomy is a group enterprise that can succeed only with the support and participation of the broad community of mycologists. Proponents of unitary taxonomy worked effectively as a community to repeal dual nomenclature and are now organizing themselves to resolve the correct names of scores of pleomorphic fungal species. Supporters of sequence-based taxonomy have not been so unified, however. The publication of *P. cryptodigmaticus* demonstrates that it is 'legally' possible, under the code, to describe new species based on sequences (as long as a nominal type is deposited somewhere), but community effort will be needed to develop the broadly accepted protocols required for a mass movement towards sequence-based taxonomy.

At least one difficult issue appears to have been resolved: the internal transcribed spacer (ITS) region of the nuclear rRNA gene has been proposed as the fungal barcode locus[22] and is being used for sequence-based species delimitation in environmental surveys for many groups of fungi. However, other key issues remain problematic. Longer reads that provide sequences for the ITS and the phylogenetically tractable large subunit (LSU) rRNA cannot be obtained until there are improvements in next-generation sequencing. The gold standard for species delimitation in fungi is the genealogical concordance method, which uses multiple genetic loci to assess the limits of recombination[36]. Such approaches are not applicable in environmental data sets, which usually use single loci amplified from pooled DNAs. Moreover, in order to carry out species delimitation in environmental samples, the consequences of intragenomic heterogeneity in multicopy rRNA genes, as well as error owing to gene tree versus species tree conflict, will have to be determined empirically in relation to multigene data sets. The names of species known only from environmental sequences might require a new taxonomic category comparable to the *Candidatus* status for bacteria and archaea[5], or an identifying suffix (for example, ENAS (environmental nucleic acid sequence) or eMOTU (environmental molecular operational taxonomic unit))[6]. The reality of sequencing errors might prevent naming until the same sequence is found a second time and by a different research group. Finally, mycological databases such as MycoBank must prepare for a massive influx of new species, especially if automated work flows are developed to describe fungi from environmental nucleic acid sequences. Given the rate of species discovery, mycologists do not have another 25 years to ponder the problem.

David S. Hibbett is at The Biology Department, Clark University, Worcester, Massachusetts 01610, USA.

John W. Taylor is at The Department of Plant and Microbial Biology, University of California, Berkeley, California 94720, USA.

Correspondence to D.S.H.
e-mail: dhibbett@clarku.edu

doi:10.1038/nrmicro2942
Published online 3 January 2013

1. Kirk, P. *et al*. (eds) *Dictionary of the Fungi* 10th edn (CABI, 2008).
2. Bass, D. & Richards, T. A. Three reasons to re-evaluate fungal diversity "on Earth and in the ocean". *Fungal Biol. Rev.* **25**, 159–164 (2011).

3. McNeill, J. *et al.* (eds) *International Code of Botanical Nomenclature (Vienna Code)* (Gantner, 2006).
4. Hawksworth, D. L. *et al.* The Amsterdam declaration on fungal nomenclature. *IMA Fungus* **2**, 105–112 (2011).
5. Hibbett, D. S. *et al.* Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. *Fungal Biol. Rev.* **25**, 38–47 (2011).
6. Taylor, J. W. One fungus = one name: DNA and fungal nomenclature twenty years after PCR. *IMA Fungus* **2**, 113–120 (2011).
7. Houbraken, J., Frisvad, J. C. & Samson, R. A. Taxonomy of *Penicillium citrinum* and related species. *Fungal Divers.* **44**, 117–133 (2010).
8. Crous, P. W. *et al.* Phylogenetic lineages in the *Botryosphaeriaceae. Stud. Mycol.* **55**, 235–253 (2006).
9. Gräfenhan, T., Schroers, H.-J., Nirenberg, H. I. & Seifert, K. A. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora, Acremonium, Fusarium, Stilbella,* and *Volutella. Stud. Mycol.* **68**, 79–113 (2011).
10. Norvell, L. L. Fungal nomenclature. 1. Melbourne approves a new Code. *Mycotaxon* **116**, 481–490 (2011).
11. Hawksworth, D. A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. *IMA Fungus* **2**, 155–162 (2011).
12. Weresub, L. K. & Pirozynski, K. A. in *The Whole Fungus: The Sexual-Asexual Synthesis* (ed. Kendrick, B.) 17–30 (National Museum of Natural Sciences and National Museums of Canada, 1979).
13. Tulasne, L.-R. & Tulasne, C. *Selecta Fungorum Carpologia.* Vol. 1 (Imperial Typographer, 1861).
14. Kendrick, B. (ed.) *The Whole Fungus: The Sexual-Asexual Synthesis.* (National Museum of Natural Sciences and National Museums of Canada, 1979).
15. Houbraken, J. & Samson, R. A. Phylogeny of *Penicillium* and the segregation of Trichocomaceae into three families. *Stud. Mycol.* **70**, 1–51 (2011).
16. Hawksworth, D. L. & Taylor, J. W. One fungus = which name? *IMA Fungus* **3**, 10–14 (2012).
17. Rosling, A. *et al.* Archaeorhizomycetes: unearthing an ancient class of ubiquitous soil fungi. *Science* **333**, 876–879 (2011).
18. James, T. Y. & Berbee, M. L. No jacket required – new fungal lineage defies dress code: recently described zoosporic fungi lack a cell wall during trophic phase. *Bioessays* **34**, 94–102 (2012).
19. Jones, M. D. *et al.* Discovery of novel intermediate forms redefines the fungal tree of life. *Nature* **474**, 200–203 (2011).
20. Kirk, P. M. Index Fungorum no. 2. *Index Fungorum* [online] http://www.indexfungorum.org/Publications/Index%20Fungorum%20no.2.pdf (2012).
21. Bidartondo, M. I. Preserving accuracy in GenBank. *Science* **319**, 1616 (2008).
22. Schoch, C. L. *et al.* Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl Acad. Sci. USA* **109**, 6241–6246 (2012).
23. Abarenkov, K. *et al.* The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytol.* **186**, 281–285 (2010).
24. Tindall, B. J., Rossello-Mora, R., Busse, H. J., Ludwig, W. & Kampfer, P. Notes on the characterization of prokaryote strains for taxonomic purposes. *Int. J. Syst. Evol. Microbiol.* **60**, 249–266 (2010).
25. De Vos, P., Trüper, H. G. & Tindall, B. J. Judicial Commission of the International Committee on Systematics of Prokaryotes; Xth International (IUMS) Congress of Bacteriology and Applied Microbiology; Minutes of the meetings, 28, 29 and 31 July and 1 August 2002, Paris, France. *Int. J. Syst. Evol. Microbiol.* **55**, 525–532 (2005).
26. Lapage, S. *et al.* (eds) *International Code Of Nomenclature Of Bacteria.* (American Society for Microbiology, 1992).
27. Houbraken, J., Frisvad, J. C. & Samson, R. A. Fleming's penicillin producing strain is not *Penicillium chrysogenum* but *P. rubens. IMA Fungus* **2**, 87–95 (2011).
28. Henk, D. A. *et al.* Speciation despite globally overlapping distributions in *Penicillium chrysogenum*: the population genetics of Alexander Fleming's lucky fungus. *Mol. Ecol.* **20**, 4288–4301 (2011).
29. Whitaker, R. J., Grogan, D. W. & Taylor, J. W. Geographic barriers isolate endemic populations of hyperthermophilic archaea. *Science* **301**, 976–978 (2003).
30. Cadillo-Quiroz, H. *et al.* Patterns of gene flow define species of thermophilic Archaea. *PLoS Biol.* **10,** e1001265 (2012).
31. Gevers, D. *et al.* Re-evaluating prokaryotic species. *Nature Rev. Microbiol.* **3**, 733–739 (2005).
32. Rainey, F. A. & Oren, A. in *Methods in Microbiology.* Vol. 38 (eds Rainey, F. & Oren, A.) 1–5 (Academic, 2011).
33. Murray, R. G. E. & Stackebrandt, E. Taxonomic note: implementation of the provisional status *Candidatus* for incompletely described procaryotes. *Int. J. Syst. Bacteriol.* **45**, 186–187 (1995).
34. Murray, R. G. E. & Schleifer, K. H. Taxonomic notes: a proposal for recording the properties of putative taxa of procaryotes. *Int. J. Syst. Bacteriol.* **44**, 174–176 (1994).
35. Euzéby, J. P. Some names included in the category *Candidatus. List of Prokaryotic Names with Standing in Nomenclature* [online], http://www.bacterio.cict.fr/candidatus.html (2012).
36. Taylor, J. *et al.* Phylogenetic species recognition and species concepts in fungi. *Fungal Genet. Biol.* **31**, 21–32 (2000).

Acknowledgments
The authors thank their colleagues and members of their laboratories for discussions on the principles of fungal taxonomy, and the International Mycological Association and the Centraalbureau voor Schimmelcultures for hosting meetings addressing the revolution in fungal nomenclature. Research in the authors' laboratories is supported by the Open Tree of Life Project (http://opentreeoflife.org/), the US National Science Foundation (grant DEB-1208719 to D.S.H.), the US National Institutes of Health (grant U54-AI65359 to J.T.W.) and the Energy Biosciences Institute (grant EBI 001J09 to J.W.T.).

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
David S. Hibbett's homepage: http://www.clarku.edu/faculty/dhibbett/
John W. Taylor's homepage: http://taylorlab.berkeley.edu/
GenBank: http://www.ncbi.nlm.nih.gov/genbank/
IndexFungorum: http://www.indexfungorum.org/
MycoBank: http://www.mycobank.org/
RefSeq: http://www.ncbi.nlm.nih.gov/RefSeq/
UNITE: http://unite.ut.ee/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

ISSN (print) 0093-4666 ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/120.477

Volume 120, pp. 477–492 *April–June 2012*

Entomophthoromycota: a new phylum and reclassification for entomophthoroid fungi

Richard A. Humber

USDA-ARS Biological Integrated Pest Management Research, Robert W. Holley Center for Agriculture and Health, Tower Road, Ithaca, New York 14853-2901, U.S.A.

Correspondence to: *richard.humber@ars.usda.gov*

Abstract — One result of the recent phylogenetically based rejection of the phylum *Zygomycota* was the description of the subphylum *Entomophthoromycotina* (not assigned to any phylum) for fungi traditionally treated in the order *Entomophthorales*. More extensive gene-based analyses of these fungi suggest that they represent a monophyletic lineage distinct from all other fungi that deserves now to be recognized at the level of a new fungal phylum. These molecular data and further analyses of more traditional taxonomic criteria lead to this reclassification that still treats these fungi in six families but recognizes the new classes *Basidiobolomycetes*, *Neozygitomycetes*, and *Entomophthoromycetes* as well as the new order *Neozygitales*. *Ballocephala* and *Zygnemomyces* are excluded from *Entomophthorales* (*Meristacraceae*) and should be reclassified among the *Kickxellomycotina*.

Key words — *Zygomycetes*, sexuality, homothallism

Introduction

The reclassification of fungi by Hibbett et al. (2007) as the complement to a kaleidoscopic phylogenetic study all major fungal groups (James et al. 2006) validated the long-accepted sense that *Zygomycota* was polyphyletic, and recognized five taxa to replace this phylum: The phylum *Glomeromycota* accommodates arbuscular mycorrhizal fungi, and all other zygomycetes were distributed among subphyla *Entomophthoromycotina*, *Kickxellomycotina*, *Mucoromycotina*, and *Zoopagomycotina* without assignment to any phylum. It was assumed that subsequent research would determine whether any of these subphyla should be regrouped as part of an effort that would necessarily result in the recognition of one to four new phyla for these fungi.

The major characters traditionally used to classify the *Entomophthorales* have been thoroughly reviewed (summarized in Humber 1975, 1981, 1982,

1984) and were applied to six families in the last major reclassification of *Entomophthorales* (Humber 1989); this taxonomy is widely accepted despite a few minor differences in the treatments of some entomopathogenic genera (Bałazy 1993; Keller 1987, 1991, 1997; Keller & Petrini 2005). Until now, however, there have not been phylogenetic studies on a sufficiently broad range of their genes and taxa to propose a more contemporary revision.

The fungi in *Entomophthoromycotina* pose a few mycological puzzles (discussed below) for refining their current classification: Both the ultrastructure of the mitosis-associated organelle and early phylogenetic studies suggested that *Basidiobolus* has affinities with chytrid fungi and might better be excluded from *Entomophthorales.* Further, significant gaps in the gene-based understandings of entomophthoroid fungi exist because many taxa are very rarely collected and/or resist growth in vitro. Among these understudied taxa, *Neozygites* and related species represent the largest and most important 'black boxes' for which needed data remain unavailable.

The gene sequences now conceded to have taxonomic value for many fungi (nuclear rDNA genes, translation-elongation factor, β-tubulin, etc.) have been used in studies of a few entomophthoraleans in a more diverse set of fungi (Nagahama et al. 1995, Jensen et al. 2001, White et al. 2006) and for narrower studies of entomophthoralean species or species complexes (Jensen & Eilenberg 2001, Nielsen et al. 2001). A markedly different molecular approach comparing amino acid sequences for proteins (including some of the same proteins whose DNA sequences are widely used) has placed *Entomophthorales* outside of the true fungi (Einax & Voigt 2004, Liu & Voigt 2010, Voigt & Kirk 2011). While some skepticism about the meaning of such results based on amino acid sequences must be maintained, these findings do demonstrate the existence of distinct differences between all other groups of zygomycete fungi and *Entomophthoromycotina.* The amino-acid sequence-based 'exclusion' of *Entomophthorales* from the fungi echoes the hypotheses about placing *Basidiobolus* with watermolds but such 'anomalous' conclusions also suggest that molecular analyses based on highly limited inputs can yield results that have little sensibility from the broader perspective of the overall biology of the organisms analyzed.

A series of phylogenetic studies on entomophthoraleans (being prepared by A. Gryganskyi, R. Vilgalys, R. Humber, and other authors) incorporated more genes and a much broader range of entomophthoroid taxa than any earlier studies. These new analyses confirm the finding by James et al. (2006) that entomophthoroid fungi are a monophyletic group and that this group does include *Basidiobolus* and *Basidiobolaceae*. A reasonable integration of all results of traditional and phylogenetic analyses of entomophthoroid fungi suggests that they are distinct from all other fungi (including those in the other

unaffiliated zygomycete subphyla; Hibbett et al. 2007) and may occupy the most basal position among all non-flagellate fungi. The best-supported, most appropriate conclusion about the status of these fungi is, therefore, to recognize them as a new phylum in kingdom *Fungi*.

Materials & methods

Cultures and specimens used for the analyses discussed here are primarily from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF; http://www.ars.usda.gov/Main/docs.htm?docid=12125) and its associated herbarium. Unpublished molecular results of analyses of various genes are based on sequences of cultures obtained from ARSEF or isolated from nature and, in most cases, subsequently deposited in ARSEF. Other results involving reports on fungi and analyses using other isolates and specimens were completed at the Zoology Section of the Department of Agriculture and Ecology, University of Copenhagen, or at Agroscope FAL Reckenholz Eidgenössische Forschungsanstalt für Agrarökologie und Landbau (Zürich).

Major taxonomic issues affecting this reclassification

'Linkage' of *Basidiobolus* with flagellate fungi

The nuclei of entomophthoralean fungi and the details of their mitoses present a comparatively richer number and variety of characters than in most other fungal groups, and these nuclear characters are taxonomically informative, especially above the generic rank (Humber 1975, 1981, 1982, 1984, 1989). Mitosis by the huge nuclei of *Basidiobolus* species is sufficiently unusual to have been studied repeatedly (Olive 1907, Robinow 1963, Sun & Bowen 1972, Tanaka 1978). Among other surprises, the location of the mitosis-associated organelle in this genus is not fixed at the spindle poles and can even occur in the plane of the metaphase plate (Sun & Bowen 1972). The real controversies about this mitosis arose, however, when this organelle proved to be a short cylinder with a ring of 11–12 singlet microtubules (McKerracher & Heath 1985), and comparisons between this structure and centrioles were used to question the phylogenetic placement of this fungus; *Basidiobolus* remains the ONLY organism producing no flagellum in its life history for which microtubules are proven to be present in a mitosis-associated organelle. Nonetheless, any hypothesis that this organelle in *Basidiobolus* (but whose existence, location and ultrastructure remain unconfirmed from other fungi in its family) is linked with or derived from centrioles seems neither credible nor responsible: The 9×3 microtubular arrangement in centrioles and kinetosomes is invariant among ALL phyla of eukaryotes having flagella; conversely, no organelle with microtubules arranged like those in *Basidiobolus* is known from any other organism.

Later findings of gene sequence similarities between *Basidiobolus* and several chytrid and blastocladian watermolds (Nagahama et al. 1995, Jensen et al. 1998, Tanabe et al. 2000, White et al. 2006) have also been used to suggest

that *Basidiobolus* might not belong in *Entomophthorales*. However, other studies allied *Basidiobolus* with kickxelloid fungi from *Harpellales* (Keeling 2003, Tanabe et al. 2004) and one placed *Conidiobolus coronatus* (whose inclusion in *Entomophthorales* was never disputed) on a branch with the blastocladialean genus *Allomyces* (Tanabe et al 2005). These divergent findings underscore the peril of suggesting phylogenetic relationships among major fungal groups after comparing very limited sequence data and very sparse samplings of taxa within large and inherently diverse groups of fungi.

The recent survey of phylogenetic relationships within kingdom *Fungi* (James et al. 2006) supported the continued placement of *Basidiobolus* in *Entomophthorales*. Regardless of molecular or ultrastructural rationales to the contrary, hypotheses that *Basidiobolus* is not entomophthoroid are nonsensical if one considers the overall biology of these fungi. *Basidiobolus* and its relatives share many novel features in common with other entomophthoroid taxa despite the scant few pieces of data suggesting otherwise. It is necessary to bring a broader perspective to the uncertainties about *Basidiobolus*: Despite any and all evidence to the contrary, if this genus does not belong among the entomophthoroid fungi, then JUST WHERE AMONG FLAGELLATE (OR ANY OTHER) FUNGI SHOULD IT BE CLASSIFIED? The lack of any comparably well-supported answers for this question should quash any residual doubts about where *Basidiobolus* belongs.

Neozygitaceae: a special 'problem' in data gathering

The status of taxa in *Neozygitaceae* also presents a (temporary) problem for reclassifying the *Entomophthorales*. Very few cultures of *Neozygites* species are available in vitro, and, sadly, all current cultures of *Neozygites* are of mite pathogens with rough-walled, nearly globose zygospores. No gene-based data are available for *N. fresenii* [=*N. aphidis*, the type species] or other hemipteran pathogens that form ovoid, smooth-walled resting spores. A further taxonomic frustration is that these morphological and host differences suggest that *Neozygites* may eventually be split into two or more genera, but molecular data will be required to support such a decision. DNA-based evaluations of fungi from *Neozygitaceae* is encumbered by difficulties experienced in multiple laboratories to obtain clean DNA useful for amplifying and sequencing the genes needed to determine their phylogenetic relationships. While the recognition of *Neozygitomycetes* as a new class without supporting gene-based evidence may be dismissed by some as premature, such a treatment is the most reasonable for these fungi at this time, based on their known organismal biology (that integrates and represents a vastly larger proportion of the genome than those few genes now so widely treated as sufficient to complete high-level, phylogenetically sound reclassifications of virtually all other fungal groups).

Distinct differences between neozygitoid fungi and either basidioboloid or entomophthoroid taxa supports the description of three classes in this new phylum: As in basidioboloid fungi, neozygitoid fungi exert strong control in vegetative cells over nuclear number (usually four in *Neozygites*), have a central mitotic metaphase plate (Butt & Humber 1989), and, perhaps most significantly, a round of mitosis in gametangial cells precedes conjugation and zygosporogenesis while only one nucleus from each gametangium enters each zygospore (Keller 1997). As in entomophthoroid fungi, all neozygitoid taxa are obligate pathogens of insects or mites, and the nuclear membrane remains intact throughout mitosis. On the other hand, the chromosomes of neozygitoid fungi differ from basidioboloid and entomophthoroid fungi in being vermiform and of moderate size, condensing during mitosis but uncoiled (euchromatic) during interphase. *Neozygites* mitoses (Butt & Humber 1989) resemble those in animal or plant cells more closely than those in any other entomophthoroid fungi.

The presence of many novel characteristics shared among all of the fungi traditionally classified in order *Entomophthorales* underscores the need to keep these fungi together in a phylogenetically supported, coherent group and to pursue further studies to obtain more vital data about the genes, development, pathobiology, and other aspects for a better understanding of these fungi that can be very important naturally occurring biological control agents. Because these fungi occupy a very ancient position of the fungal tree of life it is also important to note that a better understanding should help to understand more about the enigmatic transition of fungi (as also occurred with plants and animals) from waterborne to terrestrial life forms.

Taxonomy

Entomophthoromycota Humber, **phyl. nov.** [Table 1]

MycoBank MB 564375

Vegetative growth as hyphae, hyphal bodies, or yeast-like; cells broad, walled or protoplastic. Conidiophores simple or digitate, each branch forming one conidiogenous cell and one conidium. Primary spores conidia, uni- to multinucleate, usually forcibly discharged; usually forming one or more types of secondary conidia. Resting spores homothallic zygospores or azygospores. Habit mostly as arthropod pathogens, but some saprobes or specialized phytopathogens.

Type genus: *Entomophthora* Fresen. 1856.

Conidiophores rise from mycelium or from body of host, usually with positive phototropic orientation, simple or apically (digitately) branched, with single conidiogenous cell on each branch giving rise to a single conidium or simple erect conidiophore becomes septate and each cell forms a single conidium. Primary conidia (not sporangia) with wall layers continuous with those on

Table 1. Proposed new classification for *Entomophthoromycota*.
New taxa described here are listed in ***boldface italics***.

Phylum *Entomophthoromycota* Humber, phyl. nov.
Class *Basidiobolomycetes* Humber, cl. nov.
Order *Basidiobolales* Caval.-Sm., Biol. Rev. 73: 246. 1998.
Family *Basidiobolaceae* Claussen, Syllab. Pflanzenfam., Edn 9 & 10: 45. 1924.
Basidiobolus Eidam, Beitr. Biol. Pflanz. 4: 194. 1886.
Other new, undescribed genera (R.A. Humber, B. Huang & K. Hodge, unpublished).

Class *Neozygitomycetes* Humber, cl. nov.
Order *Neozygitales* Humber, ord. nov.
Family *Neozygitaceae* Ben-Ze'ev, R.G. Kenneth & Uziel, Mycotaxon 28: 321. 1987.
Apterivorax S. Keller, Sydowia 57: 47. 2005.
Neozygites Witlaczil, Arch. Mikr. Anat. 24: 601. 1885.
Thaxterosporium Ben-Ze'ev & R.G. Kenneth, Mycotaxon 28: 323. 1987.

Class *Entomophthoromycetes* Humber, cl. nov.
Order *Entomophthorales* G. Winter, Rabenh. Krypt.-Fl., Edn 2, 1(1): 74. 1880.
Family *Ancylistaceae* J. Schröt., Nat. Pflanzenfam. 1(1): 92. 1893.
Ancylistes Pfitzer, Monatsb. Königl. Preuss. Akad. Wiss. Berlin: 396. 1872.
Conidiobolus Bref., Untersuch. Gesammtgeb. Mykol. 6: 37. 1884.
Macrobiotophthora Reukauf, Centrabl. Bakt., Abt 1, 63: 390. 1912.
Family *Completoriaceae* Humber, Mycotaxon 34: 453. 1989.
Completoria Lohde, Tagebl. Versamml. Deutsch. Naturf. Aertze 47: 206. 1874.
Family *Entomophthoraceae* Nowak., Bot. Ztg. 35: 35. 1877.
Subfamily *Entomophthoroideae* S. Keller, Sydowia 57: 28. 2005.
Batkoa Humber, Mycotaxon 34: 446. 1989.
Entomophaga A. Batko, Bull. Polon. Acad. Sci. Sér. Biol. Sci. 12: 325. 1964.
Entomophthora Fresen., Bot. Ztg. 14: 883. 1856.
Eryniopsis Humber, Mycotaxon 21: 258. 1984, pro parte.
Massospora Peck, Rep. New York State Mus. 31: 44. 1879.
Subfamily *Erynioideae* S. Keller, Sydowia 57: 33. 2005.
Erynia (Nowak. ex A. Batko) Remaud. & Hennebert, Mycotaxon 11: 333. 1980.
Eryniopsis Humber, Mycotaxon 21: 258. 1984, pro parte.
Furia (Batko) Humber, Mycotaxon 34: 450. 1989.
Orthomyces Steinkraus, Humber & J.B. Oliv., J. Invertebr. Pathol. 72: 5. 1998.
Pandora Humber, Mycotaxon 34: 451. 1989.
Strongwellsea A. Batko & Weiser, J. Invertebr. Pathol. 7: 463. 1965.
Zoophthora A. Batko, Bull. Polon. Acad. Sci. Sér. Biol. Sci. 12: 323. 1964.
Family *Meristacraceae* Humber, Mycotaxon 34: 456. 1989.
Meristacrum Drechsler, J. Wash. Acad. Sci. 30: 250. 1940.
Tabanomyces Couch, RV Andrejeva, Laird & Nolan, Proc. Natl. Acad. Sci. USA 76: 2302. 1979.

conidiogenous cells, inner wall layer invaginating to form two-layered septum between conidium and conidiogenous cell; almost always forcibly discharged (several possible mechanisms are known). Secondary conidia formed by most taxa: if forcibly discharged from short secondary conidiophore then usually similar in shape to primary conidium; if passively dispersed from long, thin (capillary) secondary conidiophore then usually distinctly differing in morphology from primary conidium. Resting spores (when mature) with thick, distinctly 2-layered walls, colored or hyaline, outer layer surface smooth or variously decorated; formed as zygospores (after gametangial conjugation) or azygospores (with no conjugation) either in the axis of the parental cells or budded off laterally; nuclear number in mature spores varies from 2 (from initiation or progressively reducing to 2) to multiple; germinating directly by forming germ conidiophore and germ conidium (usually resembling a secondary spore type) or indirectly by forming a small germ mycelium and then germ conidia (usually like primary conidia). Habits: saprobes in soil or litter, primary pathogens of arthropods (insect, mites, spiders) or other soil invertebrates (nematodes, tardigrades), or highly specific phytopathogens (e.g., of desmid algae or fern gametophytes). Arthropod pathogens may form specialized organs: rhizoids with or without differentiated holdfasts may anchor host to substrate, and cystidia may perforate host cuticle and facilitate emergence of conidiophores.

Primary and secondary conidia are the major spore forms in this phylum and constitute the primary basis for the taxonomy of these fungi. The resting spores are formed much less commonly than are conidia. The majority of species are pathogens of arthropods although pathogens of other soil invertebrates (nematodes and tardigrades) or of plants (desmid algae or fern gametophytes) are rare. The primary habit (especially in *Basidiobolaceae* and *Ancylistaceae*) may be in soil and plant detritus, but some species in these groups are best known as colonists of amphibian and reptile guts (*Basidiobolus*) or as facultative or obligate entomopathogens (*Conidiobolus*).

Any continued use of subphylum *Entomophthoromycotina* Humber (Hibbett et al. 2007: 517) is now superfluous until any future decision divides *Entomophthoromycota* into subphyla. This reclassification does not take up the phylum *Basidiobolomycota* Doweld (2001; LXXVII) because Doweld's name was proposed as part of a general reclassification of all fungi that does not agree with current understandings of fungal biology and relationships and, as circumscribed, *Basidiobolomycota* and the class *Basidiobolomycetes* Doweld (2001: LXXVII) used fragmentary knowledge of characters that may not apply to all taxa intended to be included while failing to account in any way for most taxa specifically included in this circumscription of phylum *Entomophthoromycota*; also see discussion below for class *Basidiobolomycetes*.

Basidiobolomycetes Humber, **cl. nov.**

MycoBank MB 564376

Differs from *Entomophthoromycetes* and *Neozygitomycetes* by unusually large nuclei (often ≥10 µm long) with a large central nucleolus that is the major feature of uninucleate cells. Mitoses involve barrel-shaped spindles, mitotic organelles incorporating microtubules (but not centrioles) but not always located at the spindle poles, and the nuclear content isolated from the cytoplasm by a layer of nuclear and cytoplasmic membrane fragments.

Type genus: *Basidiobolus* Eidam 1886.

Vegetative cells uninucleate, as regularly septate mycelium or yeast-like cells cleaved from contents of a parental cells (e.g., so-called 'Darmform' growth). Mitosis begins with fragmentation of nuclear membrane and aggregation of these and other membranes around a nuclear zone; chromosomes numerous, tiny, condensed and aligned on central metaphase plate (usually embedded inside the nucleolus) in association with a barrel-shaped spindle, chromosomes uncoil during interphase. Conidiogenous cell (conidiophore) simple but with bulbous apical swelling immediately below developing conidium. Conidia uninucleate, globose, with small conical basal papilla (projecting into spore body but everting during discharge), unitunicate (wall layers not separable). Conidia discharge forcibly by rocket-like ejection when central circumscissile weakness of the subconidial swelling ruptures; the upper portion of the swelling discharges together with conidium but may detach during flight. Secondary conidia (if formed) usually elongate, often curved, with or without an apical mucoid droplet, formed apically on an elongated capillary conidiophore, passively dispersed. Resting spores (RS) usually zygospores, formed homothallically in axis of parental cells; gametangial nuclei undergo mitosis before conjugation but only one nucleus from each cell enters the zygospore. Mature zygospores have thick, bi-layered walls; RS germinate by direct formation of germ conidium (usually a secondary conidial type: elongate, passively dispersed from a capillary conidiophore).

The foremost diagnostic character for basidioboloid fungi is their huge nucleus (often ≥10 µm in length) with a prominent central nucleolus that is the major feature of uninucleate cells (either as a broad, septate mycelium or cells cleaving internally in yeast-like growth mode). There is no staining of interphasic nuclei (nor, in any obvious manner, of mitotic chromosomes) in *Basidiobolomycetes* in aceto-orcein or other nuclear stains. The individual volumes of these nuclei may be many times greater than the entire cells of most ascomycete yeasts), and their mitoses are unusual for more than just the microtubular nucleus-associated organelle (McKerracher & Heath 1985): As mitosis begins, the nuclear envelope breaks down but the endoplasmic reticulum and other membranous cell components cluster around the nuclear zone so that the

spindle and chromosomes remain well isolated from the cytoplasm despite the fragmentation of the nuclear envelope; the corollary effect of this membrane organization is that mitotic nuclei 'disappear' when viewed with light microscopy (Robinow 1963).

Zygosporogenesis in *Basidiobolus* (Eidam 1886) is also very distinctive as short beak-like, lateral projections form at the septum between gametangial cells; gametangial nuclei move into the beaks, undergo mitosis, and the (uninucleate) beak cells are walled off before the septum dissolves and zygosporogenesis proceeds; remnants of these 'beaks' often remain visible on mature zygospores.

As noted in the discussion for the new phylum, two available names for this new class were not adopted: *Bolomycetes* Cav.-Smith (Cavalier-Smith 1998: 243) was based mainly on the microtubular mitotic organelle and 'beaked' zygospores in *Basidiobolus*. This mitotic organelle is not confirmed as present in all taxa in the *Basidiobolaceae* (including at least two still undescribed new genera), and the zygospores of some basidiobolaceous fungi are not 'beaked' as in *Basidiobolus*. *Basidiobolomycetes* Doweld (2001: LXXVII) was proposed as a nomen novum for *Bolomycetes* and cited Cavalier-Smith's description for this class; Doweld neither placed nor mentioned other entomophthoralean fungi in any rank in his general reclassification of fungi.

Neozygitomycetes Humber, **cl. nov.**

MycoBank MB 564377

Differs from *Basidiobolomycetes* and *Entomophthoromycetes* by vermiform, moderately sized chromosomes that condense during mitosis on a central metaphase plate but uncoil during interphase. Nuclear numbers in vegetative cells and conidia are low and apparently controlled at (3)-4-(5).

Type genus: *Neozygites* Witlaczil 1885.

Vegetative cells are rod-like hyphal bodies, walled or protoplastic, usually with 4 (3–5) nuclei, elongating until ± synchronous mitosis; daughter cells separate by splitting of septum. Nuclear number in all cell types strongly regulated; usually 4 (3–5) in vegetative cells and conidia, 2 in resting spores. Mitoses intranuclear, ± synchronous in any cell; nuclei fusoid at metaphase with central, fusoid spindle; no nucleus-associated mitotic organelle observed; chromosomes uncoil (euchromatic) during interphase. Conidiophores simple; forming apical conidiogenous cell and one conidium. Primary conidia subglobose to broadly ovoid, basal papilla short, comparatively flat; forcibly discharged to short distance by papillar eversion. Secondary conidia usually form quickly after primary conidial discharge, most commonly form as capilliconidia (that are the primary infective units). Resting spores bud from short conjugation bridge between rounded-up hyphal bodies (gametangia)

after preconjugational mitosis in contacting gametangia; zygospore receives one nucleus from each gametangium; only outer wall layer is melanized. Mature resting spores with two adjacent round fenestrae ('holes' through outer wall layer) and raised ridge of gametangial wall remnants between them.

Melanization of all spore types is a major feature of *Neozygitomycetes.* Primary and secondary conidia are pale, smoky gray; individual resting spores are much more strongly colored, and dark gray to black in mass.

Neozygitales Humber, **ord. nov.**

MycoBank MB

Order having all characteristics of class *Neozygitomycetes*.

Type genus: *Neozygites* Witlaczil 1885.

This order has all characters of class *Neozygitomycetes* (which includes only a single order and family).

Entomophthoromycetes Humber, **cl. nov.**

MycoBank MB 564381

Differs from *Basidiobolomycetes* by lack of uniformly uninucleate cells, nuclear morphology, details of mitoses, and modes of zygosporogenesis; and from *Neozygitomycetes* by cells not having uniformly small numbers of nuclei, details of mitoses, and lack of melanization of all spore types.

Type genus: *Entomophthora* Fresen. 1856.

Vegetative growth as coenocytic mycelium or rod-like to variably shaped hyphal bodies, walled or naturally protoplastic; if wall-less, rod-like to highly variable in shape and/amoeboid. Conidiophores simple or digitately branched, each branch with a single apical conidiogenous cell, or (in *Meristacraceae*) an unbranched erect, septate conidiophore forming one conidium per cell. Conidia unitunicate (wall layers not separating in liquid) or bitunicate (with separable outer wall layer); variously shaped, uni- to multinucleate, with basal papilla flat, conical or rounded; forcibly discharged by papillar eversion in most genera. Secondary conidia more or less similar in shape to primary conidia and forcibly discharged if formed on short secondary conidiophore, or elongate and passively dispersed if formed on elongated capillary secondary conidiophore. Nuclei (interphase) with small nucleolus, interphasic heterochromatin present in *Entomophthoraceae* but absent in all other families; mitoses intranuclear, with small lateral metaphase plate lateral; interphasic chromosomes are partly condensed (heterochromatic) and stain readily in *Entomophthoraceae* but euchromatic (uncoiled and nonstaining) in other families. Resting spores globose to subglobose, formed as zygospores or azygospores. Habit obligately pathogenic for invertebrates (*Entomophthoraceae*, *Meristacraceae*, some *Ancylistaceae*), saprobic (some *Ancylistaceae*), or phytopathogenic (*Completoria* [*Completoriaceae*] and *Ancylistes* [*Ancylistaceae*]).

This class includes all members of *Ancylistaceae*, *Entomophthoraceae*, *Completoriaceae*, and *Meristacraceae* but omits those entomophthoralean taxa reassigned here to *Basidiobolomycetes* or *Neozygitomycetes* (Table 1) or removed from *Entomophthoromycota* as noted below.

Genera incertae sedis:

Eryniopsis Humber, Mycotaxon 21: 258. 1984.

All species are in *Entomophthoraceae* but would appear to be a mix of taxa representing both subfamilies *Erynioideae* and *Entomophthoroideae*; the type species, *E. lampyridarum*, has morphological characters suggestive of both subfamilies and cannot be placed in either without molecular studies.

Tarichium Cohn, Beitr. Biol. Pflanzen 1: 69. 1870.

This form genus for species known only from resting spores apparently represents a mix of species attributable to *Neozygitaceae* (especially species pathogenic to mites) and *Entomophthoraceae*. No new species should be added to this genus; DNA-based studies and morphological re-evaluations should allow most species to be recognized as synonyms of other species or transferred to other genera in *Entomophthoraceae* and *Neozygitaceae*.

Taxa inadoptata vel excludenda:

Massosporoideae S. Keller, Sydowia 57: 44. 2005.

This subfamily (accommodating only the genus *Massospora*) seems not to be supported by phylogenetic evidence and is treated as a synonym of subfamily *Entomophthoroideae*.

Ballocephala Drechsler, Bull. Torrey Bot. Club 78: 199. 1951.

Zygnemomyces K. Miura, Rep. Tottori Mycol. Inst. 10: 520. 1973.

These two genera are excluded from *Meristacraceae* and reassigned to *Kickxellomycotina* Benny (Hibbett et al. 2007) based on the bifurcate septa with lenticular plugs in their vegetative hyphae (Saikawa 1989; Saikawa et al. 1997).

Discussion

The terms 'mitospore' and 'meiospore' are not used in characterizing taxa of *Entomophthoromycota*. They were originally adopted to describe ascomycete and basidiomycete spores, and are not applicable to entomophthoroid fungi because the reproductive products and life histories of entomophthoroid fungi are not strictly comparable with those of the *Dikarya*: The thin-walled primary conidia (the basis for entomophthoroid taxonomy) are produced by the vegetative cells of these fungi, usually forcibly discharged, and usually able to produce one or more subsequent forms of secondary conidium if conidia do not germinate by producing a germ tube. Entomophthoroid resting spores may be conventionally sexual in nature (zygospores in which it may be assumed, although not yet proven, that karyogamy and meiotic

divisions occur) in *Basidiobolomycetes* and *Neozygitomycetes*; for taxa in the *Entomophthoromycetes*, and especially those in *Entomophthoraceae*, it was noted that the MORPHOLOGICAL events of sexuality (the presence or absence of gametangial conjugations define zygo- and azygosporogenesis, respectively) and the GENETIC events of sexuality (karyogamy and meiosis, that presumably happen during resting spore germination) may be completely independent processes (Humber 1981, McCabe et al. 1984). Entomophthoroid fungi may be the only fungi in which the morphological and genetic definitions of sexuality (or their absences) are present in all possible permutations and without the routine linkage between the morphological and genetic events of sexuality that is taken for granted in virtually all other types of organisms.

No zygomycetous or flagellate fungi produce spores that can accurately be referred to as meiospores in the sense of basidiospores and ascospores. The proven or presumably 'sexual' spores of fungi below the subkingdom *Dikarya* are thick-walled, environmentally resistant spores (zygospores, azygospores, resistant sporangia, etc.) that go through a quiescent dormancy before germinating to undergo a type of sporulation that is neither functionally nor developmentally comparable to the basidiospores and ascospores that are the direct and obligatory developmental products of the cells in which karyogamy and meiosis occur.

To obtain clean DNA and good sequence data from entomophthoroid fungi may be more difficult than for many, much more extensively studied fungal groups. Part of this difficulty might involve the physical organization of the genome in these fungi that might lead to overlapping but divergent sequences for some 'needed' genes. Chromosomal counts for *Basidiobolus* (which was long treated as including only one species, *B. ranarum*) have ranged from about 60 (Olive 1907) to several hundred (Sun & Bowen 1972) based on kinetochore counts in serial sections for transmission electron microscopy. These high numbers suggest that polyploidization events may have occurred repeatedly in *Basidiobolus*. This possibility seems to be verified by genetic studies showing multiple, genetically distinct allelic forms in *B. ranarum* for elongation-translation factor genes that usually occur in single copies in the genome (Henk & Fisher 2012). The few chromosome counts for entomophthoraceous entomopathogens also suggest a tendency to towards polyploidy: While the nature of chromosomes and mitoses may not facilitate chromosomal counts in *Entomophthoraceae* (Olive 1906, Humber 1975), the few published numbers in various taxa are 8, 12 (or more), 16, and 32 (see Humber 1982). No genetic studies like those of Henk & Fisher (2012) are available for *Entomophthoraceae* but no genetic studies of this family with techniques ranging from allozyme polymorphisms (Hajek et al. 1990; B. May, personal communication) to the latest gene sequencing efforts suggest that these fungi simultaneously harbor

multiple allelic variants at single loci; there is no indication that vegetative nuclei of these fungi are either diploid or include heterologous sets of chromosomes.

The interpretation of such seemingly numerous chromosomes in some taxa in *Entomophthoromycota* becomes more problematic in the absence of evidence suggesting that any putatively sexual reproduction in this phylum is heterothallic rather than strictly homothallic. No data support the invocation of heterothallic sexuality (even if outbreeding events were extremely rare) to explain Henk & Fisher's (2012) conclusions about the *Basidiobolus* genome. Except for gametangial fusions during zygosporogenesis, no cellular fusions (even between the naturally protoplastic vegetative cells of some of the pathogenic taxa) are known from cultures or natural collections of any entomophthoroid fungus; such fastidious behavior by these fungi precludes consideration of heterokaryosis and parasexuality as a mechanism to increase or to sustain gene flow among taxa in *Entomophthoromycota*.

It is important to note again that the *Entomophthorales* as traditionally recognized (Humber 1989) is the same group reclassified here except for the removal of *Ballocephala* and *Zygnemomyces* to *Kickxellomycotina* based on the bifurcate, plugged septa in their vegetative hyphae (Saikawa 1989, Saikawa et al. 1997). Despite earlier doubts about retaining *Basidiobolus* in *Entomophthorales*, molecular studies of more genes and a broader spectrum of entomophthoraleans (A. Gryganskyi, R. Vilgalys, and R. Humber, unpublished) confirm that this order, as historically treated, is monophyletic. These fungi exemplify yet another major group for which the traditional, pre-molecular classification has been fundamentally confirmed (although amplified and adjusted) rather than overturned by phylogenetic analyses. Phylogenetic techniques must not be allowed to override or to supplant the existing knowledge about groups of organisms despite the vital inputs, seductively authoritative-looking dendrograms, and current pre-eminence among taxonomy methodologies. The best role for phylogenetic techniques should be as partners with the much broader (and usually older) perspectives gained by a thorough understanding of the overall biology as the means to determine the most sensible and best supported organismal classifications.

Acknowledgments

Special thanks are due to Andrii Gryganskyi, Annette Bruun Jensen, Kathie T. Hodge, and Bo Huang for the molecular results that underpin this new classification, to Walter Gams and Rytas Vilgalys for critical reviews, and to James L. Reveal for stimulating discussion of nomenclatural issues.

Literature cited

Bałazy S. 1993. *Entomophthorales*. Flora of Poland (Flora Polska), Fungi (Mycota) 24: 1–356. Polish Acad Sci, W Szafer Inst Botany, Kraków.

Butt TM, Humber RA. 1989. An immunofluorescence study of mitosis in a mite-pathogen, *Neozygites* sp. *(Zygomycetes: Entomophthorales)*. Protoplasma 151: 115–123. http://dx.doi.org/10.1007/BF01403448

Cavalier-Smith T. 1998. A revised six-kingdom system of Life. Biol. Rev. 73: 203–266. http://dx.doi.org/10.1017/S0006323198005167

Doweld AB. 2001. Prosyllabus Tracheophytorum, Tentamen Systematis Plantarum Vascularium (Tracheophyta). Geos, Moscow.

Eidam E. 1886. *Basidiobolus*, eine neue Gattung der *Entomophthoraceen*. Beitr. Biol. Pflanzen 4: 181–241. http://dx.doi.org/10.1016/0022-2011(86)90060-1

Einax E, Voigt K. 2004. Oligonucleotide primers for the universal amplification of β-tubulin genes facilitate phyogenetic analyses in the regnum *Fungi*. Org. Divers. Evol. 3: 185–194. http://dx.doi.org/10.1078/1439-6092-00069

Fresenius G. 1856. Notiz, Insecten-Pilze betreffrend. Bot. Zeitg. 14: 882.

Hajek AE, Humber RA, Elkinton JS, May B, Walsh SRA, Silver JC. 1990. Allozymes and restriction fragment length polymorphism analyses confirm *Entomophaga maimaiga* responsible for 1989 epizootics in North American gypsy moth populations. Proc. Natl. Acad. Sci. USA 87: 6979–6982. http://dx.doi.org/10.1073/pnas.87.18.6979

Henk DA, Fisher MC. 2012. The gut fungus *Basidiobolus ranarum* has a large genome and different copy numbers of putatively functionally redundant elongation factor genes. PLoS ONE 7: e31268. http://dx.plos.org/10.1371/journal.pone.0031268

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Lumbsch HT, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Kõljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schüßler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N. 2007. A higher-level phylogenetic classification of the *Fungi*. Mycol. Res. 111: 509–547. http://dx.doi.org/10.1016/ j.mycres.2007.03.004

Humber RA. 1975. Aspects of the biology of an insect-parasitic fungus, *Strongwellsea magna* (*Zygomycetes*: *Entomophthorales*). PhD dissertation, University of Washington, Seattle.

Humber RA. 1981. An alternative view of certain taxonomic criteria used in the *Entomophthorales* (*Zygomycetes*). Mycotaxon 13: 191–240.

Humber RA. 1982. *Strongwellsea* vs. *Erynia*: the case for a phylogenetic classification of the *Entomophthorales* (*Zygomycetes*). Mycotaxon 15: 167–184.

Humber RA. 1984. Foundations for an evolutionary classification of the *Entomophthorales* (*Zygomycetes*). 166–183, in: Q Wheeler, M Blackwell (eds). Fungus/insect relationships: perspectives in ecology and evolution. Columbia University Press, New York.

Humber RA. 1989. Synopsis of a revised classification for the *Entomophthorales* (*Zygomycotina*). Mycotaxon 34: 441–460.

James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, Cox CJ, Celio G, Geuidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüßler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MW, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman A, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker

RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R. 2006. Reconstructing the early evolution of *Fungi* using a six-gene phylogeny. Nature 443: 818–822. http://dx.doi.org/10.1038/nature05110

Jensen AB, Eilenberg J. 2001. Genetic variation with the insect-pathogenic genus *Entomophthora*, focusing on *the E. muscae* complex, using PCR-RFLP of the ITS II and the LSU rDNA. Mycol. Res. 105: 307–312. http://dx.doi.org/10.1017/S0953756201003434

Jensen AB, Gargas A, Eilenberg J, Rosendahl S. 1998. Relationships of the insect-pathogenic order *Entomophthorales* (*Zygomycota*, *Fungi*) based on phylogenetic analyses of nuclear small subunit ribosomal DNA sequences (SSU rDNA). Fung. Genet. Biol. 24: 325–334. http://dx.doi.org/10.1006/fgbi.1998.1063

Keeling PJ. 2003. Congruent evidence from α-tubulin and β-tubulin gene phylogenies for a zygomycete origin of microsporidia. Fung. Genet. Biol. 38: 298–309. http://dx.doi.org/10.1016/S1087-1845(02)00537-6

Keller S. 1987. Arthropod-pathogenic *Entomophthorales* of Switzerland. I. *Conidiobolus*, *Entomophaga*, and *Entomophthora*. Sydowia 40: 122–167.

Keller S. 1991. Arthropod-pathogenic *Entomophthorales* of Switzerland. II. *Erynia*, *Eryniopsis*, *Neozygites*, *Zoophthora*, and *Tarichium*. Sydowia 43: 39–122.

Keller S. 1997. The genus *Neozygites* (*Zygomycetes*, *Entomophthorales*) with special reference to species found in tropical regions. Sydowia 49: 118–146.

Keller S, Petrini O. 2005. Keys to the identification of the arthopod pathogenic genera of the families *Entomophthoraceae* and *Neozygitaceae* (*Zygomycetes*), with descriptions of three new subfamilies and a new genus. Sydowia 57: 23–53.

Liu X-Y, Voigt K. 2010. Molecular characters of zygomycetous fungi. In: Molecular identification of Fungi. Y Gherbawy, K. Voigt (eds). Springer-Verlag, Berlin. http://dx.doi.org/10.1007/978-3-642-05042-8_20

McCabe DE, Humber RA, Soper RS. 1984. Observation and interpretation of nuclear reductions during maturation and germination of entomophthoralean resting spores. Mycologia 76: 1104–1107. http://dx.doi.org/10.2307/3793025

McKerracher LJ, Heath IB. 1985. The structure and cycle of the nucleus-associated organelle in two species of *Basidiobolus*. Mycologia 77: 412–417. http://dx.doi.org/10.2307/3793197

Nahaghama T, Sato H, Shimazu M, Sugiyama J. 1995. Phylogenetic divergence of the entomophthoralean fungi: Evidence from nuclear 18S ribosomal RNA gene sequences. Mycologia 87: 203–209. http://dx.doi.org/10.2307/3760906

Nielsen C, Sommer C, Eilenberg J, Hansen KS, Humber RA. 2001. Characterization of aphid pathogenic species in the genus *Pandora* by PCR techniques and digital image analysis. Mycologia 93: 864–874. http://dx.doi.org/10.2307/3761752

Olive EW. 1906. Cytological sudies on the *Entomphthoreae*. II. Nuclear and cell division of *Empusa*. Bot. Gaz. 41: 229–261. http://dx.doi.org/10.1086/328797

Olive EW. 1907. Cell and nuclear division in *Basidiobolus* Ann. Mycol. 5: 404–418.

Robinow CF. 1963. Observations on cell growth, mitosis, and division in the fungus *Basidiobolus ranarum*. J. Cell Biol. 17: 123–152. http://dx.doi.org/10.1083/jcb.17.1.123

Saikawa M. 1989. Ultrastructure of the septum in *Ballocephala verrucospora* (*Entomophthorales*, *Zygomycetes*). Can. J. Bot. 67: 2484–2488. http://dx.doi.org/10.1139/b89-318

Saikawa M, Oguchi M, Castañeda Ruiz RF. 1997. Electron microscopy of two nematode-destroying fungi, *Meristacrum asterospermum* and *Zygnemomyces echinulatus* (*Meristacraceae*, *Entomophthorales*). Can. J. Bot. 75: 762–768. http://dx.doi.org/10.1139/b97-086

Sun NC, Bowen CC. 1972. Ultrastructural studies of nuclear division in *Basidiobolus ranarum* Eidam. Caryologia 25: 471–494.

Tanabe Y, O'Donnell K, Saikawa M, Sugiyama J. 2000. Molecular phyogeny of parasitic *Zygomycota* (*Dimargaritales*, *Zoopagales*) based on nuclear small subunit ribosomal DNA sequences. Mol. Phylogenet. Evol. 16: 253–262. http://dx.doi.org/10.1006/mpev.2000.0775

Tanabe Y, Saikawa M, Watanabe MM, Sugiyama J. 2004. Molecular phylogeny of *Zygomycota* based on EF-1α and RPB1 sequences: limitations and utility of alternative markers to rDNA. Mol. Phylogenet. Evol. 30: 438–449. http://dx.doi.org/10.1016/S1055-7903(03)00185-4

Tanabe Y, Watanabe MM, Sugiyama J. 2005. Evolutionary relationships among basal fungi (*Chytridiomycota* and *Zygomycota*): Insights from molecular phylogenetics. J. Gen. Appl. Microbiol. 51: 267–276. http://dx.doi.org/10.2323/jgam.51.267

Tanaka K. 1978. Mitosis in the fungus *Basidiobolus ranarum* revealed by electron microscopy. Protoplasma 70: 423–440. http://dx.doi.org/10.1007/BF01275768

Voigt K, Kirk PM. 2011. Recent developments in the taxonomic affiliation and phylogenetic positioning of fungi: impact in applied microbiology and environmental biotechnology. Appl. Microbiol. Biotechnol. 90: 41–57. http://dx.doi.org/10.1007/s00253-011-3143-4

White MM, James TY, O'Donnell K, Cafaro MJ, Tanabe Y, Sugiyama J. 2006. Phylogeny of the *Zygomycota* based on nuclear ribosomal sequence data. Mycologia 98: 885–895. http://dx.doi.org/10.3852/mycologia.98.6.872

NOTE ADDED IN PROOF: Since the acceptance of this article, the bibliographic citations for the two molecularly based papers that underpin and justify this new classification of entomophthoroid fungi have become available:

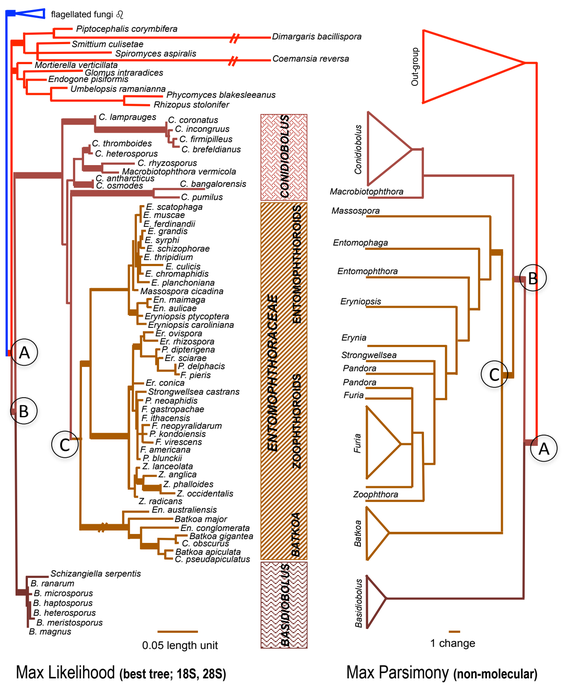
Gryganskyi AP, Humber RA, Smith ME, Miadlikovska J., Wu S, Voigt K, Walther G, Anishchenko IM, Vilgalys R. 2012. Molecular phylogeny of the Entomophthoromycota. Mol. Phylog. Evol. 65: 682-694. http://dx.doi.org/10.1016/j.ympev.2012.07.026

Gryganskyi AP, Humber RA, Smith ME, Hodge K, Huang B, Voigt K, Vilgalys R. 2012. Phylogenetic lineages in Entomophthoromycota. Persoonia: in press.

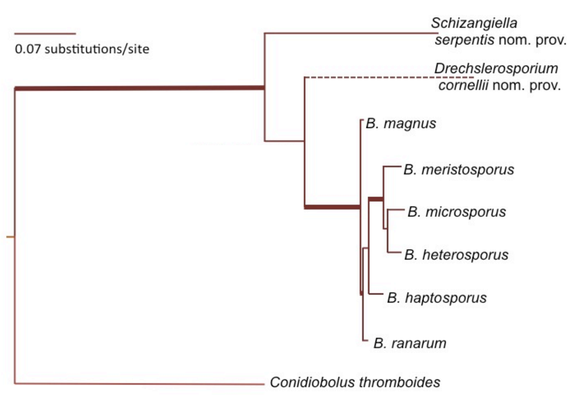
Phylogenetic reclassification raises new respect–and a new phylum!– for *Entomophthorales*

Richard A. Humber richard.humber@ars.usda.gov
USDA-ARS Biological Integrated Pest Management
RW Holley Center for Agriculture & Health
538 Tower Road, Ithaca, NY 14853, USA

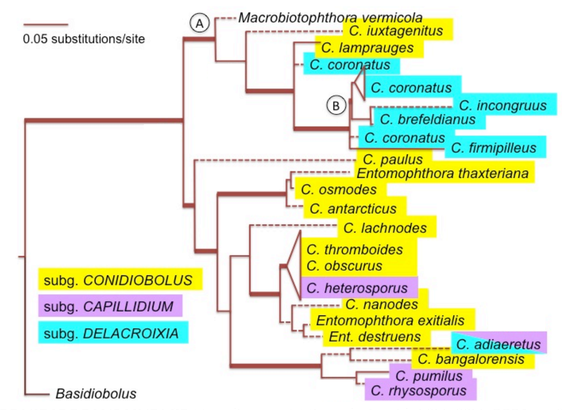
Andrii P. Gryganskyi apg10@duke.edu
Rytas Vilgalys fungi@duke.edu
Dept. of Biology, Duke University
Durham, NC 27708, USA



THE MONOPHYLETIC PHYLUM ENTOMOPHTHOROMYCOTA. Comparison of trees from molecular (L) and morphological (R) character data. Thick branches are statistically supported. Major nodes show separation of entomophthoroid fungi (A) from all other fungi; *Basidiobolomycetes* (B) are basal to all other fungi in the phylum, and separation of the exclusively entomopathogenic *Entomophthoraceae* (C) from mostly saprobic taxa in *Ancylistaceae*. *B=Basidiobolus*, *C=Conidiobolus*, *E=Entomophthora*, *En=Entomophaga*, *Er=Erynia*, *F=Furia*, *P=Pandora*, *Z=Zoophthora*. (Gryganskyi et al. 2012a)



***BASIDIOBOLUS* LINEAGE** (*Basidiobolomycetes*) ML analysis with LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. All taxa are united by cells with one large nucleus with a prominent central nucleolus and distinctive mitotic mechanism. (Gryganskyi et al. 2012b)



***CONIDIOBOLUS* LINEAGE** (*Ancylistaceae*) ML analysis with LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. *Conidiobolus* is paraphyletic, and its current subgeneric taxonomy is not supported. *Macrobiotophthora* (A) is basal to *Conidiobolus*; *C. adiaeretus* produces all three types of secondary conidia and indicates microconidiogenesis may be a paraphyletic character. (Gryganskyi et al. 2012b)

ABSTRACT: The recent phylogenetic studies and reclassifications produced by the global All-Fungal Tree of Life study recognized the *Entomophthorales* (as historically treated, with *Basidiobolus* remaining in this order) as a new subphylum, *Entomophthoromycotina*, that was not placed in any phylum. Subsequent phylogenetic analyses of the broadest range of entomophthoroid taxa and more genes than in any previous studies confirmed the monophyletic nature of these fungi and their distinctness from all other groups formerly classified in the phylum *Zygomycota*. As a lead-in to the publications of these molecular and traditional taxonomic analyses, the subphylum is now formally raised to phylum level as the *Entomophthoromycota*, and its included fungi are distributed among the classes *Basidiobolomycetes*, *Neozygitomycetes*, and *Entomophthoromycetes*; the genera *Ballocephala* and *Zygnemomyces* were removed from the family *Meristacraceae* (*Entomophthorales*) and reassigned to the subphylum *Kickxellomycotina*.

The systematics of entomophthoralean fungi has changed dramatically since the time when nearly all entomopathogens in this group (except, notably, for the cicada-pathogenic species of *Massospora*—were treated as *Entomophthora* species. The pace of taxonomic improvements heated up dramatically in the mid-1960's when the Batkoan classification offered so many radical changes from the existing taxonomy of these fungi that his scheme was effectively ignored in print by all other students of these fungi, and barely even mentioned in conversations despite the recognition that the Batkoan classification included many good decisions as well as some that were clearly wrong. Batko's classification was ignored—neither praised, condemned, nor corrected—until entomophthoralean taxonomy boiled over in the 1980's with the publication of several competing large-scale attempts to reclassify these fungi (by Remaudière and colleagues in Europe, by Humber and, separately, Tucker in the US, and by Ben-Ze'ev and Kenneth in Israel) that spawned serious disagreements in print, and also some loud ones in person. After the dust settled and tempers cooled, a six-family classification with a number of new and modified genera (Humber 1989) gained nearly universal acceptance.

The Entomophthorales entered the era of phylogenetic systematics with an immediate challenge to the integrity of the order thanks to single-gene analyses that suggested the *Basidiobolus* was more closely related to chytrid fungi than to the *Entomophthorales* (Nagahama et al 1995). The addition of more genes to the analyses, particularly under the global All-Fungal Tree of Life project, led James et al. (2006) to suggest that *Basidiobolus* was, indeed, a member of *Entomophthorales*. Molecular studies on these fungi were uniformly based until this year on limited numbers of genes and on very limited samplings of taxa from among entomophthoroid fungi. New analyses (Gryganskyi et al. 2012a,b) using multiple genes and an unprecedented number of entomophthoralean taxa unambiguously confirm several key points about the systematics of these fungi:

- The Entomophthorales as traditionally classified and as recognized by Hibbett et al. (2007) as the subphylum *Entomophthoromycotina* is a monophyletic group distinct from all other fungi.
- This confirmation justifies their treatment as a new phylum and the newly adjusted classification (Humber 2012) at the right.
- No cultures of other material was available for *Completoriaceae* or *Meristacraceae*. Few data were available for *Neozygitaceae* but their pertinent sequences, while difficult to obtain from the few cultured taxa, clearly exclude these fungi from the three well studied families.
- *Basidiobolus* (and all *Basidiobolaceae*) occupy a basal position in the phylum. *Conidiobolus* (the major genus of *Ancylistaceae*) proved to be paraphyletic and needs a new gene-based classification to replace its current, unsupported subgenera; *Ancylistaceae* is basal to the large, complex, and wholly entomopathogenic family *Entomophthoraceae*.
- Within the *Entomophthoraceae*, a separate subfamily for *Massospora* subfamily (Keller & Petrini 2005) is not supported. *Batkoa* may (or may not) represent a new subfamiliy. The currently recognized generic limits among zoophthoroid genera (subfamily *Erynioideae*) are proven to need more intensive study and, except for *Zoophthora*, are unsupported as currently recognized.

REFERENCES

Gryganskyi AP, Humber RA, Smith ME, Miadlikovska J, Wu S, Voigt K, Walter G, Anischenko IM, Vilgalys R. 2012. Molecular phylogeny of *Entomophthoromycota*. *Mol. Phylog. Evol.*: in press.

Gryganskyi AP, Humber RA, Smith ME, Hodge K, Huang B, Voigt K, Vilgalys R. 2012. Phylogenetic lineages in *Entomophthoromycota*. *Persoonia*: in press.

Hibbett DS et al. (65 co-authors) 2007. A high-level phylogenetic classification of the *Fungi*. *Mycol. Res.* 111: 509-547.

Humber RA. 1989. Synopsis of a revised classification for the *Entomophthorales* (*Zygomycotina*). *Mycotaxon* 34: 441-460.

Humber RA. 2012. *Entomophthoromycota*: a new phylum and reclassification for entomophthoroid fungi. *Mycotaxon 120*: in press.

James et al. (69 co-authors) 2006. Reconstructing the early evolution of the fungi using a six gene phylogeny. *Nature* 4443: 818-822.

Keller S,Petrini O. 2005. Keys to the identification of the arthropod pathogenic genera of the families *Entomophthoraceae* and *Neozygitaceae* (*Zygomycetes*), with descriptions of three new subfmilies and a new genus. *Sydowia* 57: 23-53.

Saikawa M. 1989. Ultrastructure of the septum in *Ballocephala verrucospora* (*Entomophthorales*, *Zygomycetes*). *Can. J. Bot.* 67: 2484–2488.

Saikawa M, Oguchi M, Castañeda Ruiz RF. 1997. Electron microscopy of two nematode-destroying fungi, *Meristacrum asterospermum* and *Zygnemomyces echinulatus* (*Meristacraceae*, *Entomophthorales*). *Can. J. Bot.* 75: 762–768.

Nagahama T, Sato H, Shimazu M, Sugiyama J, 1995. Phylogenetic divergence of the entomophthoralean fungi: evidence from nuclear 18S ribosomal RNA gene sequences. *Mycologia* 87: 203-209.

PHYLUM *Entomophthoromycota* Humber, phyl. nov.

CLASS *Basidiobolomycetes* Humber, cl. nov.

ORDER *Basidiobolales* Cavalier-Smith

FAMILY *Basidiobolaceae* Claussen

Basidiobolus Eidam
Schizangiella Humber, B. Huang & Hodge (unpubl. new genus)
Drechslerosporium B. Huang, Humber & Hodge (unpubl. new genus)

CLASS *Neozygitomycetes* Humber, cl. nov.

ORDER *Neozygitales* Humber, ord. nov.

FAMILY *Neozygitaceae* Ben-Ze'ev, R.G. Kenneth & Uziel

Apterivorax S. Keller
Neozygites Witlaczil
Thaxterosporium Ben-Ze'ev & R.G. Kenneth

CLASS *Entomophthoromycetes* Humber, cl. nov.

ORDER *Entomophthorales* G. Winter

FAMILY *Ancylistaceae* J. Schröter

Ancylistes Pfitzer
Conidiobolus Brefeld
Macrobiotophthora Reukauf

FAMILY *Completoriaceae* Humber

Completoria Lohde

FAMILY *Entomophthoraceae* Nowakowski

SUBFAMILY *Entomophthoroideae* S. Keller

Batkoa Humber
Entomophaga A. Batko
Entomophthora Fresenius
Eryniopsis Humber (in part; see subfam. *Erynioideae*)
Massospora Peck

SUBFAMILY *Erynioideae* S. Keller

Erynia (Nowakowski ex A. Batko) Remaudière & Hennebert
Eryniopsis Humber (in part; see subfam. *Entomophthoroideae*)
Furia (Batko) Humber
Orthomyces Steinkraus, Humber & J.B. Oliver
Pandora Humber
Strongwellsea A. Batko & Weiser
Zoophthora A. Batko

FAMILY *Meristacraceae* Humber

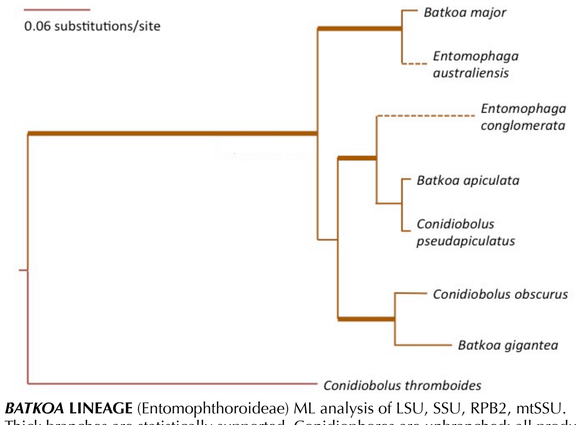
Meristacrum Drechsler
Tabanomyces Couch, RV Andrejeva, Laird & Nolan

Taxa with uncertain status, not accepted, or excluded from *Entomophthoromycota*:

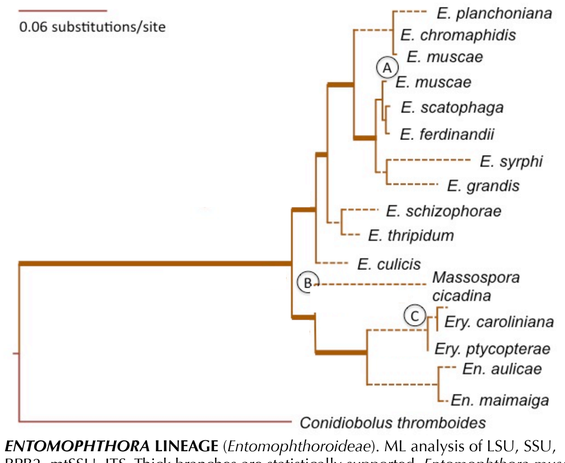
Subfamily *Massosporoideae* Keller – Not accepted; without molecular support
Tarichium Cohn – A form-genus; mixture of species of *Entomophthoraceae* and *Neozygitaceae*
Eryniopsis Humber – Heterogeneous; species probably belong in separate subfamilies
Ballocephala Drechsler } { Moved from *Meristacraceae* to subphylum *Kickxellomycotina*
Zygnemomyces Miura } { due to septal ultrastructure (Saikawa 1989; Saikawa et al. 1997)

The earliest phylogenetic studies including entomophthoroid taxa suggested that they were not homogeneous. The much more extensive sampling and use of more genes here than in previous analyses confirm that the classically defined Entomophthorales is both monophyletic and distinct from all other zygomycetes. This justified raising the subphylum to phylum rank. There is an acute need for a kaleidoscopic survey and phylogenetic review of *Conidiobolus*. The existing subgeneric scheme for this genus is not supported, and we dare not guess about the number or circumscriptions of genera that will result from its reclassification.

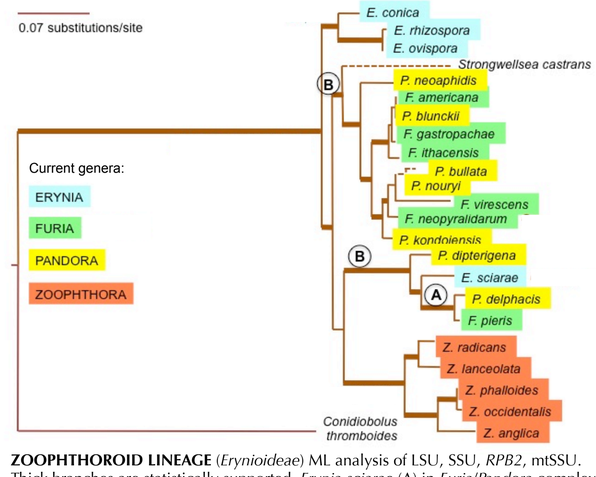
The *Entomophthoraceae* is the largest and most taxonomically complex family of this phylum. More data (and better underlying identifications of a number of strains) will be needed to determine the extent to which the *Batkoa* lineage may be separate from the rest of the subfamily *Entomophthoroideae*. More species and isolates of several genera must be studied (especially specis of *Eryniopsis*). The genera *Erynioideae* represent the most difficult problem using the current molecular data. Zoophthora is clearly supported as a separate genus, it is possible that Erynia might be supported, and Pandora and Furia may need to be combined; only the use of more collections and more genes will be able to resolve these questions.



***BATKOA* LINEAGE** (Entomophthoroideae) ML analysis of LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. Conidiophores are unbranched; all produce secondary conidia; no cystidia are formed. Mixed identifications of taxa indicate strains identified before description of *Batkoa*. (Gryganskyi et al. 2012b)



***ENTOMOPHTHORA* LINEAGE** (*Entomophthoroideae*). ML analysis of LSU, SSU, RPB2, mtSSU, ITS. Thick branches are statistically supported. *Entomophthora muscae* complex (A) is paraphyletic; *Massospora* (B) belongs in *Entomophthoroideae*; status of *Eryniopsis* (C) needs re-examination. (Gryganskyi et al. 2012b)

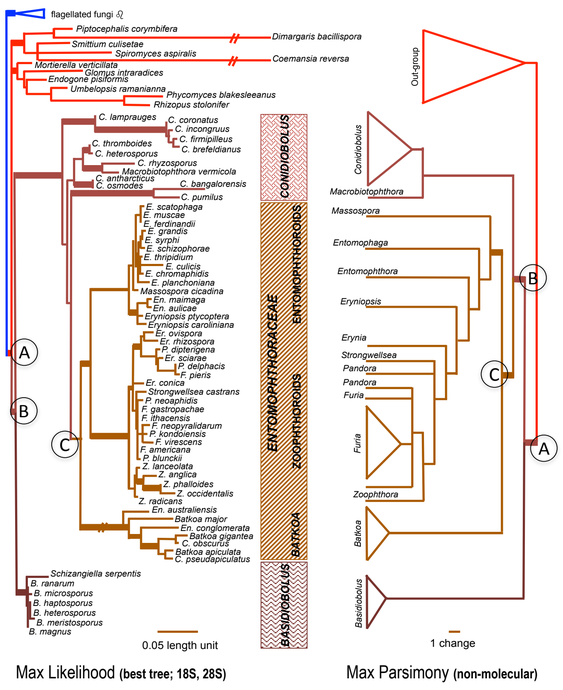


ZOOPHTHOROID LINEAGE (*Erynioideae*) ML analysis of LSU, SSU, *RPB2*, mtSSU. Thick branches are statistically supported. *Erynia sciarae* (A) in *Furia/Pandora* complex may reflect misidentification; *Furia* and *Pandora* (B) do not have molecular support; *Zoophthora* does seem to have good molecular support . (Gryganskyi et al. 2012b)

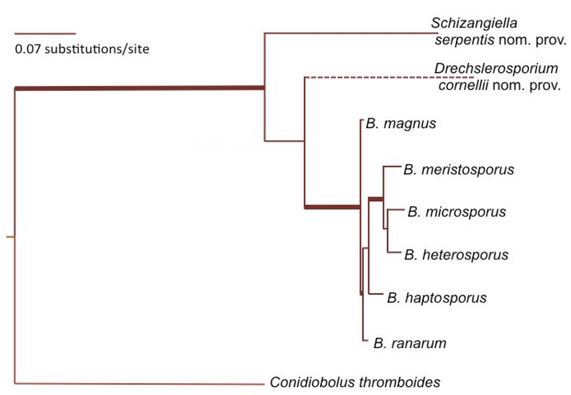
Phylogenetic reclassification raises new respect–and a new phylum!– for *Entomophthorales*

Richard A. Humber richard.humber@ars.usda.gov
USDA-ARS Biological Integrated Pest Management
RW Holley Center for Agriculture & Health
538 Tower Road, Ithaca, NY 14853, USA

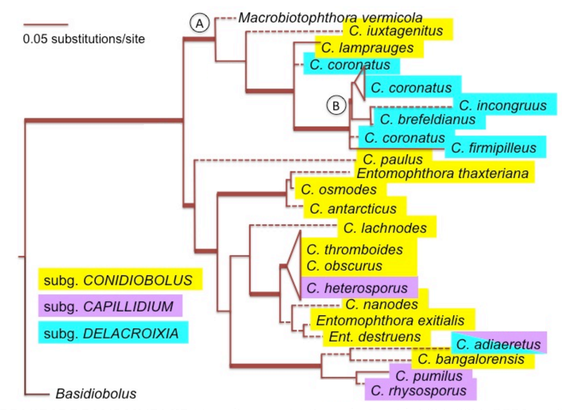
Andrii P. Gryganskyi apg10@duke.edu
Rytas Vilgalys fungi@duke.edu
Dept. of Biology, Duke University
Durham, NC 27708, USA



THE MONOPHYLETIC PHYLUM ENTOMOPHTHOROMYCOTA. Comparison of trees from molecular (L) and morphological (R) character data. Thick branches are statistically supported. Major nodes show separation of entomophthoroid fungi (A) from all other fungi; *Basidiobolomycetes* (B) are basal to all other fungi in the phylum, and separation of the exclusively entomopathogenic *Entomophthoraceae* (C) from mostly saprobic taxa in *Ancylistaceae*. *B=Basidiobolus*, *C=Conidiobolus*, *E=Entomophthora*, *En=Entomophaga*, *Er=Erynia*, *F=Furia*, *P=Pandora*, *Z=Zoophthora*. (Gryganskyi et al. 2012a)



***BASIDIOBOLUS* LINEAGE** (*Basidiobolomycetes*) ML analysis with LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. All taxa are united by cells with one large nucleus with a prominent central nucleolus and distinctive mitotic mechanism. (Gryganskyi et al. 2012b)



***CONIDIOBOLUS* LINEAGE** (*Ancylistaceae*) ML analysis with LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. *Conidiobolus* is paraphyletic, and its current subgeneric taxonomy is not supported. *Macrobiotophthora* (A) is basal to *Conidiobolus*; *C. adiaeretus* produces all three types of secondary conidia and indicates microconidiogenesis may be a paraphyletic character. (Gryganskyi et al. 2012b)

ABSTRACT: The recent phylogenetic studies and reclassifications produced by the global All-Fungal Tree of Life study recognized the *Entomophthorales* (as historically treated, with *Basidiobolus* remaining in this order) as a new subphylum, *Entomophthoromycotina*, that was not placed in any phylum. Subsequent phylogenetic analyses of the broadest range of entomophthoroid taxa and more genes than in any previous studies confirmed the monophyletic nature of these fungi and their distinctness from all other groups formerly classified in the phylum *Zygomycota*. As a lead-in to the publications of these molecular and traditional taxonomic analyses, the subphylum is now formally raised to phylum level as the *Entomophthoromycota*, and its included fungi are distributed among the classes *Basidiobolomycetes*, *Neozygitomycetes*, and *Entomophthoromycetes*; the genera *Ballocephala* and *Zygnemomyces* were removed from the family *Meristacraceae* (*Entomophthorales*) and reassigned to the subphylum *Kickxellomycotina*.

The systematics of entomophthoralean fungi has changed dramatically since the time when nearly all entomopathogens in this group (except, notably, for the cicada-pathogenic species of *Massospora*—were treated as *Entomophthora* species. The pace of taxonomic improvements heated up dramatically in the mid-1960's when the Batkoan classification offered so many radical changes from the existing taxonomy of these fungi that his scheme was effectively ignored in print by all other students of these fungi, and barely even mentioned in conversations despite the recognition that the Batkoan classification included many good decisions as well as some that were clearly wrong. Batko's classification was ignored—neither praised, condemned, nor corrected—until entomophthoralean taxonomy boiled over in the 1980's with the publication of several competing large-scale attempts to reclassify these fungi (by Remaudière and colleagues in Europe, by Humber and, separately, Tucker in the US, and by Ben-Ze'ev and Kenneth in Israel) that spawned serious disagreements in print, and also some loud ones in person. After the dust settled and tempers cooled, a six-family classification with a number of new and modified genera (Humber 1989) gained nearly universal acceptance.

The Entomophthorales entered the era of phylogenetic systematics with an immediate challenge to the integrity of the order thanks to single-gene analyses that suggested the *Basidiobolus* was more closely related to chytrid fungi than to the *Entomophthorales* (Nagahama et al 1995). The addition of more genes to the analyses, particularly under the global All-Fungal Tree of Life project, led James et al. (2006) to suggest that *Basidiobolus* was, indeed, a member of *Entomophthorales*. Molecular studies on these fungi were uniformly based until this year on limited numbers of genes and on very limited samplings of taxa from among entomophthoroid fungi. New analyses (Gryganskyi et al. 2012a,b) using multiple genes and an unprecedented number of entomophthoralean taxa unambiguously confirm several key points about the systematics of these fungi:

- The Entomophthorales as traditionally classified and as recognized by Hibbett et al. (2007) as the subphylum *Entomophthoromycotina* is a monophyletic group distinct from all other fungi.
- This confirmation justifies their treatment as a new phylum and the newly adjusted classification (Humber 2012) at the right.
- No cultures of other material was available for *Completoriaceae* or *Meristacraceae*. Few data were available for *Neozygitaceae* but their pertinent sequences, while difficult to obtain from the few cultured taxa, clearly exclude these fungi from the three well studied families.
- *Basidiobolus* (and all *Basidiobolaceae*) occupy a basal position in the phylum. *Conidiobolus* (the major genus of *Ancylistaceae*) proved to be paraphyletic and needs a new gene-based classification to replace its current, unsupported subgenera; *Ancylistaceae* is basal to the large, complex, and wholly entomopathogenic family *Entomophthoraceae*.
- Within the *Entomophthoraceae*, a separate subfamily for *Massospora* subfamily (Keller & Petrini 2005) is not supported. *Batkoa* may (or may not) represent a new subfamiliy. The currently recognized generic limits among zoophthoroid genera (subfamily *Erynioideae*) are proven to need more intensive study and, except for *Zoophthora*, are unsupported as currently recognized.

REFERENCES

Gryganskyi AP, Humber RA, Smith ME, Miadlikovska J, Wu S, Voigt K, Walter G, Anischenko IM, Vilgalys R. 2012. Molecular phylogeny of *Entomophthoromycota*. *Mol. Phylog. Evol.*: in press.

Gryganskyi AP, Humber RA, Smith ME, Hodge K, Huang B, Voigt K, Vilgalys R. 2012. Phylogenetic lineages in *Entomophthoromycota*. *Persoonia*: in press.

Hibbett DS et al. (65 co-authors) 2007. A high-level phylogenetic classification of the *Fungi*. *Mycol. Res.* 111: 509-547.

Humber RA. 1989. Synopsis of a revised classification for the *Entomophthorales* (*Zygomycotina*). *Mycotaxon* 34: 441-460.

Humber RA. 2012. *Entomophthoromycota*: a new phylum and reclassification for entomophthoroid fungi. *Mycotaxon 120*: in press.

James et al. (69 co-authors) 2006. Reconstructing the early evolution of the fungi using a six gene phylogeny. *Nature* 4443: 818-822.

Keller S,Petrini O. 2005. Keys to the identification of the arthropod pathogenic genera of the families *Entomophthoraceae* and *Neozygitaceae* (*Zygomycetes*), with descriptions of three new subfmilies and a new genus. *Sydowia* 57: 23-53.

Saikawa M. 1989. Ultrastructure of the septum in *Ballocephala verrucospora* (*Entomophthorales*, *Zygomycetes*). *Can. J. Bot.* 67: 2484–2488.

Saikawa M, Oguchi M, Castañeda Ruiz RF. 1997. Electron microscopy of two nematode-destroying fungi, *Meristacrum asterospermum* and *Zygnemomyces echinulatus* (*Meristacraceae*, *Entomophthorales*). *Can. J. Bot.* 75: 762–768.

Nagahama T, Sato H, Shimazu M, Sugiyama J, 1995. Phylogenetic divergence of the entomophthoralean fungi: evidence from nuclear 18S ribosomal RNA gene sequences. *Mycologia* 87: 203-209.

PHYLUM *Entomophthoromycota* Humber, phyl. nov.

CLASS *Basidiobolomycetes* Humber, cl. nov.

ORDER *Basidiobolales* Cavalier-Smith

FAMILY *Basidiobolaceae* Claussen

Basidiobolus Eidam
Schizangiella Humber, B. Huang & Hodge (unpubl. new genus)
Drechslerosporium B. Huang, Humber & Hodge (unpubl. new genus)

CLASS *Neozygitomycetes* Humber, cl. nov.

ORDER *Neozygitales* Humber, ord. nov.

FAMILY *Neozygitaceae* Ben-Ze'ev, R.G. Kenneth & Uziel

Apterivorax S. Keller
Neozygites Witlaczil
Thaxterosporium Ben-Ze'ev & R.G. Kenneth

CLASS *Entomophthoromycetes* Humber, cl. nov.

ORDER *Entomophthorales* G. Winter

FAMILY *Ancylistaceae* J. Schröter

Ancylistes Pfitzer
Conidiobolus Brefeld
Macrobiotophthora Reukauf

FAMILY *Completoriaceae* Humber

Completoria Lohde

FAMILY *Entomophthoraceae* Nowakowski

SUBFAMILY *Entomophthoroideae* S. Keller

Batkoa Humber
Entomophaga A. Batko
Entomophthora Fresenius
Eryniopsis Humber (in part; see subfam. *Erynioideae*)
Massospora Peck

SUBFAMILY *Erynioideae* S. Keller

Erynia (Nowakowski ex A. Batko) Remaudière & Hennebert
Eryniopsis Humber (in part; see subfam. *Entomophthoroideae*)
Furia (Batko) Humber
Orthomyces Steinkraus, Humber & J.B. Oliver
Pandora Humber
Strongwellsea A. Batko & Weiser
Zoophthora A. Batko

FAMILY *Meristacraceae* Humber

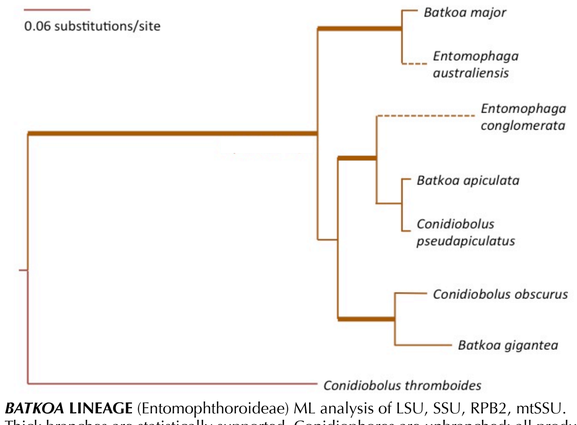
Meristacrum Drechsler
Tabanomyces Couch, RV Andrejeva, Laird & Nolan

Taxa with uncertain status, not accepted, or excluded from *Entomophthoromycota*:

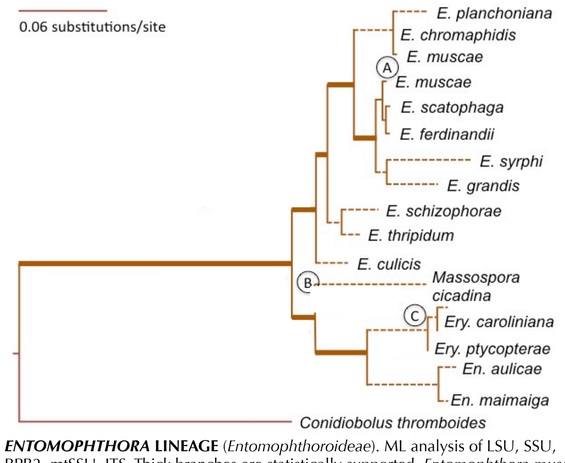
Subfamily *Massosporoideae* Keller – Not accepted; without molecular support
Tarichium Cohn – A form-genus; mixture of species of *Entomophthoraceae* and *Neozygitaceae*
Eryniopsis Humber – Heterogeneous; species probably belong in separate subfamilies
Ballocephala Drechsler } { Moved from *Meristacraceae* to subphylum *Kickxellomycotina*
Zygnemomyces Miura } { due to septal ultrastructure (Saikawa 1989; Saikawa et al. 1997)

The earliest phylogenetic studies including entomophthoroid taxa suggested that they were not homogeneous. The much more extensive sampling and use of more genes here than in previous analyses confirm that the classically defined Entomophthorales is both monophyletic and distinct from all other zygomycetes. This justified raising the subphylum to phylum rank. There is an acute need for a kaleidoscopic survey and phylogenetic review of *Conidiobolus*. The existing subgeneric scheme for this genus is not supported, and we dare not guess about the number or circumscriptions of genera that will result from its reclassification.

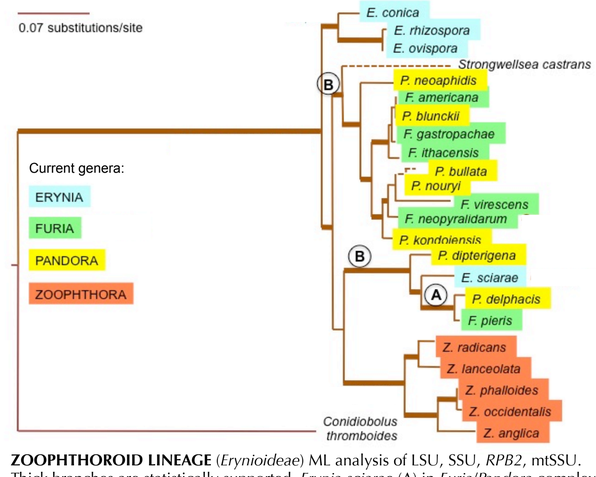
The *Entomophthoraceae* is the largest and most taxonomically complex family of this phylum. More data (and better underlying identifications of a number of strains) will be needed to determine the extent to which the *Batkoa* lineage may be separate from the rest of the subfamily *Entomophthoroideae*. More species and isolates of several genera must be studied (especially specis of *Eryniopsis*). The genera *Erynioideae* represent the most difficult problem using the current molecular data. Zoophthora is clearly supported as a separate genus, it is possible that Erynia might be supported, and Pandora and Furia may need to be combined; only the use of more collections and more genes will be able to resolve these questions.



***BATKOA* LINEAGE** (Entomophthoroideae) ML analysis of LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. Conidiophores are unbranched; all produce secondary conidia; no cystidia are formed. Mixed identifications of taxa indicate strains identified before description of *Batkoa*. (Gryganskyi et al. 2012b)



***ENTOMOPHTHORA* LINEAGE** (*Entomophthoroideae*). ML analysis of LSU, SSU, RPB2, mtSSU, ITS. Thick branches are statistically supported. *Entomophthora muscae* complex (A) is paraphyletic; *Massospora* (B) belongs in *Entomophthoroideae*; status of *Eryniopsis* (C) needs re-examination. (Gryganskyi et al. 2012b)



ZOOPHTHOROID LINEAGE (*Erynioideae*) ML analysis of LSU, SSU, *RPB2*, mtSSU. Thick branches are statistically supported. *Erynia sciarae* (A) in *Furia/Pandora* complex may reflect misidentification; *Furia* and *Pandora* (B) do not have molecular support; *Zoophthora* does seem to have good molecular support . (Gryganskyi et al. 2012b)

Review

Cell PRESS

Evolutionary Microbiology

The search for the fungal tree of life

David J. McLaughlin[1], David S. Hibbett[2], François Lutzoni[3], Joseph W. Spatafora[4] and Rytas Vilgalys[3]

[1] Department of Plant Biology, University of Minnesota, St. Paul, MN 55108, USA
[2] Department of Biology, Clark University, Worcester, MA 01610, USA
[3] Department of Biology, Duke University, Durham, NC 27708-0338, USA
[4] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, USA

The *Fungi* comprise a diverse kingdom of eukaryotes that are characterized by a typically filamentous but sometimes unicellular vegetative form, and heterotrophic, absorptive nutrition. Their simple morphologies and variable ecological strategies have confounded efforts to elucidate their limits, phylogenetic relationships, and diversity. Here we review progress in developing a phylogenetic classification of *Fungi* since Darwin's *On the Origin of Species*. Knowledge of phylogenetic relationships has been driven by the available characters that have ranged from morphological and ultrastructural to biochemical and genomic. With the availability of multiple gene phylogenies a well-corroborated phylogenetic classification has now begun to emerge. In the process some fungus-like heterotrophs have been shown to belong elsewhere, and several groups of enigmatic eukaryotic microbes have been added to the *Fungi*.

Fungal diversity and antiquity

Fungi make up a remarkably diverse kingdom whose species interact with a broad array of other organisms. Their compact genomes have been completely sequenced in more than 70 species. Nevertheless, the phylogenetic relationships of the *Fungi* remain incompletely known because of the challenges presented by the antiquity of fungal lineages and the incomplete documentation of extant species. Improved sequencing methods, expanded datasets and sophisticated phylogenetic algorithms, coupled with community-wide collaborations, are now contributing to the emergence of a well-supported phylogeny and classification for the kingdom *Fungi*.

Roles and antiquity of *Fungi*

Fungi interact extensively with plants, animals, bacteria and other organisms. Their heterotrophic, absorptive nutrition, aided by their filamentous and occasionally unicellular growth forms, allows them to play major roles as decomposers, mutualists and parasites [1]. They form symbioses with cyanobacteria and algae in lichens, and with the roots and aerial parts of most plants as mycorrhizae and endophytes, respectively. In animals these mutualisms may be external and aerobic, as in ant-fungal gardens, internal and aerobic in insect gut, or anaerobic in the rumen or caecum of herbivorous mammals. Parasitism of both plants and animals has a significant impact on humans and ecosystems.

The ages of fungal clades have been estimated from fossils and molecular sequence data. The fossil record is very incomplete but the data suggest that most fungal phyla were present at least 400 to 500 mya although their actual ages might be much greater [1,2].

Numbers of *Fungi*

The number of extant species of *Fungi* is unknown. The most widely cited estimate of 1.5 million [3] has been supported by the data of Schmit and Mueller [4] that suggest about 700,000 species as a conservative lower limit. This estimate is based primarily on the ratio of

Glossary

Ascomycetes: *Fungi* that produce filaments or yeasts, and reproduce sexually with spores formed internally in an ascus.
Basidiomycetes: *Fungi* that produce filaments or yeasts, and reproduce sexually with spores formed externally on a basidium.
Chytrids: an informal term for *Fungi* with flagellated cells at some point in the life cycle.
Flagellar apparatus: the region of a zoospore comprised of the kinetosome, transition zone and flagellum.
Homology: two genes are said to be homologs if they derive from a single gene in a common ancestor.
Monophyletic group: a group of species that includes an ancestor and all of its descendants, a clade.
Ontologies: controlled structured vocabularies.
Ortholog identification: a method to detect a homologous gene among species.
Orthologous genes: homologous gene copies in two or more species that arose by speciation.
Paralogous genes: homologous gene copies in one or more genomes that arose by gene duplication.
Paraphyletic group: a group of species that includes the most recent common ancestor and some of its descendants.
Phylogenomics: phylogenetic analysis using whole genomes of species.
Polytomy: unresolved branching in a phylogenetic tree resulting in multiple branches arising at a branch point reflecting uncertainty about the order of cladogenesis.
Synapomorphy: a shared derived character that unites species in a monophyletic group.
Septal pore: opening in the cross wall between adjacent cells of a filament.
Spindle pole body: a structure that forms spindle and astral microtubules in *Fungi* that lack flagella.
Spitzenkörper: a fungal-specific hyphal tip organization.
Supermatrix: multigene phylogenetic dataset in which not all taxa are represented by the same genes.
Water molds: filamentous, fungal-like species that produce biflagellate cells; relatives of the brown and golden algae.
Zygomycetous fungi, or zygomycetes: coenocytic, filamentous species that lack complex fruiting bodies.

Corresponding author: McLaughlin, D.J. (davem@umn.edu)

0966-842X/$ – see front matter doi:10.1016/j.tim.2009.08.001 Available online 24 September 2009

Fungi to plant species for several ecologically defined groups from different regions of the world. There are approximately 100,000 described species and this number is increasing at about 1.2% per year [5]. Knowing the number of species of *Fungi*, and their phylogenetic distribution, is important for the understanding of the pattern and tempo of fungal diversification, as well as the complexity of ecosystems. Moreover, species-rich phylogenies assist in taxon identification in molecular ecology studies [6–10]. These phylogenies have practical application in ecosystem management, agriculture, drug discovery and medicine.

Search for the missing *Fungi*

Like other microorganisms, *Fungi* still harbor many undescribed and undiscovered lineages. Many of these represent species that have never been cultured or collected previously by fungal taxonomists. The number of unidentified fungal sequences of environmental origin in public databases has grown significantly in the past 10 years [8–12], suggesting that a large number of fungal lineages remain undiscovered [13,14]. Many of the undescribed species of *Fungi* are probably inconspicuous or microscopic forms that do not produce fruiting bodies, such as yeasts, molds, endophytic or arbuscular mycorrhizal (AM) fungi, and particularly those that live in obscure, poorly-explored habitats. For example, new yeast species obtained from beetle digestive tracts have increased the number of known yeasts by more than 20% [4,15]. However, common habitats such as plant leaves (phylosphere) are known to host a hyperdiversity of unknown fungal species [8–10,12–14]. Molecular environmental studies have revealed unknown major clades of *Fungi*, some of whose species are winter active and grow beneath the snow at high elevations [7]. One of these clades, known only from molecular sequences, is a basal clade in the Ascomycota and is thus important in understanding the evolution of the phylum; this clade is distributed on three continents and might require metagenomic analysis to understand its role in ecosystems [16]. In addition, multiple lineages of undescribed *Fungi* have been encountered repeatedly within taxa previously thought to contain only a single species. Examples of such cryptic diversity have been found in a wide variety of fungal groups, including chytrids (*Rhizophydium*) [17], molds (*Trichoderma*), animal pathogens (*Pneumocystis*), and mushrooms (*Armillaria, Cantharellus*) [18].

In this review we trace how the relationships among *Fungi* have been viewed since Darwin's *On the Origin of Species*, the current state of fungal systematics, and future prospects for reconstructing the Fungal Tree of Life (FToL). Highlights in the development of a phylogenetic classification of the *Fungi* will be presented.

Evolving knowledge of fungal phylogeny and classification

First century following Darwin's On the Origin of Species: mid-19th to mid-20th century

The publication of Darwin's *On the Origin of Species* in 1859 resulted in the rapid introduction of evolutionary thought into the study of fungi. Anton de Bary in his 1866 textbook was the first to introduce evolution into fungal classification [19]. He based his classification of the basal fungi on similarities in morphology between certain algae and aquatic and zygomycetous fungi, and considered other fungal groups – ascomycetes and basidiomycetes – to be more derived. By the second edition of the textbook in 1884 his tentative classification resembled that used until the second half of the 20th century (Figure 1, Box 1). In this period the characters used for phylogenies were morphological, anatomical and chemical.

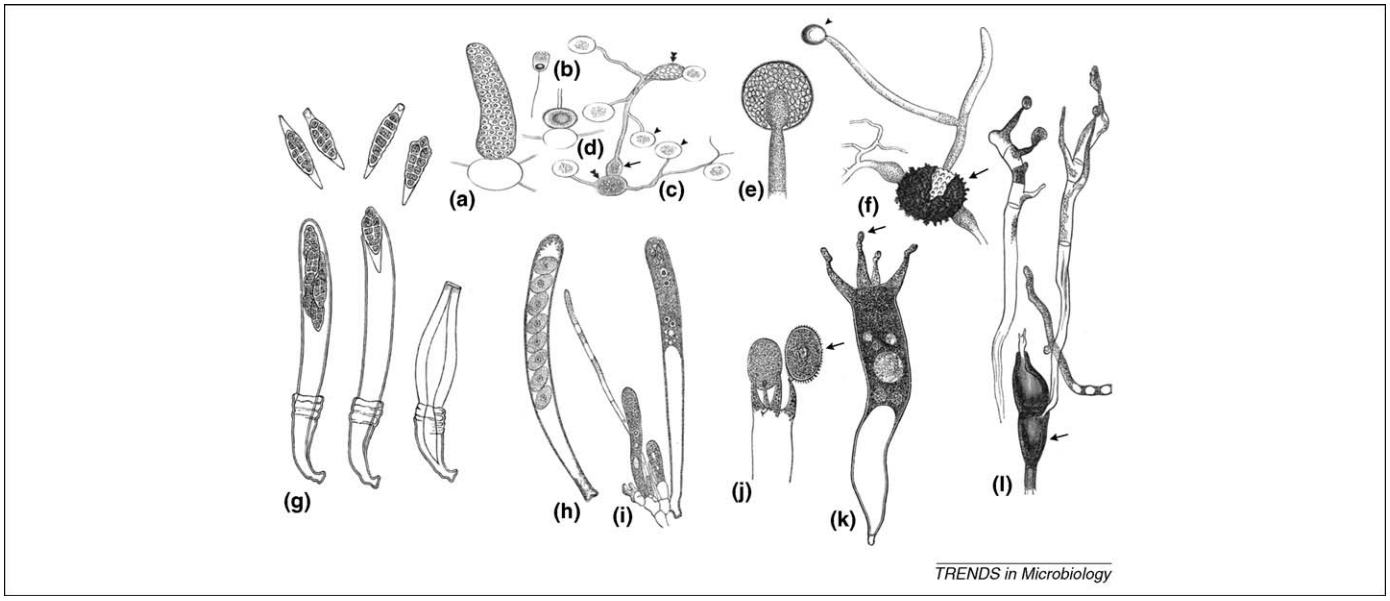


Figure 1. The defining features of the major groups of *Fungi*. These illustrations from the 1880s by de Bary and his students [72] are fully informative for characterizing taxa today. **(a–d)** Chytridiomycota, *Polyphagus euglenae*: **(a)** zoosporangium with discharge vesicle, **(b)** uniflagellate zoospore, **(c)** conjugating thalli (double arrowheads) initiating a resting spore (arrow) and attached to parasitized *Euglena* cysts (arrowheads), **(d)** maturing resting sporangium. **(e,f)** Zygomycetous fungi, *Mucor mucedo*: **(e)** sporangium and **(f)** germinating zygosporangium (arrow) between suspensors with germ sporangium (arrowhead). **(g–i)** Ascomycota: **(g)** *Macrospora scirpi* and **(h,i)** *Pyronema confluens* with **(g)** bitunicate asci before, during and after ascospore discharge and **(i)** unitunicate asci forming and **(h)** mature. **(j–l)** Basidiomycota: **(j,k)** *Aleurodiscus amorphus* and **(l)** *Puccinia graminis* with basidia with **(k)** asymmetrically forming and **(j)** mature basidiospores (arrows) or **(l)** arising from the overwintered teliospore (arrow).

Box 1. Classification of the fungi and slime molds by Anton de Bary

Groups that diverge from a class or are of doubtful position are indicated by an asterisk (*). Except for the uncertain placement of Protomyces and Ustilagineae, this classification from the 1880s [72] remained accepted for much of the 20th century.

Fungi

- Phycomycetes
  - Peronosporeae
  - Saprolegnieae
  - Mucorinii or Zygomycetes
  - Entomophthoreae
  - *Chytrideae
  - *Protomyces and Ustilagineae
- Ascomycetes
- Uredineae
  - *Basidiomycetes

Mycetozoa

- Myxomycetes
- Acrasieae

The class Phycomycetes – fungi with algal characteristics – was introduced by de Bary. This included aquatic and nonaquatic taxa, chytrids, water molds and their relatives, and zygomycetes. The class persisted for about 100 years. Subdivision of the aquatic fungi by Sparrow [20] based on motile cell structure began the unraveling of aquatic members of the *Fungi* from those species more closely related to algal groups, such as the Oomycota. However, the zygomycetous fungi, although also a paraphyletic group, could not be sorted out until much later when molecular data became available [21].

By the 1960s cell wall chemistry and biochemical pathways began to clarify relationships among fungi (Figure 2) [22]. *Fungi* were defined by amino acid biosynthesis via the diaminopimelic acid pathway and cell walls of chitin and often β-glucan, while fungus-like organisms used the aminoadipic acid pathway in amino acid synthesis and had different cell wall compositions. With these advances the modern outlines of the *Fungi* as a monophyletic group began to emerge.

Fungi and the kingdoms of the eukaryotes: mid-19th century to present

Early classifications divided all organisms into two major groups, the plant and animal kingdoms. Fungi were included in the plant kingdom by de Bary because of their morphological similarities, although this point of view was not universally accepted [19]. Whitaker [23] was first to recognize *Fungi* as a distinct kingdom. He based his classification on cell structure, levels of tissue organization and nutritional mode. Although Whitaker's classification was heavily influenced by ecological considerations it had a major impact on thinking about fungi. A monophyletic kingdom of *Fungi* and its alignment with *Animalia* emerged in the 1990s with molecular sequence data [1,24]. The inclusion of animals and *Fungi* in the Opisthokonta is supported by all large datasets with broad species coverage and by a limited number of cellular synapomorphies; these include flattened mitochondrial cristae, a single posterior flagellum on motile cells, and similarities in the flagellar apparatus [24]. Although formerly treated

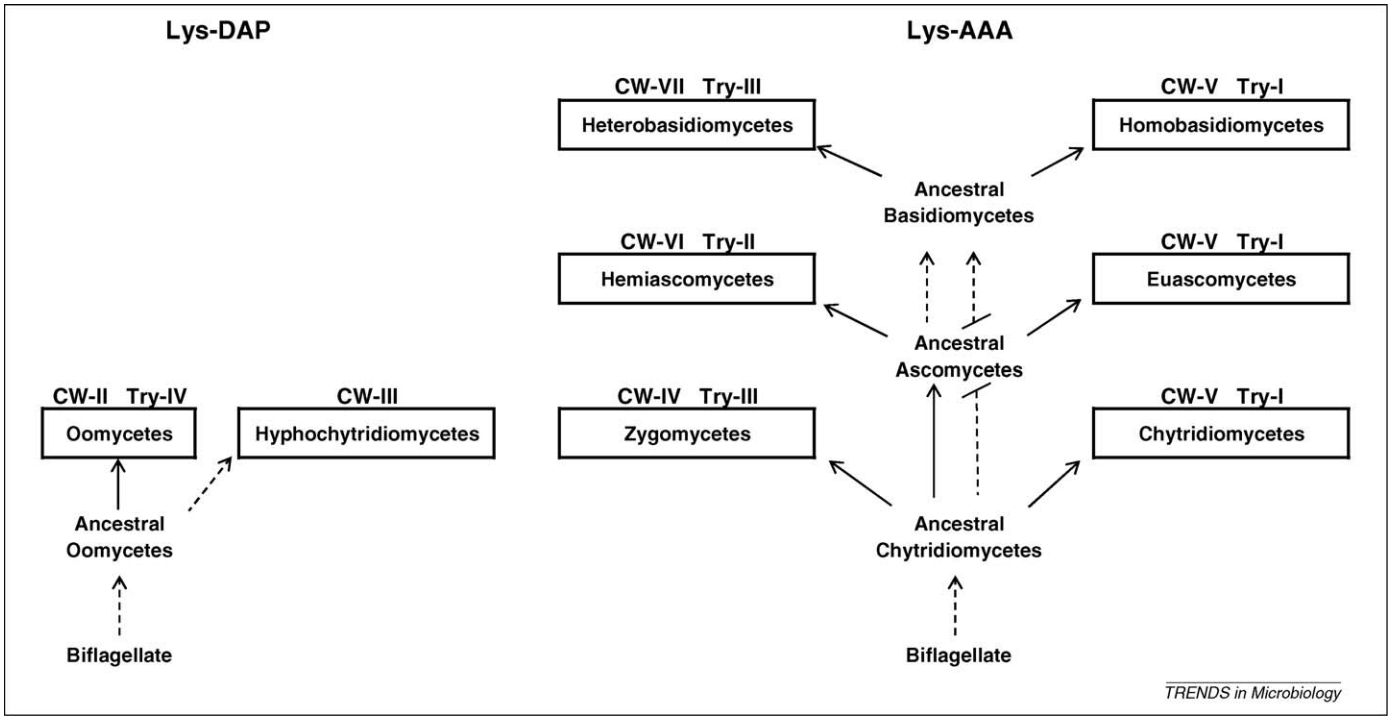


Figure 2. Hypotheses of fungal evolutionary relationships from 1969. Relationships are based on cell wall composition and biosynthetic pathways from Bartnicki-Garcia [22]. Lysine synthesis (Lys) can occur via the diaminopimelic acid (DAP) or aminoadipic acid (AAA) pathways. Cell wall (CW) types range from type II to VII: II, cellulose–β-glucan; III, cellulose–chitin; IV, chitin–chitosan; V, chitin–β-glucan; VI, mannan–β-glucan; VII, chitin–mannan. There are also four types of sedimentation patterns of tryptophan biosynthesis enzymes (Tryl–IV). Reproduced with permission of Academic Press [22].

Box 2. What do we mean when we use 'fungi' and '*Fungi*'?

What do we mean by 'fungi'? When the term 'fungi' is used it conveys a historical meaning of all groups that have fungal or fungal-like characteristics. Thus, besides the species in the monophyletic kingdom *Fungi*, it includes the water molds and white rusts (i.e. Oomycota), some orders previously included in the Trichomycetes (i.e. the zygomycetous fungi that form symbiotic relationships with aquatic invertebrates) and slime molds. The organisms that fall outside kingdom *Fungi* are now classified in other kingdoms. Pseudofungi has been proposed as a subphylum for Oomycetes and Hyphochytriomycetes [73] but the term 'pseudofungi' can be applied to any of these fungal-like organisms; this term is not needed. These fungi are no more 'pseudofungi' than the non-monophyletic organisms that comprise the algae or bacteria are pseudo-members of each of these groups. In these cases the name has an ecological meaning, not a systematic one. A possible solution to the confusion caused by 'fungi' is to qualify the term as is done with the algae and use 'true fungi', 'chromistan fungi', etc. To avoid confusion *Eumycota* has been introduced for *Fungi*, but the preference of most mycologists is to retain the better-known term *Fungi* for this kingdom [40].

as fungi by many authors, the cellular and acellular slime molds (Mycetozoa), together with lobose amoebae (Lobosa), form the sister clade to the Opisthokonta [24]. Taken together these clades have been referred to as the Unikonts (Keeling et al., 2005). The relationship among the *Eukarya* continues to be refined; however, placement in the Unikonts is a reasonably supported hypothesis of the relationships of the *Fungi* [24,25]. Clarification of which taxa belong in the kingdom *Fungi* has led to a nomenclatural problem that continues to cause confusion (Box 2).

Ultrastructural and molecular data and phylogenies: 1950s to present

The advent of ultrastructural data in the late 1950s and of molecular data in the 1990s has clarified the distinctions between fungal groups and revealed numerous cases of parallel or convergent evolution (homoplasy). But neither type of data has fully resolved the FToL. Structural data are incomplete with only a limited number of species studied in any phylum; new subgroups of *Fungi* revealed by molecular phylogenetic studies [26] are only now being examined structurally. Molecular data are similarly limited and have yet to resolve fully the deeper nodes of the FToL.

The types of cellular structures that have proven phylogenetically informative among fungal phyla include septal pore organization, nuclear division and spindle pole body (SPB) form, and the organization of motile cells (Figure 3). These characters have been used in phylogenetic analyses [27] but are often incompletely known within phyla [28]. Until the basal branches of the FToL are fully resolved it may be difficult to interpret the evolution of some structural characters, such as SPBs. The multiple losses of centrioles in basal fungi [26] could imply multiple independent origins of SPB structure in basal groups, but not necessarily in the Ascomycota and Basidiomycota that are sister clades. Bioinformatics is an essential tool for utilizing both structural and molecular data in phylogenetic reconstruction. Comparison of structural characters is best achieved with scientific community input into a common database, for which the Structural and Biochemical Database (see http://aftol.umn.edu) has been developed to provide character and character state data in an exportable format for use in phylogenetic analysis programs [28]. This database reveals the limitations of the available data and will guide future data acquisition.

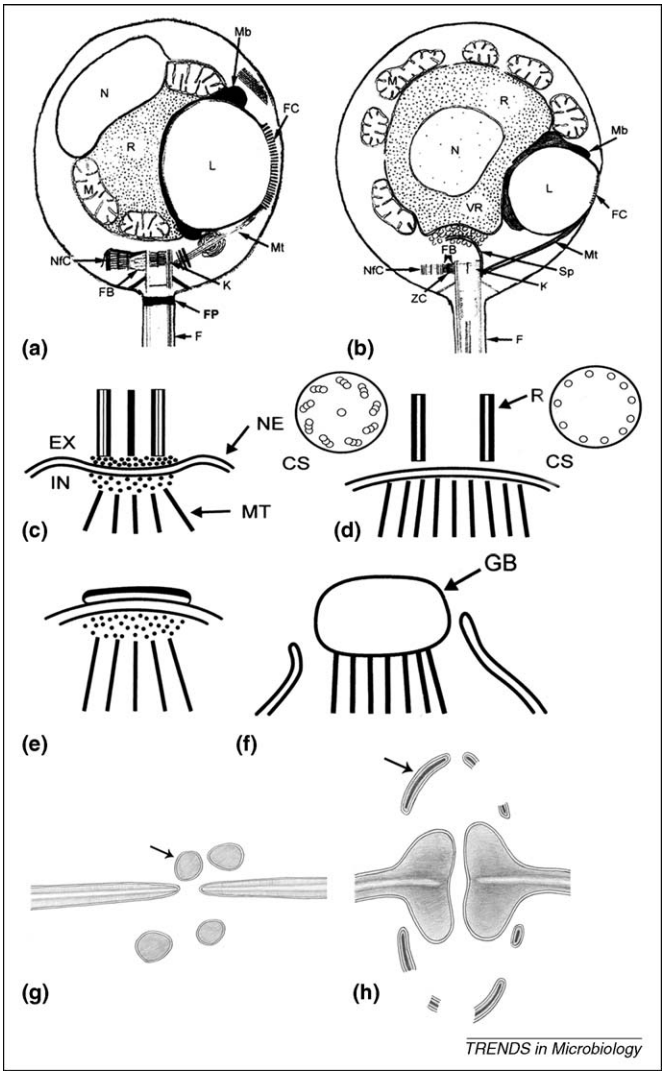


Figure 3. Examples of phylogenetically informative subcellular structures. These structures were used for elucidating fungal clades at the ordinal to subphylum or phylum level [17,28]. **(a,b)** Zoospore types in Chytridiomycota: **(a)** Chytridiales and **(b)** Rhizophydiales. Abbreviations: F, flagellum; FB, fibrillar bridge; FC, fenestrated cisterna; FP, flagellar plug; K, kinetosome; L, lipid globule; M, mitochondrion; Mb, microbody; Mt, microtubular root; N, nucleus; NfC, nonflagellated centriole; R, ribosomes; Sp, spur; VR, vesiculated region; ZC, zone of convergence. **(c–f)** Spindle pole body forms at metaphase–anaphase and their relationship to the nuclear envelope in **(c)** Blastocladiomycota, **(d)** zygomycetous fungi, **(e)** Ascomycota, and **(f)** Basidiomycota. Abbreviations: CS, cross section of the kinetosome; EX, extranuclear area; GB, globoid spindle pole body; IN, intranuclear area; MT, spindle microtubules; NE, nuclear envelope; R, ring with microtubules but lacking nine-fold symmetry. **(g,h)** Septa and septal pore organization in hyphae of **(g)** Ascomycota with Woronin bodies (arrow) and **(h)** Basidiomycota with septal pore cap (arrow) and pore swelling. Reproduced from Refs. [17] **(a,b)** and [28] **(c–h)** with permission.

Molecular phylogenies of the *Fungi* initially were based on single locus trees of nuclear ribosomal DNA (rDNA). Two-locus trees of *Fungi* began to appear soon after (in 1992), but it took until 1997 for these phylogenetic studies to be based on three loci and an additional three years before more than four loci were used [29]. Indeed, more

than 75% of all fungal trees published each year until 2003 were still based on a single locus. Recently, the availability of whole genomes has permitted the application of phylogenomics to fungal phylogeny. The complete genomes of *Saccharomyces* species were used to determine the number of genes needed to develop a robust phylogeny [30,31]. Phylogenomics is now being extended to a broader sampling of taxa [32,33] for phylogenetic reconstruction across the *Fungi*. The large number of genes now available for phylogenetic studies of the *Fungi* has provided several new bioinformatic challenges, including the need for interactive databases with increasing levels of sophistication (e.g. Provenance, Ref. [34]), large scale data set assembly and visualization (such as WASABI, Ref. [35]; and Mesquite, http://mesquiteproject.org), phylogenetic search methods that can be implemented on supermatricies of thousands of taxa (e.g. RaxML, Ref. [36]), and efficient bioinformatic tools to visualize large-scale phylogenetic trees (such as PhyloWidget, Ref. [37]) and the information they contain (e.g. the database *mor*, Ref. [38]).

The FToL in the 21st century

Fungal systematics received a boost early in the 21st century from two National Science Foundation-sponsored projects, the Deep Hypha Research Coordination Network (RCN) and the AFTOL1 (Assembling the Fungal Tree of Life) project [39]. Deep Hypha supported a series of meetings of fungal systematists from 2001 to 2006 that enabled the community to share information and plan research. However, Deep Hypha did not directly support data-gathering activities. Plans for AFTOL1 were developed in the context of Deep Hypha, and benefited greatly from the community network that was formed through the RCN. The AFTOL1 proposal included a very large number of supporting letters, most from Deep Hypha participants, and the project adopted a policy that all donors of material would be invited to be coauthors on publications that reported new data derived from those materials. This policy recognizes the significant mycological expertise required to find and identify organisms and to archive voucher specimens and cultures. As a consequence, many of the AFTOL1 publications have numerous coauthors, examples being Lutzoni *et al.* [29], James *et al.* [26], and Hibbett *et al.* [40] respectively with 44, 70 and 67 coauthors.

AFTOL1 sought to generate molecular data of seven loci [nuclear large and small subunit and 5.8S ribosomal RNA genes, subunits 1 and 2 of RNA polymerase II (*rpb1*, *rpb*2), elongation factor 1-α, and mitochondrial ATP synthetase (*atp6*)] from about 1500 species representing all groups of *Fungi*, as well as ultrastructural characters from selected taxa. Molecular data from AFTOL1, including primer sequences and reference alignments, are available through a web-accessible database (http://aftol.org/data.php). Most of the AFTOL1 molecular data have been published and are in the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/index.html) that includes 4478 nucleotide sequences from 1106 species that can be retrieved with the keyword AFTOL.

Much of the output of AFTOL1 is summarized in four key references, including two kingdom-wide multilocus analyses [26,29], a collection of phylogenetic studies on diverse groups of *Fungi* in the Deep Hypha issue of *Mycologia* [2,21,28,39,41–60], and a novel higher-level phylogenetic classification of the *Fungi* [40] that has been adopted by the mycological community and beyond, thus facilitating scientific communication.

The analysis of James and colleagues [26] included six of the seven AFTOL1 target loci (excluding only *atp6*) that were sampled in 199 species. The major conclusions of this study concerned the phylogenetic disposition of the 'basal fungal lineages', a paraphyletic assemblage containing multiple clades of chytrids and zygomycetes. The analysis also suggested that the Glomeromycota (traditional zygomycetes, including arbuscular mycorrhizal fungi) is the sister group of the Dikarya (a clade containing Basidiomycota and Ascomycota that is named from the synapomorphy of dikaryotic hyphae), although support for the Glomeromycota–Dikarya clade was weak.

One of the most contentious issues addressed by James *et al.* [26] concerns the number of losses of the flagellum among the *Fungi*. Several clades of chytrids form a paraphyletic assemblage at the base of the *Fungi* that is consistent with the view that the presence of flagella is an ancestral character state in the *Fungi*. Two groups of non-flagellated taxa appear to be nested among the chytrids and probably represent independent losses of the flagellum. One is *Hyaloraphidium curvatum*, an enigmatic planktonic organism that was first shown to be a member of the *Fungi* by Ustinova and coworkers [61]. The analysis of James *et al.* [26] suggests that *H. curvatum* is nested in a clade that includes free-living chytrids (Chytridiomycota *sensu stricto*) and anaerobic rumen symbionts (Neocallimastigomycota). The other group of non-flagellated taxa that appears to be nested among the basal chytrids is the Microsporidia, which are obligate intracellular parasites notable for their highly reduced genomes, degenerate mitochondria, and accelerated rates of molecular evolution [62]. The analysis of James *et al.* [26] suggests that a clade containing Microsporidia and the chytrid *Rozella allomycis* (an endoparasite of other chytrids) is the sister group of all other *Fungi*. Several other studies have suggested that the Microsporidia are nested within the *Fungi* or could be the sister group of the *Fungi* [24,63,64]. The apparent number of losses of the flagellum is also influenced by the position of *Olpidium brassicae*, a soil-dwelling chytrid that is a pathogen of plant roots. Surprisingly, *O. brassicae* was placed as a close relative of the zygomycete *Basidiobolus ranarum*, a filamentous species that functions as an animal pathogen or saprotroph.

Considering its complexity it is unlikely that the eukaryotic flagellum could be regained after having been lost. Applying this principle, the optimal trees produced by James *et al.* [26] imply five independent losses of the flagellum, two on the lineages leading to *H. curvatum* and Microsporidia, and three among the zygomycetes (owing to the position of *O. brassicae*). However, alternative placements of Microsporidia and *O. brassicae* resulted in trees that imply only two or three losses, and these could not be rejected. An analysis of data on *rpb1* and *rpb*2 published at about the same time as the James *et al.* study suggested that the Microsporidia are the sister group of the

Fungi and that the traditional zygomycetes are monophyletic, and therefore concluded that there was only a single loss of the flagellum in fungal evolution [63]. However, this analysis did not include *R. allomycis, H. raphidium,* or *O. brassicae*.

One of the major goals of AFTOL1 was to formalize our understanding of fungal phylogeny by the introduction of new classifications. At the time that AFTOL1 and Deep Hypha were initiated there were substantial differences among the major classifications for *Fungi*, with different names often being applied to the same clades and some taxa lacking monophyly. Examples of the competing classifications included the *Dictionary of the Fungi* series [5] and the classification employed by GenBank. Under the auspices of Deep Hypha and AFTOL1 a consensus classification containing only strongly supported monophyletic groups was developed, with reference to 102 phylogenetic studies published between 1998 and 2007. Again, this was a community-based endeavor, including experts on diverse groups and the authors and administrators of major taxonomic resources [40]. The 'AFTOL classification', that includes 129 orders as its terminal taxa, is now embodied in the current *Dictionary of the Fungi* [5], the GenBank classification, the Tree of Life Web Project (http://tolweb.org/tree/), the Myconet classification of Ascomycota (http://www.fieldmuseum.org/myconet/), and the Catalogue of Life annual checklist (http://www.catalogueoflife.org/annual-checklist/search.php). Reflecting uncertainty about the earliest branching events in the *Fungi*, the classification has a large polytomy at its base, including Dikarya, Glomeromycota, and eight other groups containing chytrids, zygomycetes, and Microsporidia (Figure 4).

Future prospects for fungal phylogeny

The immediate future of phylogenetics of the kingdom *Fungi* involves the analyses of genomic and subcellular data to address hypotheses pertaining to long-standing, enigmatic questions regarding the FToL. Major hypotheses to be addressed include (i) the placement of Microsporidia among the *Fungi*, (ii) resolution of the early diverging lineages of *Fungi* traditionally classified as chytrids and zygomycetes, (iii) more definitive ancestral character reconstruction associated with multiple losses of the flagellum, (iv) the placement of the Glomeromycota relative to other major clades of terrestrial, plant-associated *Fungi*, and (v) resolution of several problematic internal nodes of the Ascomycota and Basidiomycota that are crucial to the understanding of the diversification of fungal structure and ecology. All of these hypotheses represent questions in fungal evolutionary biology that have eluded traditional approaches using standard molecular systematics and observational studies of subcellular traits; novel approaches will be necessary to develop robust and testable explanations successfully.

Based on results from AFTOL1 (Figure 4), a second phase of AFTOL (AFTOL2) recently proposed a targeted set of taxa for sampling that will explicitly address problematic nodes and the hypotheses summarized above. Importantly, sampling of subcellular and genomic characters will overlap for a core set of taxa so as to maximize the explanatory power of the combined data. Subcellular characters to be sampled include septa of vegetative hyphae and meiosporangia, the nuclear division apparatus, SPB cycle, and the Spitzenkörper. In addition to the collection of subcellular data for target taxa, AFTOL2 is developing ontologies for these characters so that homologies can be communicated more accurately across disparate groups of taxa.

Advancements in genome sequencing technologies have resulted in a rapid increase in the availability of genomic data for *Fungi* [65] (see http://fungalgenomes.org/genome), setting the stage for the convergence of the fields of phylogenetics and genomics [66,67]. These studies include evolutionary analyses of genome organization that have recently provided additional support for placement of Microsporidia among the *Fungi* [64], and the phylogenetic analyses of a large amount of primary nucleotide or amino acid data [33,68]. The accurate determination of orthologous sequence data is central to the phylogenetic analyses of genomic data. The problem of paralogy and misinterpretation of homology is significantly higher with genomic data as compared to PCR-directed gene sequencing. Numerous analytical approaches have recently been developed for determination of orthologous sequences, and Kuzniar *et al.* [69] provided a comprehensive review of the strengths and weaknesses of currently available programs and databases. In addition to ortholog determination, early phylogenomic studies also observed potential conflicts among gene trees [30,68], systematic biases associated with taxon and character sampling [31], and difficulty in the assessment of nodal support [33,67,68]. Guided by these preliminary studies, AFTOL2 initiated a study to identify a kingdom-wide set of orthologous markers and facilitate acquisition and analyses of these data.

AFTOL2 identified a core set of 71 genes that are ubiquitously distributed across the *Fungi* and are good candidates (e.g. length of predicted proteins, sequence variability, single or low copy-number gene family) for large-scale phylogenomic analyses (see http://www.aftol.org). Twenty-five of these genes have been included in other phylogenomic studies [30,70] or tree of life projects (http://atol.sdsc.edu/projects), and provide cross-reference data points for global studies of the Tree of Life. The remaining 46 genes were identified by AFTOL2 using a Markov clustering approach [33] and target the FToL. To facilitate working with such large datasets AFTOL2 developed a semi-automated PERL wrapper to integrate and articulate existing algorithms for ortholog identification, multiple protein alignments, model of evolution assessment, and phylogenetic analyses of individual and concatenated super alignments (*Hal*: see http://aftol.org/pages/Halweb3.htm; beta versions of *Hal* are available from J.S. upon request). This approach not only uses data from completely sequenced genomes but it is also able to incorporate identified orthologs from heterogeneous genome resources such as expressed sequence tag (EST) libraries. The result will be a supermatrix whereby some genes are missing for some taxa, but will permit a broader and more inclusive approach to taxon sampling. In addition, to facilitate the rapid expansion of additional phylogenetic markers for use in fungal phylogenetics, AFTOL2 is also

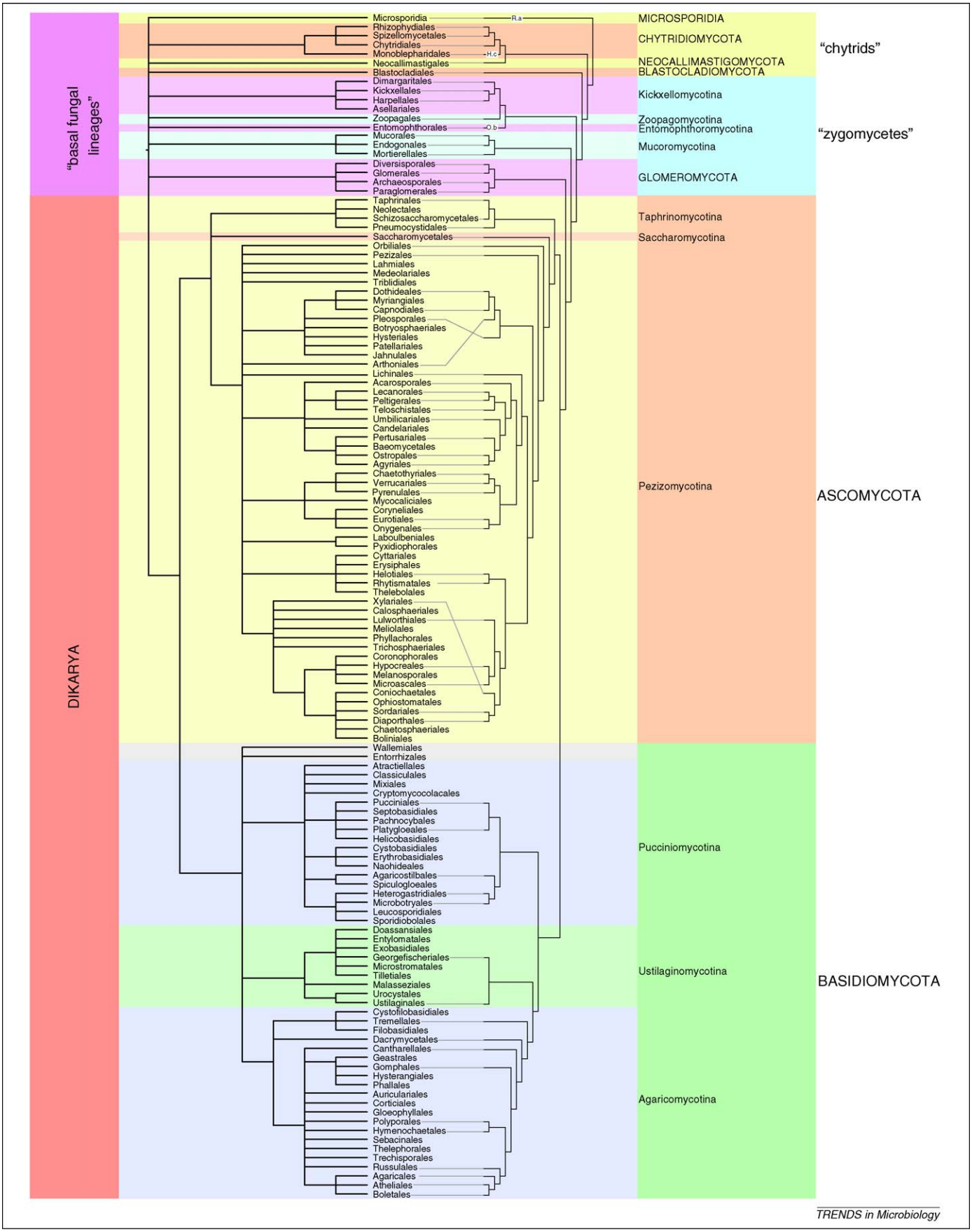


Figure 4. Phylogeny and classification of *Fungi*. The tree on the left represents the AFTOL classification. Only nodes corresponding to formally named taxa are resolved. Phyla (suffix -mycota), subphyla (-mycotina) and subkingdom-level taxa (Dikarya) are labeled. Names in quotation marks are informal, non-monophyletic groups. The tree on the right reflects taxon sampling and tree topology from James *et al.* [26] (the AFTOL classification was developed with reference to many additional studies). Positions of *Rozella allomycis*, *Hyaloraphidium curvatum*, and *Olpidium brassicae* estimated by James and coworkers are indicated by R.a, H.c, and O.b., respectively.

Box 3. Outstanding questions

- **How has subcellular structure evolved in the *Fungi*?**
  The range of variation in subcellular structures within fungal phyla is unknown. Generalizations are based on minimal data (i.e. from one or a few species) but in better-studied subphyla a range of subcellular features is observed, for instance in motile cell organization in Chytridiomycota or SPB form and septal pore organization in Basidiomycota. Several SPB forms are known in zygomycetous fungi but the clades are still largely unstudied. To determine how SPB form has evolved in these fungi and its relationship to flagella loss in basal fungi a detailed analysis of nuclear division is needed for four zygomycete subphyla and the Glomeromycota. To understand subcellular evolution and characterize the genes in the many fungal genomes that are becoming available, a renewed focus will be required on fungal cytology, employing well thought-out sampling strategies. Improvements in bioinformatic resources for image labeling and storage will aid in comparative structural analyses and integration with molecular data.
- **What will be the next limiting factors for assembling the fungal tree of life?**
  Mycologists are entering a period where it will be as easy to sequence fungal genomes (often <40 Mb) as it was for prokaryotes over the last decade. The rapid sequencing of small genomes will permit finding the optimal set of genes to provide sufficient resolution to generate a FToL for all described species. The main challenges will be to obtain samples of all known species, necessitating coordination of effort and worldwide mycological expertise, as well as new bioinformatic and analytical tools. Another limiting factor will be the description and naming of the unknown fungal species, representing the great majority of the extant fungal species richness.
- **What are the key evolutionary innovations that took place during the evolution of the *Fungi* and their biological consequences?**
  For example, when and how many times did the lichen symbiosis originate? The origination of the lichen symbiosis might be associated with a rapid adaptive radiation early in the evolution of the Pezizomycotina (a subphylum representing nearly all filamentous ascomycetes). The statistical power of all current methods to infer ancestral traits using phylogenies is unknown. These methods are likely to be biased against changes occurring during rapid adaptive radiations (i.e. on very short internodes) because they all assume a constant rate of evolution across the entire phylogeny. Therefore, if lichen symbiosis originated during a rapid radiation, current methods are more likely to infer erroneously a more recent origin and, consequently, more numerous independent origins. This explains in large part (e.g. in addition to taxon sampling issues and branch length estimations) the high uncertainty associated with current estimations of the exact number of origins and their precise localization on phylogenetic trees.
- **Are current taxonomic practices adequate for describing fungal diversity and translating emerging phylogenetic hypotheses into classifications?**
  Fungal taxonomy is increasingly based on molecular phylogenies. Similarly, our knowledge of the diversity, distribution, and ecological roles of *Fungi* is expanding rapidly through molecular environmental studies. At the same time, new species descriptions and taxonomic proposals follow rules that were developed in the absence of phylogenetic perspectives, strongly emphasize morphology, and are scattered in the literature. Should current practices be enhanced or replaced by systems that emphasize phylogeny as the primary criterion for taxonomy, use centralized databases to update a global classification, and allow species descriptions based solely on sequence data?

developing PCR and sequencing primers for these target genes for use by the broader fungal systematics community.

Initial phylogenetic analyses of genome-scale data have provided increased support for controversial taxa (e.g. Taphrinomycotina) [71] and have continued to identify problematic regions of the FToL (e.g. the backbone of the Pezizomycotina) [33,68]. One limiting factor in all of these analyses, however, is taxon sampling. Although the number of sequenced genomes is rapidly increasing, most currently available genomes have been selected because they are human and plant pathogens or are central to the carbon cycle and energy concerns (e.g. mycorrhizae and wood decay fungi). Although these are important organisms for genomic sequencing, the initial result has been a bias in taxon sampling of phylogenomic analyses and there is an urgent need for genome sequencing of unsampled fungal lineages that are crucial to the understanding of deep divergences in the FToL.

In summary, in Darwin's day only a skeletal outline of the FToL was known and the fungi included unrelated taxa with similar morphologies and ecological roles. Understanding the relationships of these taxa, especially the basal taxa, took more than a century. In the second half of the 20th century, and especially in the past 20 years, the availability of biochemical, ultrastructural and genomic data has led to a sea-change in our understanding of the FToL. Recent studies have provided a well-corroborated phylogenetic tree for the *Fungi* and have permitted the development of a consensus classification. Deep branches within the FToL, as well as many internal branches, remain unresolved and are the focus of current multigene analyses; these are expected to resolve many of the uncertainties and provide guidance in interpreting character evolution and assistance in environmental studies and in identifying the probable large numbers of unknown species (Box 3).

Acknowledgements

We thank T. K. Arun Kumar and Brianna Julius for help with the figures, B. Robbertse for advice and S. Bartnicki-Garcia for Figure 2. This publication resulted in part from the Assembling the Fungal Tree of Life projects (AFTOL1 and AFTOL2), that is supported by the National Science Foundation Assembling the Tree of Life awards DEB-0228671 and DEB-0732550 to D.J.M., DEB-0228657 and DEB-0732968 to D.S.H., DEB-0228668 to F.L. and R.V., DEB-0732984 to F.L., J. Miadlikowska and R.V., and DEB-0228725 to J.W.S. We also acknowledge support from the National Science Foundation Research Coordination Networks in Biological Sciences: A Phylogeny for Kingdom Fungi, NSF-0090301 to M. Blackwell, J.W. Spatafora, and J.W. Taylor.

References

1 Taylor, J.W. *et al.* (2004) The Fungi. In *Assembling the Tree of Life* (Cracraft, J. and Donoghue, M.J., eds), pp. 171–194, Oxford University Press
2 Taylor, J.W. and Berbee, M.L. (2006) Dating divergences in the Fungal Tree of Life: review and new analyses. *Mycologia* 98, 838–849
3 Hawksworth, D.L. (1991) The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol. Res.* 95, 641–655
4 Schmidt, J.P. and Mueller, G.M. (2007) An estimate of the lower limits of global fungal diversity. *Biodiversity Conservation* 16, 99–111
5 Kirk, P.M. *et al.* (2008) *Dictionary of the Fungi*, (10th edn), CAB International
6 Moncalvo, J-M. *et al.* (2002) One hundred seventeen clades of euagarics. *Mol. Phylogen. Evol.* 23, 357–400
7 Schadt, C.W. *et al.* (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* 301, 1359–1361

8 Arnold, A.E. *et al.* (2007) Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. *Mycologia* 99, 185–206
9 Higgins, K.L. *et al.* (2007) Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. *Mol. Phylogen. Evol.* 42, 543–555
10 Arnold, A.E. *et al.* (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? *Syst. Biol.* 58, 283–297
11 O'Brien, H.E. *et al.* (2005) Fungal community analysis by large-scale sequencing of environmental samples. *Appl. Environ. Microbiol.* 71, 5544–5550
12 Arnold, A.E. and Lutzoni, F. (2007) Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* 88, 541–549
13 Arnold, A.E. *et al.* (2000) Are tropical fungal endophytes hyperdiverse? *Ecology Letters* 3, 267–274
14 Rodriguez, R.J. *et al.* (2009) Fungal endophytes: diversity and functional roles. *New Phytol.* 182, 314–330
15 Suh, S-O. *et al.* (2005) The beetle gut: a hyperdiverse source of novel yeasts. *Mycol. Res.* 109, 261–265
16 Porter, T.M. *et al.* (2008) Widespread occurrence and phylogenetic placement of a soil clone group adds a prominent new branch to the fungal tree of life. *Mol. Phylogen. Evol.* 46, 635–644
17 Letcher, P.M. *et al.* (2006) Ultrastructural and molecular phylogenetic delineation of a new order, the Rhizophydiales (Chytridiomycota). *Mycol. Res.* 110, 898–915
18 Hawksworth, D.L. (2004) Fungal diversity and its implications for genetic resource collections. *Studies Mycol.* 50, 9–18
19 Ainsworth, G.C. (1976) *Introduction to the History of Mycology*, Cambridge University Press
20 Sparrow, F.K. (1958) Interrelationships and phylogeny of the aquatic Phycomycetes. *Mycologia* 50, 797–813
21 White, M.W. *et al.* (2006) Phylogeny of the Zygomycota based on nuclear ribosomal sequence data. *Mycologia* 98, 872–874
22 Bartnicki-Garcia, S. (1970) Cell wall composition and other biochemical markers in fungal phylogeny. In *Phytochemical Phylogeny* (Harborne, J.B., ed.), pp. 81–103, Academic Press
23 Whitaker, R.H. (1969) New concepts of kingdoms of organisms. *Science* 163, 150–160
24 Baldauf, S.L. *et al.* (2004) The tree of life. In *Assembling the Tree of Life* (Cracraft, J. and Donoghue, M.J., eds), pp. 43–75, Oxford University Press
25 Keeling, P.J. *et al.* (2005) The tree of eukaryotes. *Trends Ecol. Evol.* 20, 670–676
26 James, T.Y. *et al.* (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* 443, 818–822
27 Heath, I.B. (1986) Nuclear division: a marker for protist phylogeny? *Progress Protistol.* 1, 115–162
28 Celio, G.J. *et al.* (2006) Assembling the fungal tree of life: Constructing the Structural and Biochemical Database. *Mycologia* 98, 850–859
29 Lutzoni, F. *et al.* (2004) Where are we in assembling the fungal tree of life, classifying the fungi, and understanding the evolution of their subcellular traits? *Am. J. Bot.* 91, 1446–1480
30 Rokas, A. *et al.* (2003) Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* 425, 798–804
31 Jeffroy, O. *et al.* (2006) Phylogenomics: the beginning of incongruence? *Trends Genet.* 22, 225–231
32 Kuramae, E.E. *et al.* (2006) Phylogenomics reveal a robust fungal tree of life. *FEMS Yeast Res.* 6, 1213–1220
33 Robbertse, B. *et al.* (2006) A phylogenomic analysis of the Ascomycota. *Fungal Genet. Biol.* 43, 715–725
34 Green, T.J. *et al.* (2007) Provenance semirings. *PODS* 2007, 31–40
35 Kauff, F. *et al.* (2007) WASABI: an automated sequence processing system for multi-gene phylogenies. *Syst. Biol.* 56, 523–531
36 Stamatakis, A. (2006) RaxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690
37 Jordan, G.E. and Piel, W.H. (2008) PhyloWidget: web-based visualization for the tree of life. *Bioinformatics* 24, 1641–1642
38 Hibbett, D.S. *et al.* (2005) Automated phylogenetic taxonomy: An example in the Homobasidiomycetes (mushroom-forming fungi). *Syst. Biol.* 54, 660–668
39 Blackwell, M. *et al.* (2006) Research coordination networks: a phylogeny for kingdom Fungi (Deep Hypha). *Mycologia* 98, 829–837
40 Hibbett, D.S. *et al.* (2007) A higher-level phylogenetic classification of the Fungi. *Mycol. Res.* 111, 509–547
41 James, T.Y. *et al.* (2006) A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). *Mycologia* 98, 860–871
42 Redecker, D. and Raab, P. (2006) Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* 98, 885–895
43 Aime, M.C. *et al.* (2006) An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. *Mycologia* 98, 896–905
44 Begerow, D. *et al.* (2006) A phylogenetic hypothesis of Ustilaginomycotina based on multiple gene analyses and morphological data. *Mycologia* 98, 906–916
45 Hibbett, D.H. (2006) A phylogenetic overview of the Agaricomycotina. *Mycologia* 98, 917–925
46 Larsson, K-H. *et al.* (2006) Hymenochaetales: a molecular phylogeny for the hymenochaetoid clade. *Mycologia* 98, 926–936
47 Moncalvo, J-M. *et al.* (2006) The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia* 98, 937–948
48 Hosaka, K. *et al.* (2006) Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders. *Mycologia* 98, 949–959
49 Miller, S.L. *et al.* (2006) Perspectives in the new Russulales. *Mycologia* 98, 960–970
50 Binder, M. and Hibbett, D.S. (2006) Molecular systematics and biological diversification of Boletales. *Mycologia* 98, 971–981
51 Matheny, P.B. *et al.* (2006) Major clades of Agaricales: a multilocus phylogenetic overview. *Mycologia* 98, 982–995
52 Sugiyama, J. *et al.* (2006) Early diverging Ascomycota: phylogenetic divergence and related evolutionary enigmas. *Mycologia* 98, 996–1005
53 Suh, S-O. *et al.* (2006) Phylogenetics of Saccharomycetales, the ascomycetous yeasts. *Mycologia* 98, 1006–1017
54 Spatafora, J.W. *et al.* (2006) A five-gene phylogeny of Pezizomycotina. *Mycologia* 98, 1018–1028
55 Hansen, K. and Pfister, D.H. (2006) Systematics of the Pezizomycetes – the operculate discomycetes. *Mycologia* 98, 1029–1040
56 Schoch, C.L. *et al.* (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98, 1041–1052
57 Geiser, D.M. *et al.* (2006) Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. *Mycologia* 98, 1053–1064
58 Wang, Z. *et al.* (2006) Toward a phylogenetic classification of the Leotiomycetes based on rDNA data. *Mycologia* 98, 1065–1075
59 Zhang, N. *et al.* (2006) An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia* 98, 1076–1087
60 Miadlikowska, J. *et al.* (2006) New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analysis of three ribosomal RNA- and two protein-coding genes. *Mycologia* 98, 1088–1103
61 Ustinova, I. *et al.* (2000) *Hyaloraphidium curvatum* is not a green alga, but a lower fungus; *Amoebidium parasiticum* is not a fungus, but a member of the DRIPs. *Protistology* 151, 253–262
62 Keeling, P.J. and Fast, N.M. (2002) Microsporidia: Biology and evolution of highly reduced intracellular parasites. *Ann. Rev. Microbiol.* 56, 93–116
63 Liu, Y.J. *et al.* (2006) Loss of the flagellum happened only once in the fungal lineage: phylogenetic structure of Kingdom *Fungi* inferred from RNA polymerase II subunit genes. *BMC Evol. Biol.* 6, 74
64 Lee, S.C. *et al.* (2008) Microsporidia evolved from ancestral sexual fungi. *Curr. Biol.* 18, 1675–1679
65 Galagan, J.E. *et al.* (2005) Genomics of the fungal kingdom: insights into eukaryotic biology. *Genome Res.* 15, 1620–1631
66 Eisen, J.A. and Fraser, C.M. (2003) Phylogenomics: intersection of evolution and genomics. *Science* 300, 1706–1707
67 Philippe, H. *et al.* (2005) Phylogenomics. *Annu. Rev. Ecol. Evol. Syst* 36, 541–562

68 Fitzpatrick, D.A. *et al.* (2006) A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. *BMC Evol. Biol.* 6, 99
69 Kuzniar, A. *et al.* (2008) The quest for orthologs: finding the corresponding gene across genomes. *Trends Genet.* 24, 539–551
70 Koonin, E.V. *et al.* (2004) A comprehensive evolutionary classification of proteins encoded in complete eukaryotic genomes. *Genome Biol.* 5, R7
71 Liu, Y. *et al.* (2009) Phylogenomic analyses support the monophyly of Taphrinomycotina, including Schizosaccharomyces fission yeasts. *Mol. Biol. Evol.* 26, 27–34
72 de Bary, A. (1887) *Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria*, Clarendon Press
73 Cavalier-Smith, T. (2001) What are Fungi? In *The Mycota VII: Systematics and Evolution* (Part A) (McLaughlin D.J. *et al.*, eds), pp. 1–37, Springer Verlag

OUTLINE MICOLOGIA

Drauzio Eduardo Naretto Rangel

FUNGOS – OS FILAMENTOS QUE MANTEM O ECOSSISTEMA

- When someone thing about fungi
  - Pizza
  - Mold food
  - Toes
- But in fact fungi are everywhere and affect our lives every day
- Plant helpers
- Pathogens
- Fungal biology to Control or Exploit for our own purposes
- Important roles in the ecosystem
- Associations live and dead – vital part of the links in the food web as a decomposers and pathogens.
- Scavengers on animal and plants
- Mutualists
  - 90% plants mycorhizae - P cicling
  - Cyanobacteria – nitrogen cyclin
- Detrimental -
  - Plant diseases - *Phytophthora infestans*, *Bipolaris oryzae*
  - Animal diseases – Blastomicose, Coccidiomycose, others
  - Decomposition – destroy almost every kind of manufactured good – except some plastics and some pesticides.
    - The example of textiles stains
- Mycotoxins – *Claviceps* (Ergot), *Aspergillus flavus*
- Mushroom poisons
- Alergy - *Tilletia controversa*
- Antibiotics – penicillin and cephalosporin - Cephalosporium
    - Alexander Fleming
      - There were many earlier workers who noted that there were antagonisms between microbes.
  - "I am going to tell you about the early days of penicillin, for this is the part of the penicillin story which earned me a Nobel Award. We were all taught about these inhibitions and indeed it is seldom that an observant clinical bacteriologist can pass a week without seeing in the course of his ordinary work very definite instances of bacterial antagonism
- Cyclosporin
- Heterologous gene products
  - Vaccine against hepatitis B
  - Interferon with antitumour activity
- Steroids, hormones and even birth control pills
- Edible mushrooms - cultivated and collected (festivais)
- Citric Acid
- Gourmet cheeses – Roquefort, Brie and Camembert
- Stone washed jeans are softened by Trichoderma species
- Experimental model organisms.
  - Important genetic tools "one gene one enzyme" in Neurospora (won Beadle and Tatum 1958 Nobel Prize)

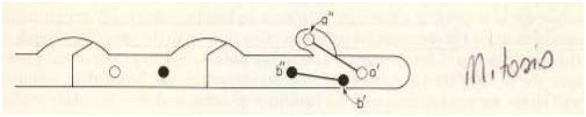
    - S. cerevisiae, the first eukaryote genome sequenced.
- Disciplines of applied mycology
    - Plant pathology
    - Human and animal pathology
    - Fermentation technology
    - Mushroom cultivation
- Fungi never fail to fascinate me
    - Entomophthora muscae
    - Pilobolus – 3 meters away.
    - Fungi are farmed by ants and termites
    - Fungi trap nematodes

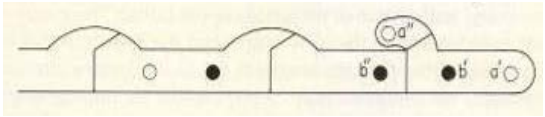
OUTLINE

- Introdução
    - Distribuição dos microorganismos na natureza
    - Importância dos fungos
- Características dos fungos
    - Estrutura geral: a hifa
    - Estrutura geral: leveduras
    - Septo
    - Componentes citoplasmáticos
    - Reprodução e Crescimento.
    - Morfologia
- Fisiologia de Fungos.
    - Nutrição, Enzimas, Respiração e Fermentação
    - Crescimento, Avaliação do Crescimento
    - Requerimento químico para o crescimento.
    - Aquisição de nutrientes por fungos, digestão e transporte.
    - O meio ambiente físico e o crescimento.
- Reprodução assexual e sexual
- Nova classificação dos fungos
    - Filo Microsporidia
    - Filo Chytridiomycota
    - Filo Neocallimastigomycota (Chytridiomycota)
    - Filo Blastocladiomycota (Chytridiomycota)
    - Filo Entomophthoromycota (Humber, 2012) (Zygomycota) (Subfilo Entomophthoromycotina).
    - Filo Glomeromycota (Zygomycota)
    - Subfilo Mucormycotina
    - Subfilo Zoopagomycotina
    - Subfilo Kickxellomycotina
    - Filo Basidiomycota
    - Filo Ascomycota
- Biologia de Fungos
    - Ciclo sexual dos principais filos dos fungos.
        - **Filo Chytridiomycota**
            - Encontrados florestas, solos agrícolas, rúmen de animais
            - Patógenos
            - Reprodução assexual – zoósporos que nascem em zoosporângio
            - Reprodução sexual – zooósporos realizam a conjugação por gametas similares mas diferente fisiologicamente.
                - Formação do zigoto

        - Zigoto germina e as hifas se fundem com as hifas de outro zigoto (somatogamia) precedendo a formação de esporos de repouso que dão origem a novos zoósporos.
- **Subfilo Mucormycotina**
    - Encontrados em vida livre e como patógenos importantes.
    - Reprodução sexual consiste na fusão de dois gametângios dando origem ao zigósporo (esporo de repouso com parede espessa) dentro de um zigosporângio. Reprodução induzida pelo ácido trispórico. Um feromônio sexual.
        - Reprodução pode ser homotálica ou heterotálica.
        - Características reprodução sexual
            - Reprodução heterotálica dois micélios se fundem
            - Progametângio
            - Gametângio
            - Zigoto
            - Zigosporângio
        - Características reprodução assexual
            - Germinação do zigósporo
            - Esporangióforo
            - Esporangio
            - Esporangiósporos
- **Filo Entomophthoromycota** (Humber, 2012) (Zygomycota) (Subfilo Entomophthoromycotina).
- **Filo Glomeromycota (Zygomycota)**
    - Endomicorrizos
    - Pouco se sabe sobre os ciclos sexuais/assexuais
- **Filo Ascomycota**
    - 2000 gêneros
    - Três subfilos:
        - Pezizomycotina
        - Saccharomycotina
        - Taphinomycotina
    - Predominantemente terrestre. vida livre, ectomicorrizas, liquens, patógenos importantes de plantas e animais, parasitas de nematóides.
    - Fase sexual tipicamente heterogâmica entre gametas morfologicamente diferentes.
        - Ascósporos dentro de ascos
        - Ascó podem nascem dentro de um ascocarpo – cleistotécio, apotécio, peritécio ou ascos nus.
        - Ascogônio (feminino) tem uma projeção Trichogine que se cresce e funde com o anterídio (masculino)
        - Após a plasmogamia o ascogônio produz hifa ascógena na condição dicariótica.
        - A hifa ascógena produz um Crosier onde ocorre inicialmente a divisão de núcleos pela mitose na condição dicariótica anterior a cariogamia. Posterior a cariogamia ocorre a meiose dividindo em 4 e 8 nucleos formando os ascósporos dentro do asco.
- **Filo Basidiomycota**
    - 34% dos fungos descritos
    - Três subfilos
        - Pucciniomycotina
        - Ustilaginomycotina

- Agaricomycotina

- Caracterizado por ter uma curta fase diplóide, ocorrendo no basídio somente, e uma prolongada fase dicariótica.
- Com exceção das classes Pucciniomycetes e Ustilaginomycetes, quase todos Basidiomycota tem virtualmente o mesmo ciclo de vida
- O basidiósporo contém um simples núcleo QUANDO GERMINA INICIA O MICELIO HAPLOIDE MONOCARIÓTICO
- A hifa monocariótica é inicialmente não septada mas depois fica dividido em células uninucleadas.
- Esta fase no ciclo de vida é geralmente curta
- Plasmogamia então ocorre. Orgaos sexuais não existem nos Basidiomycota (exceto em Puccinales) e plasmogamia ocorre pela fusão de duas hifas monocarióticas.
- Quando duas hifas monocarióticas se fundem, o núcleo de uma hifa entra na outra.
- A hifa agora tem núcleos de dois tipos genéticos e é dicariótico (N + N)
- O dicário formado após plasmogamia continua a proliferar e mantendo a condição binucleada dicariótica.
- Grampos de conexão são então formados para manter esta condição
- Os grampos de conexão jovens começam a formar uma projeção na parede da hifa entre os dois núcleos do terminal dicariótico da célula
- Ocorre a divisão mitótica de ambos os núcleos
- 

- Um septo transverso é iniciado e separa o novo terminal binucleado da célula. A célula remanescente atrás ainda contem um núcleo apenas e o outro núcleo ainda está isolado no grampo lateral
- 
- O grampo se recurva e entra em contato com a célula uninucleada e a parede celular é dissolvida com a migração do núcleo para a célula hifal, reconstituindo a condição dicariótica.
- O grampo continuará como uma parte permanente da hifa e é frequentemente usado para reconhecer o estado dicariótico da hifa.
- O basídio é inicialmente dicariótico e tem dois núcleos haploides
- Os núcleos se fundem formando um núcleo diploide CARIOGAMIA
- Meiose
- Formação de uma projeção chamada esterigma.
- Migração de núcleos haploides dentro dos basidiósporos
- Usualmente quatro basidiósporos uninucleados são formados em cada basídio

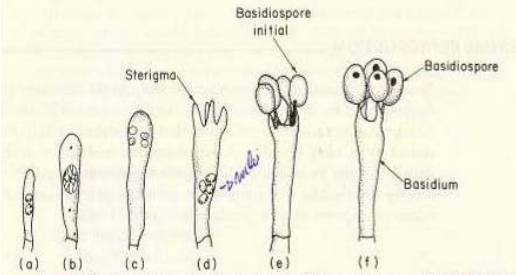
- Os eventos mais importantes que ocorrem no basídio são cariogamia e meiose
- 

Figure 6-5 Development of a typical basidium: (a) young dikaryotic basidium; (b) diploid basidium; (c) basidium with four nuclei resulting from meiosis; (d) basidium after development of sterigmata; (e) migration of nuclei into basidiospore initials; (f) mature basidium with basidiospores. [Adapted from A. H. Smith, 1934, Mycologia 26:305–331.]

PLANO DE AULA

Professor: Prof. Dr. Drauzio Eduardo Naretto Rangel
Duração: 50 minutos

Tópico: 003 MICOLOGIA

Objetivos gerais: Conhecer as estruturas celulares de fungos filamentosos e leveduras. Entender o processo de crescimento celular. Distinguir os membros dos filos do Reino Fungi e conhecer a nova classificação filogenética.

Conteúdo Programático:

- Introdução
  - Distribuição dos microorganismos na natureza
  - Importância dos fungos
- Características dos fungos
  - Estrutura geral: a hifa
  - Estrutura geral: leveduras
  - Septo
  - Componentes citoplasmáticos
  - Reprodução e Crescimento.
  - Morfologia
- Fisiologia de Fungos.
  - Nutrição, Enzimas, Respiração e Fermentação
  - Crescimento, Avaliação do Crescimento
  - Requerimento químico para o crescimento.
  - Aquisição de nutrientes por fungos, digestão e transporte.
  - O meio ambiente físico e o crescimento.
- Reprodução assexual e sexual
- Nova classificação dos fungos
  - Filo Microsporidia
  - Filo Chytridiomycota
  - Filo Neocallimastigomycota (Chytridiomycota)
  - Filo Blastocladiomycota (Chytridiomycota)
  - Filo Entomophthoromycota (Humber, 2012) (Zygomycota) (Subfilo Entomophthoromycotina).
  - Filo Glomeromycota (Zygomycota)
  - Subfilo Mucormycotina
  - Subfilo Zoopagomycotina
  - Subfilo Kickxellomycotina
  - Filo Basidiomycota
  - Filo Ascomycota
- Biologia de Fungos
  - Ciclo sexual dos principais filos dos fungos.

Conhecimentos prévios necessários:
Noções de microbiologia geral

Recursos didáticos – materiais e equipamentos – necessários:
- Data-show;
- Lousa e caneta;
- Material para atividade extraclasse: livros didáticos e artigos.

Metodologia de Ensino - Estratégias
- Aula expositiva
- Discussão em grupo

Avaliação:
- Provas

- Seminários

Bibliografia

Alexopoulos, C.J., Mims, C.W., Blackwell, M., 1996. Introdutory Mycology. John Wiley & Sons, New York.
Carlile, M.J., Watkinson, S.C., Gooday, G.W., 2001. The Fungi. Academic Press, London.
Deacon, J.W., 1997. Modern Mycology. Blackwell Science Ltd., Oxford.
Madigan, M.R., Martinko, J.M., 2006. Brock Biology of Microorganisms. Pearson Prentice Hall, Upper Saddle River.
Moore-Landecker, E., 1996. Fundamentals of the Fungi. Prentice-Hall Inc., Upper Saddle River.

Bibliografia Complementar

Gryganskyi AP et al. (2012) Molecular phylogeny of the Entomophthoromycota. Mol Phylogenet Evol 65:682-694
Hawksworth DL (2011) A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. IMA Fungus 2:155-162
Hawksworth DL et al. (2011) The amsterdam declaration on fungal nomenclature. IMA Fungus 2:105-112
Hibbett DS et al. (2007) A higher-level phylogenetic classification of the Fungi. Mycological Research 111:509-547
Hibbett DS, Taylor JW (2013) Fungal systematics: is a new age of enlightenment at hand? Nature Reviews Microbiology 11:129-133
Humber RA (2012) Entomophthoromycota: a new phylum and reclassification for entomophthoroid fungi. Mycotaxon 120:477-492
McLaughlin DJ, Hibbett DS, Lutzoni F, Spatafora JW, Vilgalys R (2009) The search for the fungal tree of life. Trends in Microbiology 17:488-497
Stajich JE et al. (2009) The Fungi. Current Biology 19:R840-R845
Taylor JW (2011) One Fungus = One Name: DNA and fungal nomenclature twenty years after PCR. IMA Fungus 2:113-120

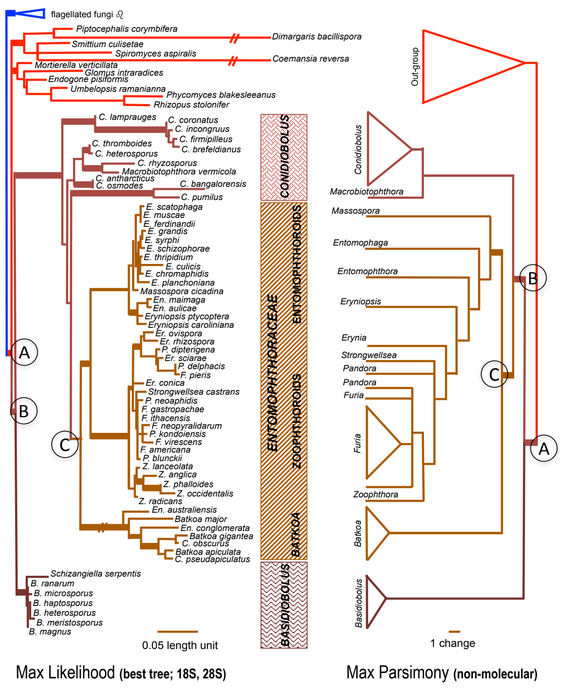
_____

Professor Drauzio Eduardo Naretto Rangel

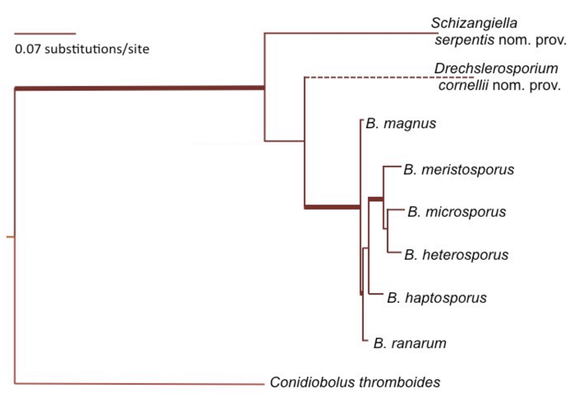
Phylogenetic reclassification raises new respect–and a new phylum!– for *Entomophthorales*

Richard A. Humber richard.humber@ars.usda.gov
USDA-ARS Biological Integrated Pest Management
RW Holley Center for Agriculture & Health
538 Tower Road, Ithaca, NY 14853, USA

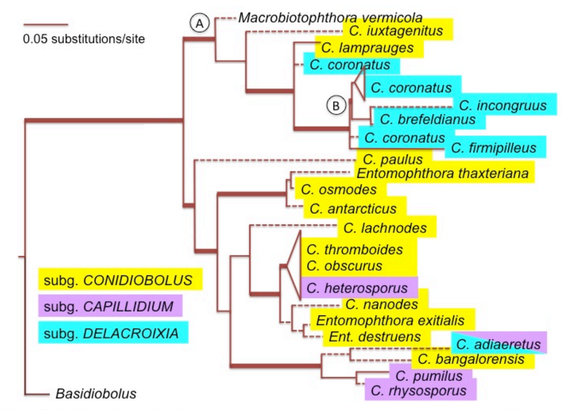
Andrii P. Gryganskyi apg10@duke.edu
Rytas Vilgalys fungi@duke.edu
Dept. of Biology, Duke University
Durham, NC 27708, USA



THE MONOPHYLETIC PHYLUM ENTOMOPHTHOROMYCOTA. Comparison of trees from molecular (L) and morphological (R) character data. Thick branches are statistically supported. Major nodes show separation of entomophthoroid fungi (A) from all other fungi; *Basidiobolomycetes* (B) are basal to all other fungi in the phylum, and separation of the exclusively entomopathogenic *Entomophthoraceae* (C) from mostly saprobic taxa in *Ancylistaceae*. *B=Basidiobolus*, *C=Conidiobolus*, *E=Entomophthora*, *En=Entomophaga*, *Er=Erynia*, *F=Furia*, *P=Pandora*, *Z=Zoophthora*. (Gryganskyi et al. 2012a)



***BASIDIOBOLUS* LINEAGE** (*Basidiobolomycetes*) ML analysis with LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. All taxa are united by cells with one large nucleus with a prominent central nucleolus and distinctive mitotic mechanism. (Gryganskyi et al. 2012b)



***CONIDIOBOLUS* LINEAGE** (*Ancylistaceae*) ML analysis with LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. *Conidiobolus* is paraphyletic, and its current subgeneric taxonomy is not supported. *Macrobiotophthora* (A) is basal to *Conidiobolus*; *C. adiaeretus* produces all three types of secondary conidia and indicates microconidiogenesis may be a paraphyletic character. (Gryganskyi et al. 2012b)

ABSTRACT: The recent phylogenetic studies and reclassifications produced by the global All-Fungal Tree of Life study recognized the *Entomophthorales* (as historically treated, with *Basidiobolus* remaining in this order) as a new subphylum, *Entomophthoromycotina*, that was not placed in any phylum. Subsequent phylogenetic analyses of the broadest range of entomophthoroid taxa and more genes than in any previous studies confirmed the monophyletic nature of these fungi and their distinctness from all other groups formerly classified in the phylum *Zygomycota*. As a lead-in to the publications of these molecular and traditional taxonomic analyses, the subphylum is now formally raised to phylum level as the *Entomophthoromycota*, and its included fungi are distributed among the classes *Basidiobolomycetes*, *Neozygitomycetes*, and *Entomophthoromycetes*; the genera *Ballocephala* and *Zygnemomyces* were removed from the family *Meristacraceae* (*Entomophthorales*) and reassigned to the subphylum *Kickxellomycotina*.

The systematics of entomophthoralean fungi has changed dramatically since the time when nearly all entomopathogens in this group (except, notably, for the cicada-pathogenic species of *Massospora*—were treated as *Entomophthora* species. The pace of taxonomic improvements heated up dramatically in the mid-1960's when the Batkoan classification offered so many radical changes from the existing taxonomy of these fungi that his scheme was effectively ignored in print by all other students of these fungi, and barely even mentioned in conversations despite the recognition that the Batkoan classification included many good decisions as well as some that were clearly wrong. Batko's classification was ignored—neither praised, condemned, nor corrected—until entomophthoralean taxonomy boiled over in the 1980's with the publication of several competing large-scale attempts to reclassify these fungi (by Remaudière and colleagues in Europe, by Humber and, separately, Tucker in the US, and by Ben-Ze'ev and Kenneth in Israel) that spawned serious disagreements in print, and also some loud ones in person. After the dust settled and tempers cooled, a six-family classification with a number of new and modified genera (Humber 1989) gained nearly universal acceptance.

The Entomophthorales entered the era of phylogenetic systematics with an immediate challenge to the integrity of the order thanks to single-gene analyses that suggested the *Basidiobolus* was more closely related to chytrid fungi than to the *Entomophthorales* (Nagahama et al 1995). The addition of more genes to the analyses, particularly under the global All-Fungal Tree of Life project, led James et al. (2006) to suggest that *Basidiobolus* was, indeed, a member of *Entomophthorales*. Molecular studies on these fungi were uniformly based until this year on limited numbers of genes and on very limited samplings of taxa from among entomophthoroid fungi. New analyses (Gryganskyi et al. 2012a,b) using multiple genes and an unprecedented number of entomophthoralean taxa unambiguously confirm several key points about the systematics of these fungi:

- The Entomophthorales as traditionally classified and as recognized by Hibbett et al. (2007) as the subphylum *Entomophthoromycotina* is a monophyletic group distinct from all other fungi.
- This confirmation justifies their treatment as a new phylum and the newly adjusted classification (Humber 2012) at the right.
- No cultures of other material was available for *Completoriaceae* or *Meristacraceae*. Few data were available for *Neozygitaceae* but their pertinent sequences, while difficult to obtain from the few cultured taxa, clearly exclude these fungi from the three well studied families.
- *Basidiobolus* (and all *Basidiobolaceae*) occupy a basal position in the phylum. *Conidiobolus* (the major genus of *Ancylistaceae*) proved to be paraphyletic and needs a new gene-based classification to replace its current, unsupported subgenera; *Ancylistaceae* is basal to the large, complex, and wholly entomopathogenic family *Entomophthoraceae*.
- Within the *Entomophthoraceae*, a separate subfamily for *Massospora* subfamily (Keller & Petrini 2005) is not supported. *Batkoa* may (or may not) represent a new subfamiliy. The currently recognized generic limits among zoophthoroid genera (subfamily *Erynioideae*) are proven to need more intensive study and, except for *Zoophthora*, are unsupported as currently recognized.

REFERENCES

Gryganskyi AP, Humber RA, Smith ME, Miadlikovska J, Wu S, Voigt K, Walter G, Anischenko IM, Vilgalys R. 2012. Molecular phylogeny of *Entomophthoromycota*. *Mol. Phylog. Evol.*: in press.

Gryganskyi AP, Humber RA, Smith ME, Hodge K, Huang B, Voigt K, Vilgalys R. 2012. Phylogenetic lineages in *Entomophthoromycota*. *Persoonia*: in press.

Hibbett DS et al. (65 co-authors) 2007. A high-level phylogenetic classification of the *Fungi*. *Mycol. Res.* 111: 509-547.

Humber RA. 1989. Synopsis of a revised classification for the *Entomophthorales* (*Zygomycotina*). *Mycotaxon* 34: 441-460.

Humber RA. 2012. *Entomophthoromycota*: a new phylum and reclassification for entomophthoroid fungi. *Mycotaxon 120*: in press.

James et al. (69 co-authors) 2006. Reconstructing the early evolution of the fungi using a six gene phylogeny. *Nature* 4443: 818-822.

Keller S,Petrini O. 2005. Keys to the identification of the arthropod pathogenic genera of the families *Entomophthoraceae* and *Neozygitaceae* (*Zygomycetes*), with descriptions of three new subfmilies and a new genus. *Sydowia* 57: 23-53.

Saikawa M. 1989. Ultrastructure of the septum in *Ballocephala verrucospora* (*Entomophthorales*, *Zygomycetes*). *Can. J. Bot.* 67: 2484–2488.

Saikawa M, Oguchi M, Castañeda Ruiz RF. 1997. Electron microscopy of two nematode-destroying fungi, *Meristacrum asterospermum* and *Zygnemomyces echinulatus* (*Meristacraceae*, *Entomophthorales*). *Can. J. Bot.* 75: 762–768.

Nagahama T, Sato H, Shimazu M, Sugiyama J, 1995. Phylogenetic divergence of the entomophthoralean fungi: evidence from nuclear 18S ribosomal RNA gene sequences. *Mycologia* 87: 203-209.

PHYLUM *Entomophthoromycota* Humber, phyl. nov.

CLASS *Basidiobolomycetes* Humber, cl. nov.

ORDER *Basidiobolales* Cavalier-Smith

FAMILY *Basidiobolaceae* Claussen

Basidiobolus Eidam
Schizangiella Humber, B. Huang & Hodge (unpubl. new genus)
Drechslerosporium B. Huang, Humber & Hodge (unpubl. new genus)

CLASS *Neozygitomycetes* Humber, cl. nov.

ORDER *Neozygitales* Humber, ord. nov.

FAMILY *Neozygitaceae* Ben-Ze'ev, R.G. Kenneth & Uziel

Apterivorax S. Keller
Neozygites Witlaczil
Thaxterosporium Ben-Ze'ev & R.G. Kenneth

CLASS *Entomophthoromycetes* Humber, cl. nov.

ORDER *Entomophthorales* G. Winter

FAMILY *Ancylistaceae* J. Schröter

Ancylistes Pfitzer
Conidiobolus Brefeld
Macrobiotophthora Reukauf

FAMILY *Completoriaceae* Humber

Completoria Lohde

FAMILY *Entomophthoraceae* Nowakowski

SUBFAMILY *Entomophthoroideae* S. Keller

Batkoa Humber
Entomophaga A. Batko
Entomophthora Fresenius
Eryniopsis Humber (in part; see subfam. *Erynioideae*)
Massospora Peck

SUBFAMILY *Erynioideae* S. Keller

Erynia (Nowakowski ex A. Batko) Remaudière & Hennebert
Eryniopsis Humber (in part; see subfam. *Entomophthoroideae*)
Furia (Batko) Humber
Orthomyces Steinkraus, Humber & J.B. Oliver
Pandora Humber
Strongwellsea A. Batko & Weiser
Zoophthora A. Batko

FAMILY *Meristacraceae* Humber

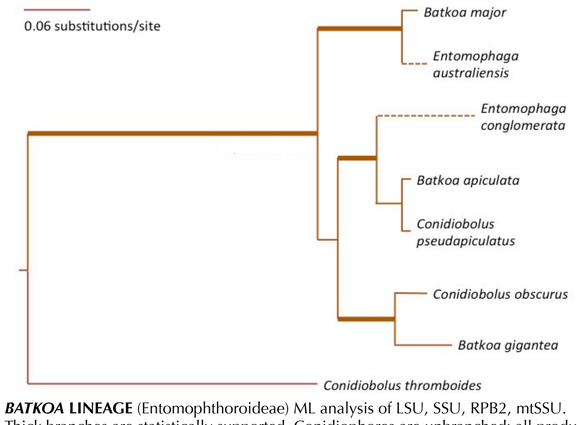
Meristacrum Drechsler
Tabanomyces Couch, RV Andrejeva, Laird & Nolan

Taxa with uncertain status, not accepted, or excluded from *Entomophthoromycota*:

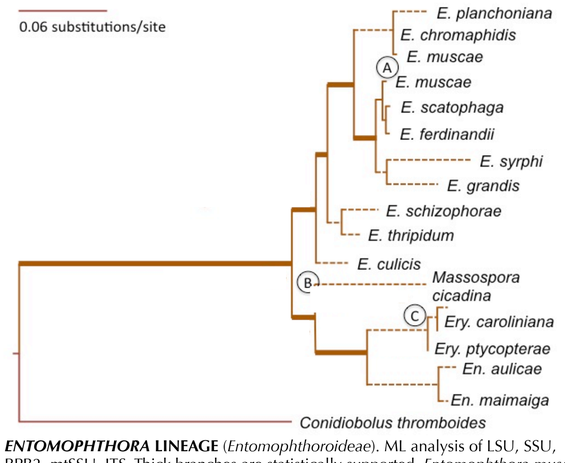
Subfamily *Massosporoideae* Keller – Not accepted; without molecular support
Tarichium Cohn – A form-genus; mixture of species of *Entomophthoraceae* and *Neozygitaceae*
Eryniopsis Humber – Heterogeneous; species probably belong in separate subfamilies
Ballocephala Drechsler } { Moved from *Meristacraceae* to subphylum *Kickxellomycotina*
Zygnemomyces Miura } { due to septal ultrastructure (Saikawa 1989; Saikawa et al. 1997)

The earliest phylogenetic studies including entomophthoroid taxa suggested that they were not homogeneous. The much more extensive sampling and use of more genes here than in previous analyses confirm that the classically defined Entomophthorales is both monophyletic and distinct from all other zygomycetes. This justified raising the subphylum to phylum rank. There is an acute need for a kaleidoscopic survey and phylogenetic review of *Conidiobolus*. The existing subgeneric scheme for this genus is not supported, and we dare not guess about the number or circumscriptions of genera that will result from its reclassification.

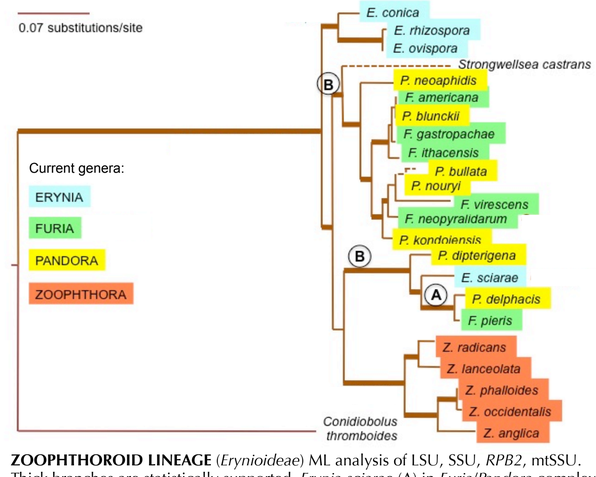
The *Entomophthoraceae* is the largest and most taxonomically complex family of this phylum. More data (and better underlying identifications of a number of strains) will be needed to determine the extent to which the *Batkoa* lineage may be separate from the rest of the subfamily *Entomophthoroideae*. More species and isolates of several genera must be studied (especially specis of *Eryniopsis*). The genera *Erynioideae* represent the most difficult problem using the current molecular data. Zoophthora is clearly supported as a separate genus, it is possible that Erynia might be supported, and Pandora and Furia may need to be combined; only the use of more collections and more genes will be able to resolve these questions.



***BATKOA* LINEAGE** (Entomophthoroideae) ML analysis of LSU, SSU, RPB2, mtSSU. Thick branches are statistically supported. Conidiophores are unbranched; all produce secondary conidia; no cystidia are formed. Mixed identifications of taxa indicate strains identified before description of *Batkoa*. (Gryganskyi et al. 2012b)



***ENTOMOPHTHORA* LINEAGE** (*Entomophthoroideae*). ML analysis of LSU, SSU, RPB2, mtSSU, ITS. Thick branches are statistically supported. *Entomophthora muscae* complex (A) is paraphyletic; *Massospora* (B) belongs in *Entomophthoroideae*; status of *Eryniopsis* (C) needs re-examination. (Gryganskyi et al. 2012b)



ZOOPHTHOROID LINEAGE (*Erynioideae*) ML analysis of LSU, SSU, *RPB2*, mtSSU. Thick branches are statistically supported. *Erynia sciarae* (A) in *Furia/Pandora* complex may reflect misidentification; *Furia* and *Pandora* (B) do not have molecular support; *Zoophthora* does seem to have good molecular support . (Gryganskyi et al. 2012b)

Mycologia, 108(5), 2016, pp. 1028–1046. DOI: 10.3852/16-042

A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data

Joseph W. Spatafora[1]
Ying Chang
Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331

Gerald L. Benny
Katy Lazarus
Matthew E. Smith
Department of Plant Pathology, University of Florida, Gainesville, Florida 32611

Mary L. Berbee
Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada

Gregory Bonito
Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, Michigan 48824

Nicolas Corradi
Department of Biology, University of Ottawa, Ottawa, Ontario, K1N 6N5 Canada

Igor Grigoriev
US Department of Energy (DOE) Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, California 94598

Andrii Gryganskyi
L.F. Lambert Spawn Co., Coatesville, Pennsylvania 19320

Timothy Y. James
Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan 48103

Kerry O'Donnell
Mycotoxin Prevention and Applied Microbiology Research Unit, NCAUR-ARS-USDA, 1815 N. University Street, Peoria, Illinois 61604

Robert W. Roberson
School of Life Sciences, Arizona State University, Tempe, Arizona 85287

Thomas N. Taylor[2]
Department of Ecology and Evolutionary Biology, and Natural History Museum and Biodiversity Research Center, University of Kansas, Lawrence, Kansas 66045

Jessie Uehling
Rytas Vilgalys
Biology Department, Box 90338, Duke University, Durham, North Carolina 27708

Merlin M. White
Department of Biological Sciences, Boise State University, Boise, Idaho 83725

Jason E. Stajich
Department of Plant Pathology & Microbiology and Institute for Integrative Genome Biology, University of California–Riverside, Riverside, California 92521

Abstract: Zygomycete fungi were classified as a single phylum, Zygomycota, based on sexual reproduction by zygospores, frequent asexual reproduction by sporangia, absence of multicellular sporocarps, and production of coenocytic hyphae, all with some exceptions. Molecular phylogenies based on one or a few genes did not support the monophyly of the phylum, however, and the phylum was subsequently abandoned. Here we present phylogenetic analyses of a genome-scale data set for 46 taxa, including 25 zygomycetes and 192 proteins, and we demonstrate that zygomycetes comprise two major clades that form a paraphyletic grade. A formal phylogenetic classification is proposed herein and includes two phyla, six subphyla, four classes and 16 orders. On the basis of these results, the phyla Mucoromycota and Zoopagomycota are circumscribed. Zoopagomycota comprises Entomophtoromycotina, Kickxellomycotina and Zoopagomycotina; it constitutes the earliest diverging lineage of zygomycetes and contains species that are primarily parasites and pathogens of small animals (e.g. amoeba, insects, etc.) and other fungi, i.e. mycoparasites. Mucoromycota comprises Glomeromycotina, Mortierellomycotina, and Mucoromycotina and is sister to Dikarya. It is the more derived clade of zygomycetes and mainly consists of mycorrhizal fungi, root endophytes, and decomposers of plant material. Evolution of trophic modes, morphology, and analysis of genome-scale data are discussed.

Key words: Entomophthoromycotina, fungi, Glomeromycotina, Kickellomycotina, Mortierellomycotina, Mucoromycota, Mucoromycotina, paraphyly, systematics, Zoopagomycota Zoopagomycotina

Introduction

Despite advances in our understanding of evolutionary relationships within Kingdom Fungi, the earliest diverging events are still poorly understood. Included among these unresolved events are the evolutionary transitions that ultimately culminated in modern diversity and in the emergence of terrestrial fungi, including subkingdom Dikarya, which comprises the phyla Ascomycota and Basidiomycota. Resolving the earliest branches in the fungal genealogy is essential to identify characteristics

Submitted 23 Feb 2016; accepted for publication 4 Jul 2016.
[1] Corresponding author. E-mail: spatafoj@oregonstate.edu
[2] Deceased 28 Apr 2016.

of the ancestral fungi, to determine what traits emerged with the dawn of terrestrial ecosystems, and to obtain an accurate assessment of the morphological and genetic homologies associated with fungal lifestyles. Central to this transition are the fungi that were once classified in the phylum Zygomycota Moreau (1954). However, because the monophyly of Zygomycota was not supported in recent phylogenetic analyses (e.g. James et al. 2006, Liu et al. 2009, Chang et al. 2015), these fungi are informally referred to herein as zygomycetes.

Zygomycetes are filamentous, nonflagellated fungi that mark the major transition away from the earliest diverging zoosporic fungi in Cryptomycota, Chytridiomycota, and Blastocladiomycota toward the rise of the nonflagellated, filamentous, multicellular Dikarya. The zygomycetes include: (i) *Phycomyces blakesleeanus* and other important model organisms; (ii) species such as *Rhizopus stolonifer* that cause economically significant pre- and postharvest diseases of fruits; (iii) members of Glomeromycota that colonize roots and form endomycorrhizal symbioses with more than 80% of land plants; and (iv) diverse and important pathogens or commensals of insects, nematodes, and other soil invertebrates (Benny et al. 2014, Redecker and Schüßler 2014). Some zygomycetes significantly benefit humans by the production of compounds such as lycopene, fatty acids, and biodiesel, but they can also cause rare and deadly human diseases such as zygomycosis (Papanikolaou and Panayotou 2007, Wang et al. 2011, Doggett and Wong 2014).

Abandonment of the phylum Zygomycota was formalized in Hibbett et al. (2007), which treated zygomycete fungi as four subphyla incertae sedis, including Entomophthoromycotina, Kickellomycotina, Mucoromycotina, and Zoopagomycotina and the phylum Glomeromycota. *Mortierella* was classified with the morphologically similar Mucorales until multigene analyses demonstrated that it was phylogenetically distinct from Mucoromycotina, resulting in the description of the subphylum Mortierollomycotina (Hoffmann et al. 2011). Results from rDNA and multigene molecular phylogenetic studies resolved these zygomycete taxa into two larger groups. One of the groups, informally known as "zygomycetes I", includes Mucoromycotina and Mortierellomycotina and in some studies, Glomeromycota (James et al. 2006, White et al. 2006, Chang et al. 2015). Mucoromycotina includes *Mucor*, *Rhizopus*, and the majority of the most common and best known zygomycetes. Many of these are fast growing, early colonizers of carbon-rich substrates, with several species used in industry for organic acid production and fermentation (Jennessen et al. 2008). Mortierellomycotina are common soil fungi that occur as root endophytes of woody plants and also are commonly isolated as saprobes (Summerbell 2005). Glomeromycota includes the arbuscular mycorrhizal fungi, which arguably comprise the most successful plant-fungal symbiosis on Earth. Glomeromycota has been a phylogenetic enigma because it lacks any known form of sexual reproduction. Morphological hypotheses placed Glomeromycota among the zygomycetes (Gerdemann and Trappe 1974, Morton and Benny 1990), whereas rDNA-based phylogenies placed this phylum as sister to Dikarya (Schüßler et al. 2001). Mitochondrial phylogenies (Nadimi et al. 2012, Pelin et al. 2012) placed Glomeromycota as sister to Mortierellomycotina, which is supported by some but not all genome-scale phylogenies (Tisserant et al. 2013, Chang et al. 2015).

The second of the larger groups, "zygomycetes II", includes Entomophthoromycota, Kickxellomycotina, and Zoopagomycotina (James et al. 2006, White et al. 2006, Sekimoto et al. 2011, Ebersberger et al. 2012, Chang et al. 2015). Zygomycetes II is more difficult of the two groups to study. In phylogenetic analyses, it has been weakly supported (James et al. 2006, Sekimoto et al. 2011) or strongly supported but based only on a couple of taxa (Chang et al. 2015). Entomophthoromycotina, the "insect destroyers", includes parasites of insects and mites, commensals of reptiles and amphibians, and poorly known parasites of desmid algae. Kickxellomycotina comprises a diverse assemblage of fungi associated with the hindgut of arthropods, saprobic species with broad substrate ranges and mycoparasites. Zoopagomycotina are either obligate mycoparasites or pathogens of invertebrates, including nematodes, rotifers, and amoebae. Members of the zygomycetes II group are almost exclusively characterized by associations with animals and fungi with essentially no associations with living plants, either as pathogens or symbionts (Benny et al. 2014).

Although the applications of multigene analysis has resulted in limited phylogenetic resolution of zygomycetes in kingdom-level analyses, they have led to significant refinement of evolutionary hypotheses for selected groups of zygomycetes, based on a combination of molecular and morphological data. These include a family-level phylogenetic classification of Mucorales (Hoffmann et al. 2013), testing of ordinal-level phylogenetic and taxonomic hypotheses for Kickxellomycotina (Tretter et al. 2014) and characterization of the major clades of Entomophthoromycota and temporal estimates of their origin in the geologic record (Gryganskyi et al. 2012). However, unlike Dikarya for which genome data and phylogenomic analyses have transformed our understanding of phylogenetic relationships and evolutionary processes (e.g. Floudas et al. 2012, Nagy et al. 2014, Kohler et al. 2015), genome data for zygomycetes have been sparse with respect to phylogenetic depth and breadth (Gryganskyi and Muszewska 2014). These gaps in

our knowledge of zygomycete evolution have manifested in a poor understanding of the homology of numerous life history traits essential to Fungi. These include characters associated with genomic, metabolic, reproductive, morphological, biochemical, and ecological traits. We attribute the limited amount of environmental data on zygomycetes to their molecular divergence, limited amplicon-based barcoding success, and paucity of well-annotated zygomycete reference data. For example, Zoopagomycotina comprises 19 genera and 228 described species worldwide, but this subphylum is only represented in GenBank by 125 DNA sequences for 17 species, 12 unnamed isolates, and seven environmental samples (NCBI nucleotide database accessed 21 Jan 2016).

Understanding zygomycete relationships from subphyla to species will provide long-awaited insight into transitions in form and function that changed as fungi colonized land, became multicellular, evolved true filamentous growth, and established intimate associations with other clades of life. A robust phylogenetic classification of zygomycetes will improve communication among biologists, ending the current use of confusing alternative names for poorly defined taxa. Here we leverage a phylogenomic approach with kingdom-wide sampling of species and genome-scale sampling of loci to resolve phylum-level relationships and propose a phylogenetic classification of the zygomycetes.

MATERIALS AND METHODS

Taxon and genome sampling.—Assembled and annotated genomes of 46 fungi were obtained from GenBank and Joint Genome Institute as part of the 1000 Fungal Genomes Project (http://1000.fungalgenomes.org) and published datasets (TABLE I). Genomes from 25 of the fungi represented all zygomycete phyla and subphyla including Mucoromycotina (12), Mortierellomycotina (2), Glomeromycota (1), Entomophthoromycotina (5), Kickellomycotina (4), and Zoopagomycotina (1). The Entomophthoromycotina fungus *Pandora formica* was included, but the accession is an assembled RNASeq of *P. formica*-infected ant and thus represents a metagenomic sample and the Zoopagomycotina fungus *Piptocephalis cylindrospora* was sequenced using a single-cell sequencing approach. Additional early diverging fungi included species from Chytridiomycota (6), Blastocladiomycota (2), and Cryptomycota (1). Five Ascomycota and four Basidiomycota genomes represented all major subphyla of the subkingdom Dikarya. Three outgroup species were included from the Metazoa, Choanozoa, and Ichthyosporea.

Phylogenetic analyses.—Phylogenetically informative proteins (markers) from the James et al. (2013) study of the placement of Cryptomycota and early branching fungi were used to analyze relationships. These conserved proteins were identified by comparing a pan-Eukaryotic set of species from plants, Metazoa, and Fungi. In total, 192 clusters of orthologous proteins (COPs) were aligned across the 39 eukaryotic species sampled in James et al. (2013) and built into Profile Hidden Markov Models (HMM) with TCOFFEE (Notredame et al. 2000) and HMMER3 (Eddy 2011). Each HMM was then searched against the predicted proteome from the 46 sampled species in this study with HMMSEARCH. For each marker, the highest scoring protein sequence in each species was selected by applying a significance cutoff of 1e-10 and binned to compose a file of fungal COPs for that marker. Alignments of sequences orthologous to their marker HMM were generated with HMMALIGN. The alignments were trimmed with TRIMAL (Capella-Gutiérrez et al. 2009) using the -strictplus parameter. The alignments were concatenated into a single super matrix alignment and analyzed using RAXML (Stamatakis 2006) with the '-f a' fast bootstrapped tree method and 100 bootstrap replicates (FIG. 1). The PROTGAMMAAUTO option was used to determine the best model of amino acid substitution across the following models with and without empirical base frequencies: DAYHOFF, DCMUT, JTT, MTREV, WAG, RTREV, CPREV, BT, BLOSUM62, MTMAM, LG, MTART, MTZOA, PMB, HIVB, HIVW, JTTDCMUT, FLU, DUMMY, and DUMMY2. As an alternative test of the organismal tree inferred from the concatenated analysis and as a measure of potential conflict among individual sequences, a protein sequence phylogeny for each COP was inferred with RAXML using the same aforementioned parameters. The maximum likelihood tree and 100 bootstrapped trees generated by RAxML for each of the 192 individual COPs were analyzed in ASTRAL (Mirarab et al. 2014) to construct a greedy consensus tree under default settings (FIG. 2). Branch support was calculated as the percentage of bootstrap replicates that contain a particular branch. The concatenated alignment and the RAxML and ASTRAL tree files are available at TreeBASE (accession No. TB2: S18957). The individual alignments, tree files, and associated scripts are available at http://zygolife.org/home/data/.

RESULTS

The final concatenated alignment comprised 60 382 amino acid positions after trimming. Individual protein alignments ranged from 57 to 1048 positions resulting in an average alignment length of 312 positions. LG with fixed base frequencies was chosen as the best model of amino acid substitution. The inferred phylogeny from the concatenated alignment supported two clades of zygomycetes (FIG. 1). The earliest diverging lineage, which we recognize below as Zoopagomycota, comprised Entomophthoromycotina, Kickxellomycotina, and Zoopagomycotina and was recovered with 100% BP support. Despite the potential for conflict due to the mixed nature of the *Pandora formica* metagenomic sample and the single cell genome data from *Piptocephalis*, strong support was recovered for their phylum-level phylogenetic placement (FIG. 1). Entomophthoromycotina and Kickxellomycotina were supported by 89% BP and 100% BP, respectively. The clade of zygomycetes including Mucoromycotina, Mortierellomycotina, and Glomeromycota, which we recognize below as Mucoromycota, was supported by 100%

TABLE I. List of taxa and genome data sources

Species	GenBank accession No./JGI Web Portal/(reference)
Allomyces macrogynus ATCC 38327 v3	ACDU00000000.1
Arthrobotrys oligospora ATCC 24927	ADOT00000000 (Yang et al. 2011)
Backusella circina FSU 941	http://genome.jgi.doe.gov/Bacci1
Batrachochytrium dendrobatidis JAM81	ADAR00000000.1
Basidiobolus heterosporus B8920 v1	JNEO00000000.1
Basidiobolus meristosporus CBS 931.73	http://genome.jgi.doe.gov/Basme2finSC
Capsaspora owczarzaki ATCC 30864 v2	ACFS00000000.2 (Suga et al. 2013)
Catenaria anguillulae PL171	http://genome.jgi.doe.gov/Catan1
Coemansia reversa NRRL 1564	JZJC00000000 (Chang et al. 2015)
Conidiobolus coronatus NRRL 28638	JXYT00000000 (Chang et al. 2015)
Conidiobolus thromboides FSU 785	http://genome.jgi.doe.gov/Conth1
Coprinopsis cinerea Okayama7_130	AACS00000000.2 (Stajich et al. 2010)
Cryptococcus neoformans JEC21	GCA_000149245.3 (Loftus et al. 2005)
Drosophila melanogaster vr6.04	http://flybase.org (Adams et al. 2000)
Gonapodya prolifera JEL478	LSZK00000000 (Chang et al. 2015)
Hesseltinella vesiculosa NRRL 3301	http://genome.jgi.doe.gov/Hesve2finisherSC
Homoloaphlyctis polyrhiza JEL142 v1	AFSM01000000.1 (Joneson et al. 2011)
Lichtheimia corymbifera FSU 9682	CBTN000000000.1
Lichtheimia hyalospora FSU 10163	http://genome.jgi.doe.gov/Lichy1
Linderina pennispora ATCC 12442	http://genome.jgi.doe.gov/Linpe1
Martensiomyces pterosporus CBS 209.56	http://genome.jgi.doe.gov/Marpt1
Monosiga brevicolis MX1 v1	ABFJ00000000.1 (King et al. 2008)
Mortierella elongata AG-77	http://genome.jgi.doe.gov/Morel2
Mortierella verticillata NRRL 6337	AEVJ00000000.1
Mucor circinelloides CBS277.49	http://genome.jgi.doe.gov/Mucci2
Neurospora crassa OR74A	AABX00000000.3 (Galagan et al. 2003)
Orpinomyces sp. C1A	ASRE00000000.1 (Youssef et al. 2013)
Pandora formicae v1	GCRV00000000.1
Phycomyces blakesleeanus NRRL 1555	http://genome.jgi.doe.gov/Phybl2 (Corrochano et al. 2016)
Piptocephalis cylindrospora RSA 2659	http://genome.jgi.doe.gov/Pipcy2/Pipcy2.home.html
Piromyces sp. E2	http://genome.jgi.doe.gov/PirE2_1
Puccinia graminis f. sp. *tritici* CRL 75-36-700-3	AAWC00000000.1 (Duplessis et al. 2011)
Ramicandelaber brevisporus CBS 109374	http://genome.jgi.doe.gov/Rambr1
Rhizophagus irregularis DAOM 181602	JARB00000000.1 (Tisserant et al. 2013)
Rhizopus delemar RA 99-880	AACW00000000.2 (Ma et al. 2009)
Rhizopus microsporus var *chinensis* CCTCC M201021	CCYT00000000.1 (Wang et al. 2013)
Rhizopus microsporus var *microsporus* ATCC 52813	http://genome.jgi.doe.gov/Rhimi1_1
Rozella allomycis CSF55	ATJD00000000.1 (James et al. 2013)
Saccharomyces cerevisiae S288C.vR64-2-1	http://yeastgenome.org/(Goffeau et al. 1996)
Saksenaea vasiformis B4078	JNDT00000000.1
Schizosaccharomyces pombe 972h-.vASM294v2	http://www.pombase.org/ (Wood et al. 2002)
Spizellomyces punctatus DAOM BR117 v1	ACOE00000000.1
Umbelopsis ramanniana NRRL 5844	http://genome.jgi.doe.gov/Umbra1
Ustilago maydis 521 v190413	AACP00000000.2 (Kamper et al. 2006)
Yarrowia lipolytica CLIB122	GCA_000002525.1 (Dujon et al. 2004)
Zoophthora radicans ATCC 208865	http://genome.jgi.doe.gov/ZooradStandDraft_FD/

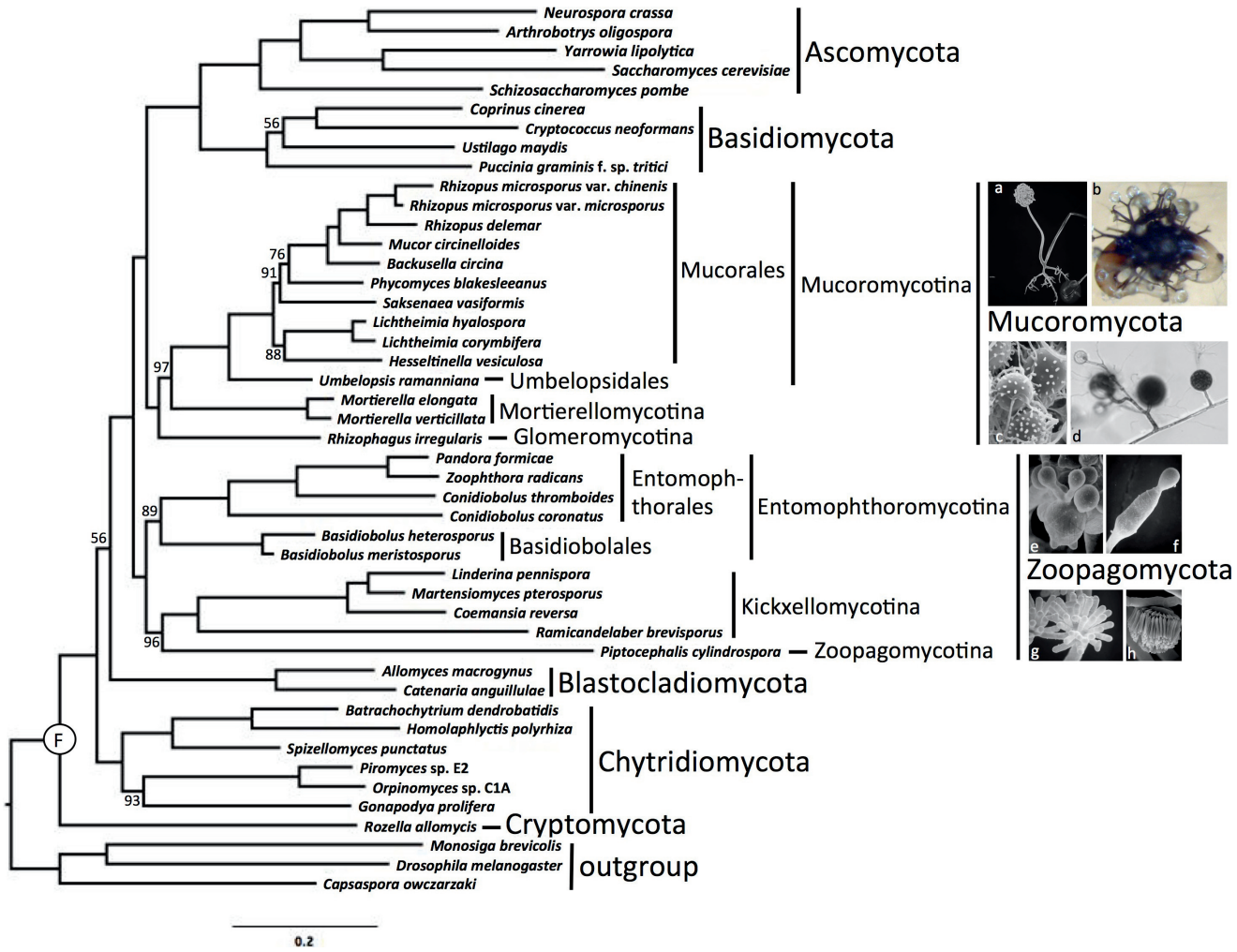


FIG. 1. RAxML phylogenetic tree of Kingdom Fungi based on the concatenated alignment of 192 conserved orthologous proteins. All branches received 100% bootstrap partitions except where noted by number above or below respective branches. Example images include: a. *Rhizopus* sporangium (SEM). b. *Phycomyces* zygospore (LM). c. *Mortierella* chlamydospores (SEM). d. *Rhizophagus* spores and hyphae (LM). e. *Conidiobolus* secondary (replicative) conidia forming on primary conidium (SEM). f. *Basidiobolus* ballistosporic conidium (SEM). g. *Piptocephalis* merosporangia (SEM). h. *Linderina* merosporangium (SEM). LM: light micrograph, SEM: scanning electron micrograph.

BP, and it was resolved as sister to Dikarya with 100% BP. Mucoromycotina and Mortierellomycotina were both supported by 100% BP, although the latter with limited taxon sampling. The arbuscular mycorrhizal species *Rhizophagus irregularis* was sister to Mucoromycotina and Mortierellomycotina with 97% BP. *Umbelopsis* was placed outside of the core Mucorales clade with 100% BP. Internal nodes pertaining to the placement of *Saksenaea* and *Hesseltinella* within Mucorales were only moderately supported by the analyses. The phylogenetic placement of Blastocladiomycota and Chytridiomycota was not strongly supported by these analyses, and their branching order is essentially interchangeable (FIGS. 1, 2).

The ASTRAL analyses provided an additional assessment of organismal phylogeny and identified nodes that may be affected by ancient incomplete lineage sorting (FIG. 2). Despite low bootstrap values, the node placing Blastocladiomycota as sister group to the nonflagellated fungi was supported by 90% ASTRAL branch support (ABS). The clades defined below as Zoopagomycota and Mucoromycota were supported by 96% and 100% ABS, respectively, and the monophyly of Mucoromycota plus Dikarya was supported by 95% ABS. Within Zoopagomycota, lower levels of ABS characterized the placement of *Piptocephalis* (60%) and the branch defining Entomophthoromycotina (82%). Within Mucoromycotina, low levels

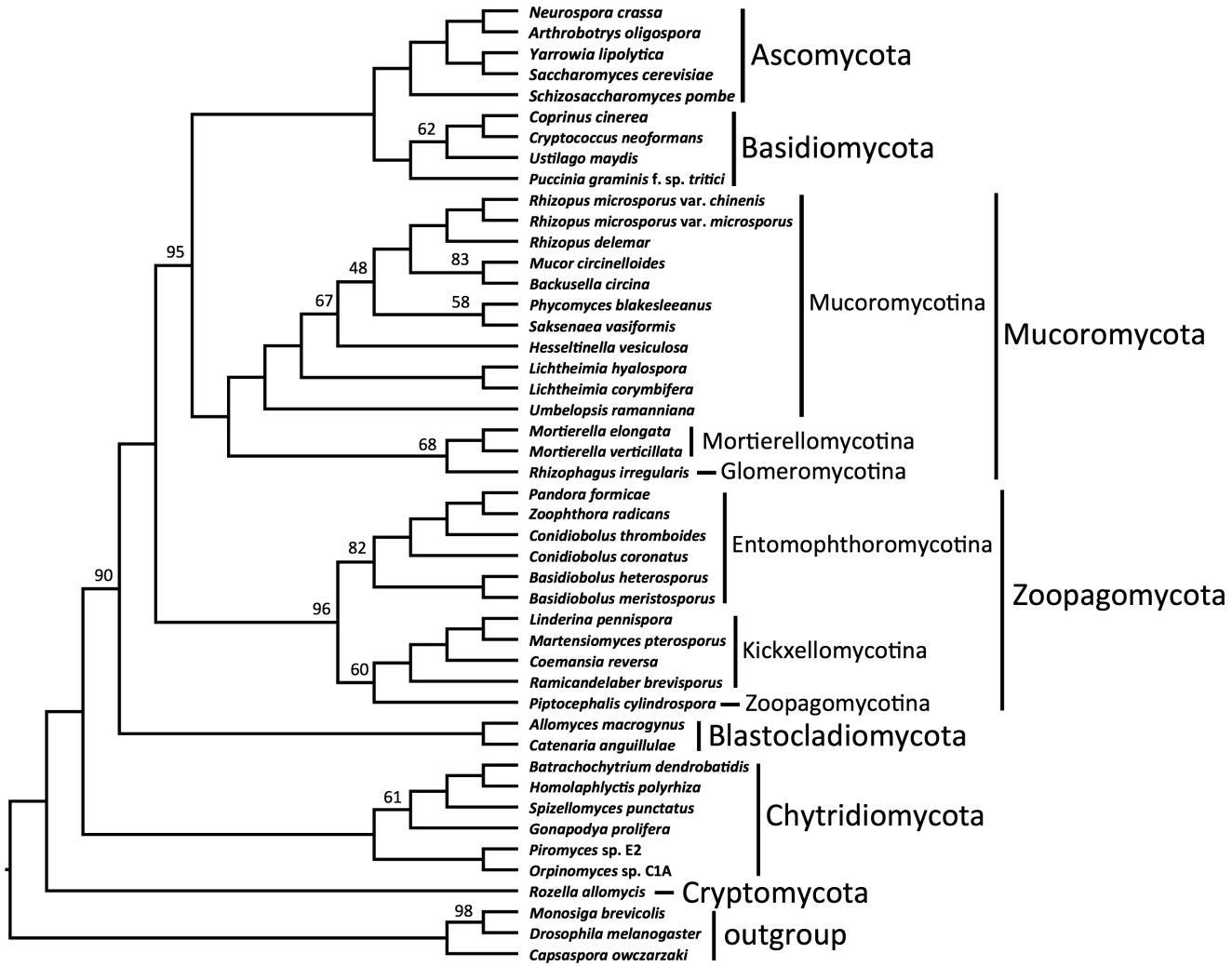


FIG. 2. ASTRAL consensus cladogram of Kingdom Fungi based on analyses of individual bootstrap trees for each of 192 conserved orthologous proteins. All branches received 100% ASTRAL branch support except where noted by number above or below respective branches.

of ABS characterized the placement of *Rhizophagus* (68%) and *Hesseltinella* and *Saksenea* within Mucorales.

TAXONOMY

Our classification follows the principles promoted in Hibbett et al.'s (2007) phylogenetic classification of Kingdom Fungi. All taxa are either demonstrated or presumed to be monophyletic and are autotypified by validly published genera. The name Zygomycota Moreau is rejected as a name for either clade of zygomycetes. Its taxonomic and nomenclatural use is in reference to the zygote, i.e. zygospore, formed through gametangial conjugation in the sexual reproductive phase. The zygospore, however, is not a synapomorphy for either clade of zygomycete fungi; rather it is a sympleisiomorphic trait inherited from the common ancestor of Zoopagomycota, Mucoromycota, and Dikarya (FIG. 1). As such, these findings support the discontinued use of Zygomycota to avoid confusion and misrepresentation of a more recent common ancestor between Zoopagomycota and Mucoromycota as opposed to Mucoromycota with Dikarya. Descriptions of new taxa follow phylogenetic nomenclature (Cantino 2010) and define the least inclusive monophyletic lineage as illustrated in a reference phylogenetic tree (FIG. 1). The classification presented here is restricted to fungi historically classified as zygomycetes, except where they have been demonstrated not to be members of Kingdom Fungi (e.g. the traditional 'trichomycete' orders Eccrinales and Amoebidiales; Benny and O'Donnell 2000, Cafaro 2005). Unnecessary intercalary taxa are avoided, and the classification does not treat taxa below the level of order. The proposed classification includes two phyla, six subphyla, four classes, and 16 orders (TABLE II).

TABLE II. Phylogenetic classification of zygomycete fungi

Mucoromycota Doweld (2001)
Glomeromycotina (C. Walker & A. Schüßler) Spatafora & Stajich, subphylum and stat. nov.
Glomeromycetes Caval.-Sm. (1998)
Archaeosporales C. Walker & A. Schüßler (2001)
Diversisporales C. Walker & A. Schüßler (2004)
Glomerales J. B. Morton & Benny (1990)
Paraglomerales C. Walker & A. Schüßler (2001)
Mortierellomycotina Kerst. Hoffm., K. Voigt & P.M. Kirk (2011)
Mortierellales Caval.-Sm. (1998)
Mucoromycotina Benny (2007)
Endogonales Moreau ex R.K. Benj. (1979)
Mucorales Fr. (1832)
Umbelopsidales Spatafora & Stajich, ord. nov.
Zoopagomycota Gryganskyi, M.E. Smith, Stajich & Spatafora, phylum nov.
Entomophthoromycotina Humber (2007)
Basidiobolomycetes Doweld (2001)
Basidiobolales Jacz. & P.A. Jacz. (1931)
Entomophoromycetes Humber (2012)
Entomophthorales G. Winter (1880)
Neozygitomycetes Humber (2012)
Neozygitales Humber (2012)
Kickxellomycotina Benny (2007)
Asellariales Manier ex Manier & Lichtw. (1978)
Dimargaritales R.K. Benj. (1979)
Harpellales Lichtw. & Manier (1978)
Kickxellales Kreisel ex R.K. Benj. (1979)
Zoopagomycotina Benny (2007)
Zoopagales Bessey ex R.K. Benj. (1979)

Phylum: Mucoromycota Doweld, Prosyllabus Tracheophytorum, Tentamen systematis plantarum vascularium (Tracheophyta): LXXVII. 2001, emend. Spatafora & Stajich.

Synonym: Zygomycota F. Moreau, Encyclopédie Mycologique 23:2035. 1954 (pro parte).

Type: *Mucor* P. Micheli ex L. (1753).

Emendation: Phylum Mucoromycota is emended here to apply to all descendants of the node defined in the reference phylogeny (FIG. 1) as the terminal Mucoromycota clade. It is the least inclusive clade containing Mucoromycotina, Mortierellomycotina, and Glomeromycotina. Characters associated with sexual reproductive states, where known, include zygospore production by gametangial conjugation. Asexual reproductive states can involve chlamydospores and spores produced in sporangia and sporangioles.

Commentary. The name Mucoromycota Doweld (2001) formally specifies the group referred to as zygomycetes I in the INTRODUCTION. It is preferred to Glomeromycota C. Walker & A. Schüßler (2001) because it is more representative of the taxa that comprise the phylum. Mucoromycota shares a most recent common ancestor with Dikarya and it is characterized by plant-associated nutritional modes (e.g. plant symbionts, decomposers of plant debris, plant pathogens etc.) and only rare or derived ecological interactions with animals (e.g. primarily associated with opportunistic infections). Zygospores tend to be globose, smooth or ornamented, and produced on opposed or apposed suspensor cells with or without appendages. Asexual reproduction typically involves the production of sporangiospores in sporangia or sporangioles, or chlamydospores. Hyphae tend to be large diameter and coenocytic with the exception of the delimitation of reproductive structures by adventitious septa.

Subphylum: **Glomeromycotina** (C. Walker & A. Schüßler) Spatafora & Stajich, subphylum and stat. nov.

MycoBank MB816301

Replaced name: Glomeromycota C. Walker & A. Schüßler, in Schüßler et al., Mycol. Res. 105:1416. 2001.

Type: *Glomus* Tul. & C. Tul. 1845.

Description: Subphylum Glomeromycotina is erected here for the least inclusive clade containing Archaeosporales, Diversisporales, Glomerales, and Paraglomerales (Redecker & Schüßler 2014). Sexual reproduction is unknown and asexual reproduction is by specialized spores that resemble azygospores or chlamydospores.

Class: Glomeromycetes Caval.-Sm., Biol. Rev. 73:246. 1998. (as "Glomomycetes").

Orders: Archaeosporales C. Walker & A. Schüßler, in Schüßler et al., Mycol. Res. 105:1418. 2001; Diversisporales C. Walker & A. Schüßler, Mycol. Res. 108:981. 2004; Glomerales J.B. Morton & Benny, Mycotaxon 37:473. 1990. (as "Glomales"); Paraglomerales C. Walker & A. Schüßler, in Schüßler et al., Mycol. Res. 105:1418. 2001.

Commentary. Glomeromycotina includes all fungi that form arbuscular mycorrhizae and *Geosiphon*, a symbiont of cyanobacteria in the genus *Nostoc*. Sexual reproduction is unknown but supported by genome evidence (Ropars et al. 2016). Asexually formed chlamydospore-like spores are borne terminally, laterally, or intercalary on specialized hyphae. Most species produce spores directly in soil or roots, but several species in different lineages make macroscopic sporocarps (Gerdemann and Trappe 1974). Arbuscules, the site of bidirectional nutrient transfer in arbuscular mycorrhizae, are modified, highly branched haustorium-like cells that are produced in cortical plant root cells. Some taxa also produce darkly staining, intercellular, and intracellular vesicles. Species of Glomeromycotina produce coenocytic hyphae that can harbor bacterial endosymbionts (Bianciotto et al. 2003, Torres-Cortés and Ghignone 2015). These fungi were previously treated as a family

within Endogonales (Glomeraceae, Gerdemann and Trappe 1974), an order within the class Zygomycetes (Glomales, Morton and Benny 1990) and as a phylum more closely related to Dikarya (Glomeromycota, Schüßler et al. 2001). Its membership in Mucoromycota is supported by genome-scale phylogenetic analyses (FIG. 1) and by gene content analyses (Tisserant et al. 2013).

Subphylum: Mortierellomycotina Kerst. Hoffm., K. Voigt & P.M. Kirk, in Hoffmann, Voigt & Kirk, Mycotaxon 115:360. 2011.

Order: Mortierellales Caval.-Sm., Biol. Rev. 73: 246. 1998.

Commentary. Mortierellomycotina reproduce asexually by sporangia that either lack or have a highly reduced columella. *Mortierella* was historically classified within Mucorales, but molecular phylogenetic (Hoffmann et al. 2011) and phylogenomic analyses (Tisserant et al. 2013) rejected this hypothesis. Rather, *Mortierella* is best treated in its own subphylum related to Mucoromycotina and Glomeromycotina (Hoffmann et al. 2011). Molecular phylogenetic analyses reveal considerable diversity within Mortierellomycotina (Wagner et al. 2013) and environmental sampling supports a diversity of taxa associated with soils, rhizosphere, and plant roots (Summerbell 2005, Nagy et al. 2011, Shakya et al. 2013). *Mortierella* species are known as prolific producers of fatty acids, especially arachidonic acid (Higashiyama et al. 2002) and they frequently harbor bacterial endosymbionts (Sato et al. 2010). Most species of Mortierellomycotina only form microscopic colonies, but at least two species in the genus *Modicella* make multicellular sporocarps (Smith et al. 2013).

Subphylum: Mucoromycotina Benny, in Hibbett et al., Mycol. Res. 111:517. 2007.

Orders: Endogonales Moreau ex R.K. Benj., in Kendrick, ed., Whole Fungus 2:599. 1979. Emend. Morton & Benny, Mycotaxon 37:473. 1990; Mucorales Fr., Syst. Mycol. 3:296. 1832; Umbelopsidales Spatafora, Stajich & Bonito, ord. nov.

Commentary. Mucoromycotina has the largest number of described species of Mucoromycota and includes the well-known model species *Mucor mucedo* and *Phycomyces blakesleeanus*. It also includes industrially important species of *Rhizopus* and other genera. Where known, sexual reproduction within Mucoromycotina is by prototypical zygospore formation and asexual reproduction typically involves the copious production of sporangia and/or sporangioles. Species are frequently isolated from soil, dung, plant debris, and sugar-rich plant parts (e.g. fruits). As such, fungi in the Mucoromycotina represent the majority of zygomycetous fungi in pure culture. Endogonales includes both ectomycorrhizal and saprobic species (Bidartondo et al. 2011). Sexual reproduction involves the production of zygospores by apposed gametangia within a simple, often sequestrate or enclosed sporocarp that may be hypogeous, embedded in heavily decayed wood, or produced among foliage of mosses or liverworts. Recent studies suggest that ectomycorrhizae have probably evolved twice within Endogonales (Tedersoo and Smith 2013). Endogonales represents an independent origin of mycorrhizae relative to the arbuscular mycorrhizae of Glomeromycotina and ectomycorrhizae of Dikarya (Bidartondo et al. 2011, Tedersoo and Smith 2013, Dickie et al. 2015) and like many of Mucoromycota, they harbor endohyphal bacteria (Desiro et al. 2014).

Order: **Umbelopsidales** Spatafora, Stajich & Bonito, ord. nov.

MycoBank MB816302

Type: *Umbelopsis* Amos & H.L. Barnett (1966)

Description: Umbelopsidales is erected here to apply to all descendants of the node defined in the reference phylogeny (FIG. 1) as the terminal Umbelopsidales clade. It is the least inclusive clade containing the genus *Umbelopsis*. Asexual reproduction is by sporangia and chlamydospores. Sporangiophores may be branched in a cymose or verticillate fashion. Sporangia are typically pigmented red or ochre, multi- or single-spored and with or without conspicuous columella. Sporangiospores are globose, ellipsoidal, or polyhedral and pigmented like sporangia. Chlamydospores are filled with oil globules and often abundant in culture. Sexual reproduction is unknown.

Commentary: Species in the Umbelopsidales were previously classified in Mucorales (e.g. *U. isabellina*) or Mortierellales (e.g. *Micromucor* [=*Umbelopsis*] *ramanniana*). Phylogenetic analyses of genome-scale data resolve this as a distant sister group to Mucorales, consistent with ordinal status. Like Mortierellales, species of Umbelopsidales are frequently isolated from rhizosphere soils, with increasing evidence that these fungi occur as root endophytes (Hoff et al. 2004, Terhonen et al. 2014).

Phylum: **Zoopagomycota** Gryganskyi, M.E. Smith, Spatafora & Stajich, phylum nov.

MycoBank MB816300

Synonym: Zygomycota F. Moreau, Encyclopédie Mycologique 23:2035. 1954 (pro parte).

Type: *Zoopage* Drechsler (1935).

Description: Phylum Zoopagomycota is erected here to apply to all descendants of the node defined in the reference phylogeny (FIG. 1) as the terminal Zoopagomycota clade. It is the least inclusive clade containing Entomophthoromycotina, Kickellomycotina, and Zoopagomycotina. Sexual reproduction, where known, involves the production of zygospores by gametangial conjugation. Morphologies associated with asexual reproductive states include sporangia, merosporangia, conidia, and chlamydospores.

Commentary. Zoopagomycota represents the earliest diverging clade of zygomycetous fungi and formally applies to the group referred to as zygomycetes II in the INTRODUCTION. It comprises three subphyla in which associations with animals (e.g. pathogens, commensals, mutualists) form a common ecological theme, although species from several lineages are mycoparasites (e.g. *Syncephalis*, *Piptocephalis*, and Dimargaritales). Because of its broader and more inclusive meaning, the name Zoopagomycota (Gr.: zoo = animal, pago = frozen, ice or unite) is preferred to other possible names for the clade including Trichomycota R.T. Moore (1994), Basidiobolomycota Doweld (2001), Entomophthoromycota Humber (2012), and Harpellomycota Doweld (2013). All of these alternative names were originally proposed to refer to a particular clade within Zoopagomycota; therefore, use of these alternative names would probably cause confusion. Although some of the fungi in Zoopagomycota can be maintained in axenic culture, most species are more difficult to maintain in pure culture than species of Mucoromycota. Accordingly, species of Zoopagomycota are most frequently observed growing in association with a host organism. Haustoria are produced by some of the animal pathogens and mycoparasites. Zoopagomycota hyphae may be compartmentalized by septa that may be complete or uniperforate; in the latter, bifurcate septa contain electron opaque lenticular plugs. Zygospore formation typically involves modified hyphal tips, thallus cells, or hyphal bodies (yeast-like cells) that function as gametangia.

Subphylum: **Entomophthoromycotina** Humber, in Hibbett et al. Mycol. Res. 111:517. 2007.

Synonym: Entomophthoromycota Humber, Mycotaxon 120:481. 2012.

Classes: Basidiobolomycetes Doweld, Prosyllabus Tracheophytorum, Tentamen systematis plantarum vascularium (Tracheophyta): LXXVII. 2001; Entomophthoromycetes Humber, Mycotaxon 120:486. 2012; Neozygitomycetes Humber, Mycotaxon 120:485. 2012.

Orders: Basidiobolales Jacz. & P.A. Jacz., *Opredelitel' Gribov*, (edn 3) I Ficomiţeti (Leningrad):8. 1931; Entomophthorales G. Winter, Rabenh. Krypt.-Fl. 1:74. 1880; Neozygitales Humber, Mycotaxon 120:486. 2012.

Commentary. Entomophthoromycotina includes three classes and three orders of saprobic and insect pathogenic fungi. The thallus may consist of coenocytic or septate hyphae, which may fragment to form hyphal bodies, or it may comprise only hyphal bodies. Asexual reproduction is by conidiogenesis from branched or unbranched conidiophores; primary conidia are forcibly discharged and secondary conidia are either forcibly or passively released. Sexual reproduction involves the formation of either zygospores by gametangial copulation, involving hyphal compartments or hyphal bodies (Humber 2012).

Subphylum: **Kickxellomycotina** Benny, in Hibbett et al. Mycol. Res. 111:518. 2007.

Synonym: Trichomycota R.T. Moore, Identification and Characterization of Pest Organisms:250. 1994 (pro parte).

Orders: Asellariales Manier ex Manier & Lichtw., Mycotaxon 7:442. 1978; Dimargaritales R.K. Benj., in Kendrick (ed.), Whole Fungus 2:607. 1979; Harpellales Lichtw. & Manier, Mycotaxon 7:441. 1978; Kickxellales Kreisel ex R.K. Benj., in Kendrick (ed.), Whole Fungus 2:610. 1979; R.K. Benj., in Kendrick, ed., Whole Fungus 2:607. 1979.

Commentary. Mycelium is regularly divided into compartments by bifurcate septa that often have lenticular occlusions. Sexual reproduction involves the formation of variously shaped zygospores by gametangial conjugation of relatively undifferentiated sexual hyphal compartments (Lichtwardt 1986). Sporophores may be produced from septate, simple, or branched somatic hyphae. Asexual reproduction involves the production of uni- or multispored merosporangia arising from a specialized vesicle (i.e. sporocladium), sporiferous branchlets, or an undifferentiated sporophore apex. Species may be saprobes, mycoparasites, and symbionts of insects; the latter includes Harpellales that are typically found within the hindguts of aquatic life history stages.

Subphylum: Zoopagomycotina Benny, in Hibbett et al. Mycol. Res. 111:518. 2007.

Order: Zoopagales Bessey ex R.K. Benj., in Kendrick, ed., Whole Fungus 2:590. 1979.

Commentary. Zoopagomycotina include mycoparasites and predators or parasites of small invertebrates and amoebae. The hyphal diameter is characteristically narrow in thalli that are branched or unbranched; sometimes specialized haustoria are produced in association with hosts. Only a handful of species have been successfully maintained in axenic culture. Sexual reproduction, where known, is by gametangial conjugation, forming globose zygospores on apposed differentiated or undifferentiated suspensor cells (Dreschler 1935). Asexual reproduction is by arthrospores, chlamydospores, conidia, or multispored merosporangia that may be simple or branched.

DISCUSSION

Overview of Kingdom Fungi.—In the concatenated RAxML analyses, we resolve and recognize seven clades that we classify as phyla of Kingdom Fungi (FIG. 1), with zoosporic fungi comprising the three earliest diverging lineages. Cryptomycota, represented by the genus *Rozella*, is the earliest diverging lineage of Fungi followed by Chytridiomycota and Blastocladiomycota. The branching order of the latter two taxa is

weakly supported and both have been resolved as sharing a most recent common ancestor (MRCA) with the nonflagellated fungi of Zoopagomycota, Mucoromycota, and Dikarya (James et al. 2006, Chang et al. 2015). Within Chytridiomycota we recognize three classes, including Chytridiomycetes Caval.-Sm. (1998), Monoblepharidomycetes J.H. Schaffner (1909), and Neocallimastigomycetes M.J. Powell (2007). The remaining phyla of Fungi include the nonflagellated phyla Zoopagomycota, Mucoromycota, Basidiomycota, and Ascomycota. Because to the absence of genomic data, we could not assess the validity of the newly erected phylum Entorrhizomycota (Bauer et al 2015).

The 192 protein clusters incorporated into these analyses are encoded by single to low-copy genes that are conserved throughout eukaryotes (James et al. 2013). As such, these genes tend to be ubiquitously distributed in Fungi and arguably less susceptible to errors associated with orthology assignment. The interpretation of bootstrap support for branches in genome-scale phylogenies is still poorly understood given that some genes within a genome may have different evolutionary histories (e.g. Salichos et al. 2014). We attempted to alleviate this problem through the use of conservative orthologs, but we cannot currently discount issues associated with ancient lineage sorting events, whole genome duplications, and inadvertent biases associated with taxon sampling (e.g. unsampled taxa, extinction events, etc.). In an attempt to characterize the effect of ancient lineage sorting events, ASTRAL analyses were performed on the bootstrap trees derived from the RAxML analyses of each protein sequence alignment. The placement of Blastocladiomycota as sister group to the nonflagellated lineages of Kingdom Fungi was supported by 56% BP and 90% ABS values, suggesting that the node is not characterized by high levels of ancient incomplete lineage sorting but low levels of phylogenetic signal present in the current dataset; a finding consistent with the results of Chang et al. (2015). The effect of adding taxa to fill the gaps among unsampled lineages is more difficult to predict, but it is reasonable to assume that it might increase support for long, relatively isolated branches, such as Blastocladiomycota (Wiens and Morrill 2011). At this time we consider the placement of Blastocladiomycota unresolved.

Paraphyly of zygomycetes and support for major clades.—Both the concatenated RAxML (FIG. 1) and the ASTRAL (FIG. 2) analyses reject zygomycete monophyly and resolve two clades, Zoopagomycota and Mucoromycota, which form a paraphyletic grade from which Dikarya are derived. Although this finding is consistent with rDNA analyses (White et al. 2006) and multigene phylogenies (James et al. 2006, Chang et al. 2015), it provides greater clarity on clade membership and relationship to other major clades of Kingdom Fungi. By not resurrecting the abandoned name Zygomycota Moreau, we propose names for each of the two monophyletic phyla and we expand the use of autotypification based on validly published genera as espoused by Hibbett et al. (2007). Because the International Code for algae, fungi, and plants (McNeill et al. 2012) does not require adherence to the principle of priority above the rank of family, we have selected names that communicate taxa or traits that are characteristic of the majority of species contained within the two phyla. In addition, the names Zoopagomycota and Mucoromycota avoid taxonomic confusion stemming from previous use of other names that are linked to alternative evolutionary hypotheses. For example, Glomeromycota has been used over the last 15 y to refer to the monophyletic group of arbuscular mycorrhizal fungi (Schüßler et al. 2001); the use of this name for a wider group of fungi would likely be problematic and confusing. Finally, we recognize the minimum number of phylum-level clades necessary to name monophyletic clades of zygomycetes to produce a classification system that is easier to teach and reduces the use of redundant taxa.

Zoopagomycota is resolved as the earliest diverging lineage of zygomycetes. Although genomic sampling included representatives from all three subphyla, a further increase in taxon sampling will undoubtedly reveal additional phylogenetic diversity. Kickxellomycotina is represented by four taxa that are all from Kickxellales. Entomophthoromycotina is represented by five taxa, three from Entomophthorales (*Conidiobolus* spp., *Pandora formicae*, *Zoophthora radicans*) and two from Basidiobolales (*B. heterosporus* and *B. meristosporus*). Branch support (BP = 89, ABS = 82) for Entomophthoromycotina is the lowest of the subphyla, which is in part a result of the topological instability of *Basidiobolus*. This finding is similar to observations in previous multigene studies (Gryganskyi et al. 2012) and suggests that more robust support for the placement of *Basidiobolus* will not be achieved by the addition of sequence data alone but will instead require additional taxon sampling, consideration of episodic events associated with rare genomic changes, and possibly the use of models of evolution that are not strictly bifurcating (Than et al. 2008). The sole representative of Zoopagomycotina is *Piptocephalis cylindrospora*, for which the sequence data were generated based on single-cell genomics methods (Rinke et al. 2013). Its membership in Zoopagomycota is strongly supported by these analyses, but its placement within the phylum is less well supported (MLBS = 96, ABS = 60). This is possibly a consequence of the nature of the data from single-cell sequencing and sparse taxon sampling for

the subphylum. As most species of Zoopagomycotina are obligate symbionts, additional sampling will require the use of advanced sequencing and computational techniques, use of dual-organism cultures and novel approaches to establish axenic cultures.

Mucoromycota is resolved as the clade of zygomycetes that diverged most recently from a shared ancestor with Dikarya. The most significant change from previous molecular-based classifications of zygomycetes (Schüßler et al. 2001) is the inclusion of Glomeromycotina in Mucoromycota. Although Glomeromycotina (=Glomeromycota) was previously resolved as more closely related to Dikarya than Mucoromycotina and Mortierellomycotina using nuclear SSU rDNA and multigene sequence data (Schüßler et al. 2001, James et al. 2006), this was not supported by the present analyses. Rather, the topology presented here is consistent with recent mitochondrial phylogenies (Nadimi et al. 2012, Pelin et al. 2012), genome-scale phylogenies, and gene content analyses (Tisserant et al. 2013, Chang et al. 2015), as well as with traditional morphology-based classifications (Gerdemann and Trappe 1974, Morton and Benny 1990). As in previous studies (Chang et al. 2015), the position of Glomeromycotina is equivocal and it appears alternatively as the earliest diverging lineage of the Mucoromycota (FIG. 1, MLBS = 97) or as a sister group to Mortierellomycotina (FIG. 2, ABS = 68). Mortierellomycotina is represented by the genomes of two species of *Mortierella*; their placement is consistent with being phylogenetically distinct from Mucoromycotina. Because of the ease of their maintenance in axenic culture, the strongly supported Mucoromycotina is sampled more and is represented by 11 taxa, two orders, and eight families. Although represented only by a single taxon, Umbelopsidales is supported as the sister clade to Mucorales, a finding consistent with multigene phylogenetic analyses (Sekimoto et al. 2011, Hoffmann et al. 2013). Suggestive of phylogenetic conflict among protein-sequence trees within the Mucorales, several nodes within the order are resolved differently between the RAxML and ASTRAL analyses. Expanding the sampling density throughout the Mucoromycota is needed to better understand processes underlying molecular evolution (e.g. possible genome duplications) around these potentially problematic nodes.

Evolution of host association and nutritional modes.—Our phylogenomic analysis shows a striking contrast between the host associations and trophic modes of Zoopagomycota and Mucoromycota (TABLE III). Most species of Zoopagomycota are pathogens, parasites, or commensals of animals and other fungi, whereas a few species are considered to be more generalized saprobes (Benny et al. 2014). Associations with living plants are rare for

TABLE III. Taxonomic distribution of selected morphological and ecological characters of zygomycete fungi

	Zoopagomycotina	Kickxellomycotina	Entomophthoromycotina	Mucoromycotina	Mortierellomycotina	Glomeromycotina
Sexual Reproduction	Zygospore	Zygospore	Zygospore	Zygospore	Zygospore	Unknown
Asexual Reproduction	Sporangia, conidia	Trichospores, sporangia, merosporangia	Conidia	Sporangia, sporangioles	Sporangia	Chlamydospore-like
Hyphae	Coenocytic	Bifurcate septa w/ lenticular plug	Complete septa, bifurcate septa, or coenocytic; hyphal bodies	Coenocytic	Coenocytic	Coenocytic
MTOC[a]	—[b]	Centriole-like	Centriole-like	Spindle pole body	—	—
Hyphal tip structure	—	AVC[c]	Spitzenkörper	AVC	AVC	AVC
Fruiting body production	Absent	Absent	Absent	Present (rare)	Present (rare)	Present (rare)
Major host/substrate	Amoeba, animal, fungi	Animal, fungi	Animal	Plant	Plant	Plant

[a] Microtubular Organizing Center.
[b] Unsampled.
[c] Apical Vesicle Crescent.

the phylum. In contrast, Mucoromycota includes multiple mycorrhizal lineages (Glomeromycotina, Endogonales; Bidartondo et al. 2011, Redecker and Schüßler 2014), root endophytes (Mortierellomycotina, Umbelopsidales; Hoff et al. 2004, Summerbell 2005, Terhonen et al. 2014) and decomposers of plant-based carbon sources (Mucorales; Benny et al. 2014). Members of both Mucoromycotina and Glomeromycotina can also form mycorrhiza-like relationships with nonvascular plants (Field et al. 2015a). All species of Mucoromycotina known as mycoparasites (e.g. *Spinellus fusiger*, *Syzygites megalocarpa*) or putative parasites of arthropods (e.g. *Sporodiniella umbellata*) are evolutionarily derived and closely related to saprobes (Hoffman et al. 2013). In rare cases when species in Mucoromycota infect humans or other animals, they are interpreted as opportunistic pathogens, typically of immunocompromised individuals.

The phylogenetic distribution of these nutritional associations illuminates two elements of fungal evolution that shape the development of evolutionary hypotheses of early diverging fungi. First, deep divergences among Zoopagomycota point to an early origin for animal- and fungus-associated nutritional relationships. Ancient associations with animals, other fungi, and non-plant organisms are poorly documented in the known fossil record (Taylor et al. 2014) and our results predict hidden fungal associations yet to be detected through analysis of animal fossils. The second major point of emphasis from these analyses is the sister-group relationship of Mucoromycota and Dikarya and the diversification of fungi in association with land plants. Dikarya clearly diversified with land plants in terrestrial ecosystems (Selosse and Le Tacon 1998, Berbee 2001). It is now reasonable to consider that nutrition from land plants had a deeper origin in fungal evolutionary history, extending back to the common ancestor of Mucoromycota and Dikarya. This is consistent with studies that considered ancient fungal relationships with algae and the land plant lineage (Chang et al. 2015, Field et al. 2015a). Furthermore, it is consistent with the record of fossil fungi from some of the earliest 407 million year old land plants. Such fossils include arbuscules characteristic of the Glomeromycotina (*Glomites rhyniensis*; Taylor et al. 1995), swellings and hyphae reminiscent of Mucoromycotina (Strullu-Derrien et al. 2014) and sporocarps suggestive of Dikarya (*Paleopyrenomycites devonicus*; Taylor et al. 2005). It has been hypothesized that symbioses with heterotrophic fungi played a role the evolution of land plants (Bidartondo et al. 2011, Field et al. 2015b). Our results specify the plant-associated, terrestrial MRCA of Mucoromycota plus Dikarya as the species that gave rise to independent and parallel origins of important plant-fungal symbioses from endophytes to mycorrhizae.

Evolution of morphology.—Interpretation of morphology in the context of this genome-scale phylogeny highlights the importance of Zoopagomycota, Mucoromycota, and their MRCA in understanding the evolution of fungal traits associated with the flagellum, hyphae, reproduction, and multicellularity. We provide a brief summary of these traits with an emphasis on development and refinement of evolutionary hypotheses, but direct readers to more comprehensive treatments for detailed discussions (Humber 2012, Benny et al. 2014, Redecker and Schüßler 2014, McLaughlin et al. 2015).

Although these analyses resolve a single loss of the flagellum in the MRCA of Zoopagomycota, Mucoromycota, and Dikarya, it should be noted that numerous lineages were not sampled here and their inclusion would indicate additional losses of the flagellum among early diverging fungi. Microsporidia are sister group to Cryptomycota and represent the loss of the flagellum in the earliest diverging lineage of Fungi (James et al. 2013). Similarly, *Hyaloraphidium* is a nonflagellated member of Chytridiomycota and represents a loss of the flagellum among the core clade of zoosporic fungi (James et al. 2006). Relevant to the zygomycete fungi is *Olpidium*, a genus of zoosporic fungi that has been hypothesized to be closely related to Zoopagomycota based on multigene phylogenies (Sekimoto et al. 2001, James et al. 2006). Analysis of genomic data for this genus is crucial to more accurately estimate the number of losses of flagellum, their placement on the fungal tree of life, and to test alternative hypotheses of a single loss of the flagellum (Liu et al. 2006). Furthermore, the placement of Zoopagomycota as the earliest diverging lineage of nonflagellated fungi is intriguing because some of its species have retained what may be relicts of a flagellum in the form of cylindrical, centriole-like organelles. Centriole-like organelles are associated with the nuclei of *Basidiobolus* of Entomophthoromycotina (McKerracher and Heath 1985, Roberson et al. 2011) and *Coemansia* of Kickxellomycotina (McLaughlin et al. 2015). In contrast to these centriole-like organelles, Mucoromycotina and Dikarya share discoidal to hemispherical spindle pole bodies. Although spindle pole bodies function as microtubule organizing centers, as do centrioles, they lack any obvious remnant of the centrioles' characteristic 9+2 microtubule arrangement (reviewed in McLaughlin et al. 2015). Broader analyses are needed, but the distribution of putative relict centrioles is consistent with flagellum loss occurring shortly before or during the diversification of Zoopagomycota.

Hyphae vary among species and clades in Mucoromycota and Zoopagomycota. Species of Zoopagomycotina typically produce small diameter coenocytic hyphae and haustoria in association with parasitism

of hosts. Species of Kickxellomycotina produce hyphae that are regularly compartmentalized by uniperforate, bifurcate septa occluded by lenticular plugs (Jeffries and Young 1979, Saikawa 1989). Species of Entomophthoromycotina produce either coenocytic hyphae, hyphae with complete septa that may disarticulate into one or two-celled hyphal bodies (reviewed in Humber 2012), or with septa similar to those of Kickxellomycotina (Saikawa 1989). Species of Mucoromycotina and Mortierellomycotina produce large diameter, coenocytic hyphae characteristic of textbook zygomycetes, as do Glomeromycotina, which in addition make highly branched, narrow hyphal arbuscules in host cells. Where septations do occur in Mucoromycota they tend to be adventitious and formed at the base of reproductive structures.

The Spitzenkörper is associated with hyphal growth in Dikarya but has been elucidated for only a few species of zygomycetes. Roberson et al. (2011) documented an apical spherical organization of microvesicles in *Basidiobolus* (Zoopagomycota) consistent with a Spitzenkörper. In contrast, hyphae of *Coemansia* (Zoopagomycota) and *Gilbertella*, *Mortierella*, and *Mucor* (Mucoromycota) (Fisher and Roberson 2016) and the germ tubes of *Gigaspora* (Mucoromycota) (Bentivenga et al. 2013) lack a classical Spitzenkörper, but instead possess a hemispherical organization of vesicles, the apical vesicle crescent, which in some taxa has been demonstrated to be mandatory for hyphal growth (Fisher and Roberson 2016).

Asexual reproduction by sporangia is present in all subphyla of Zoopagomycota and Mucoromycota with three notable exceptions (Benny et al. 2014). Entomophthoromycotina is characterized by the production of conidia with the formation of forcibly discharged primary conidia that may undergo germination to form passively dispersed secondary conidia (Humber 2012). Conidia are also described for species in Zoopagomycotina that are pathogenic to amoebae and nematodes (Dreschler 1935, 1936), but mycoparasitic lineages produce reduced sporangia, sporangioles, and merosporangia (Benny et al. 2014). Presumably, conidiogenesis in Zoopagomycota and Dikarya arose independently, but closer analysis may yet reveal homologies at the level of molecular development. Glomeromycotina are known to reproduce only asexually via unique spores that resemble chlamydospores or azygospores.

Where sexual reproduction is known in species of both Zoopagomycota and Mucoromycota, it is by the formation of zygospores via gametangial conjugation (Drechsler 1935, Lichtwardt 1986, Humber 2012). In Mucoromycota, sexual reproduction is under the control of mating type genes, *sexP* and *sexM*, which regulate the production of pheromones required for the maturation of hyphae into gametangia (Idnurm et al. 2008) and confer + and – mating-type identity, respectively (reviewed in Lee et al. 2010). Recent genomic studies have revealed numerous mating genes in the genomes of Glomeromycotina (Riley et al. 2013) and a Dikarya-like mating processes in *R. irregularis* (Ropars et al. 2016), suggesting that they may have a cryptic sexual cycle. In Zoopagomycota, the genetic basis and physiological control of mating has not been characterized. From commonalities across fungal phyla (Cassleton 2008), we assume that genetic systems in Zoopagomycota and Mucoromycota might be similar, but detailed studies are needed.

Multicellular sporocarps are not produced by Zoopagomycota and though rare, they are present within Mucoromycota through independent origins in *Endogone* (Mucoromycotina; Bidartondo et al. 2011), *Modicella* (Mortierellomycotina; Smith et al. 2013) and as aggregations of spore-producing hyphae and spores in species of Glomeromycotina (Gerdemann and Trappe 1974, Redecker and Schüßler 2014). Along with the multicellular sporocarps in Agaricomycotina (Basidiomycota) and Pezizomycotina (Ascomycota), multicellular sporocarps within Mucoromycota have been derived independently, suggesting that while the genetic and metabolic potential for complex thallus diversity did not arise until the MRCA of Mucoromycota and Dikarya, it then resulted in multiple independent origins of complex spore-producing structures involving hyphal differentiation (Stajich et al. 2009).

ACKNOWLEDGMENTS

This paper is dedicated to our colleague and coauthor Thomas N. Taylor who passed away during the final preparation of this manuscript. The authors thank the following persons for access to unpublished genomes: Santiago Torres Martínez for *Mucor circinelloides*, Teresa Pawlowska and Stephen Mondo for *Rhizopus microsporus* var. *microsporus*, Vincent Bruno for *Basidiobolus heterosporus* and *Saksenaea vasiformis*, and Francis Martin for *Mortierella elongata*. This material is based upon work supported by the National Science Foundation (DEB-1441604 to JWS, DEB-1441715 to JES, DEB-1441677 to TYJ, DEB-1441728 to RWR), the French National Research Agency through the Laboratory of Excellence ARBRE (grant No. ANR–11–LBX–0002–01 to JWS) and the Canadian National Science and Engineering Research Council grants (412318-11 and 138427-11 to MLB). Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer.

LITERATURE CITED

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, George RA, Lewis SE, Richards S, Ashburner M, Henderson SN, Sutton GG, Wortman JR, Yandell MD, Zhang Q, Chen LX, Brandon RC, Rogers YH, Blazej RG, Champe M, Pfeiffer BD, Wan KH, Doyle C, Baxter EG, Helt G, Nelson CR, Gabor GL, Abril JF, Agbayani A, An HJ, Andrews-Pfannkoch C, Baldwin D, Ballew RM, Basu A, Baxendale J, Bayraktaroglu L, Beasley EM, Beeson KY, Benos PV, Berman BP, Bhandari D, Bolshakov S, Borkova D, Botchan MR, Bouck J, Brokstein P, Brottier P, Burtis KC, Busam DA, Butler H, Cadieu E, Center A, Chandra I, Cherry JM, Cawley S, Dahlke C, Davenport LB, Davies P, de Pablos B, Delcher A, Deng Z, Mays AD, Dew I, Dietz SM, Dodson K, Doup LE, Downes M, Dugan-Rocha S, Dunkov BC, Dunn P, Durbin KJ, Evangelista CC, Ferraz C, Ferriera S, Fleischmann W, Fosler C, Gabrielian AE, Garg NS, Gelbart WM, Glasser K, Glodek A, Gong F, Gorrell JH, Gu Z, Guan P, Harris M, Harris NL, Harvey D, Heiman TJ, Hernandez JR, Houck J, Hostin D, Houston KA, Howland TJ, Wei MH, Ibegwam C, Jalali M, Kalush F, Karpen GH, Ke Z, Kennison JA, Ketchum KA, Kimmel BE, Kodira CD, Kraft C, Kravitz S, Kulp D, Lai Z, Lasko P, Lei Y, Levitsky AA, Li J, Li Z, Liang Y, Lin X, Liu X, Mattei B, McIntosh TC, McLeod MP, McPherson D, Merkulov G, Milshina NV, Mobarry C, Morris J, Moshrefi A, Mount SM, Moy M, Murphy B, Murphy L, Muzny DM, Nelson DL, Nelson DR, Nelson KA, Nixon K, Nusskern DR, Pacleb JM, Palazzolo M, Pittman GS, Pan S, Pollard J, Puri V, Reese MG, Reinert K, Remington K, Saunders RD, Scheeler F, Shen H, Shue BC, Sidén-Kiamos I, Simpson M, Skupski MP, Smith T, Spier E, Spradling AC, Stapleton M, Strong R, Sun E, Svirskas R, Tector C, Turner R, Venter E, Wang AH, Wang X, Wang ZY, Wassarman DA, Weinstock GM, Weissenbach J, Williams SM, WoodageT, Worley KC, Wu D, Yang S, Yao QA, Ye J, Yeh RF, Zaveri JS, Zhan M, Zhang G, Zhao Q, Zheng L, Zheng XH, Zhong FN, Zhong W, Zhou X, Zhu S, Zhu X, Smith HO, Gibbs RA, Myers EW, Rubin GM, Venter JC. The genome sequence of *Drosophila melanogaster*. Science 287:2185–2195, doi:10.1126/science.287.5461.2185

Bauer R, Garnica S, Oberwinkler F, Reiss K, Weiß M, Begerow D. 2015. Entorrhizomycota: a new fungal phylum reveals new perspectives on the evolution of fungi. PLoS One 10:e0128183, doi:10.1371/journal.pone.0128183

———, Humber RA, Voigt K. 2014. Zygomycetous fungi: phylum Entomophthoromycota and subphyla Kickxellomycotina, Mortierellomycotina, Mucoromycotina, and Zoopagomycotina. In: McLaughlin DJ, Spatafora JW, eds. Systematics and evolution. Part A. New York: Springer-Verlag. The Mycota VII:209–250.

Benny GL, Humber RA, Voigt K. 2014. Zygomycetous fungi: phylum Entomophthoromycota and subphyla Kickxellomycotina, Mortierellomycotina, Mucoromycotina, and Zoopagomycotina. In: McLaughlin DJ, Spatafora JW, eds. Mycota VII, part A. Systematics and evolution. New York: Springer-Verlag. p 209–250.

———, O'Donnell K. 2000. *Amoebidium parasiticum* is a protozoan, not a Trichomycete. Mycologia 92:1133–1137, doi:10.2307/3761480

Bentivenga SP, Kumar TKA, Kumar L, Roberson RW, McLaughlin DJ. 2013. Cellular organization in germ tube tips of *Gigaspora* and its phylogenetic implications. Mycologia 105:1087–1099, doi:10.3852/12-291

Berbee ML. 2001. The phylogeny of plant and animal pathogens in the Ascomycota. Physiol Mol Plant Pathol 59:165–187, doi:10.1006/pmpp.2001.0355

———, Taylor JW. 2010. Dating the molecular clock in fungi—how close are we? Fungal Biol Rev 24:1–16, doi:10.1016/j.fbr.2010.03.001

Bianciotto V, Lumini E, Bonfante P, Vandamme P. 2003. "*Candidatus Glomeribacter gigasporarum*" gen. nov., sp. nov., an endosymbiont of arbuscular mycorrhizal fungi. Int J Syst Evol Micrbiol 53:121–124, doi:10.1099/ijs.0.02382-0

Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R, Duckett JG. 2011. The dawn of symbiosis between plants and fungi. Biol Lett 7:574–577, doi:10.1098/rsbl.2010.1203

Cafaro MJ. 2005. Eccrinales (Trichomycetes) are not fungi, but a clade of protists at the early divergence of animals and fungi. Mol Phylogenet Evol 35:21–34, doi:10.1016/j.ympev.2004.12.019

Cantino P. 2010. International Code of phylogenetic nomenclature. 102 p.

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25:1972–1973, doi:10.1093/bioinformatics/btp348

Casselton LA. 2008. Fungal sex genes—searching for the ancestors. Bioessays 30:711–714, doi:10.1002/bies.20782

Chang Y, Wang S, Sekimoto S, Aerts AL, Choi C, Clum A, LaButti KM, Lindquist EA, Yee Ngan C, Ohm RA, Salamov AA, Grigoriev IV, Spatafora JW, Berbee ML. 2015. Phylogenomic analyses indicate that early fungi evolved digesting cell walls of algal ancestors of land plants. Genome Biol Evol 7:1590–1601, doi:10.1093/gbe/evv090

Corrochano LM, Kuo A, Marcet-Houben M, Polaino S, Salamov A, Villalobos-Escobedo JM, Grimwood J, Álvarez MI, Avalos J, Bauer D, Benito EP, Benoit I, Burger G, Camino LP, Cánovas D, Cerdá-Olmedo E, Cheng JF, Domínguez A, Eliáš M, Eslava AP, Glaser F, Gutiérrez G, Heitman J, Henrissat B, Iturriaga EA, Lang BF, Lavín JL, Lee SC, Li W, Lindquist E, López-García S, Luque EM, Marcos AT, Martin J, McCluskey K, Medina HR, Miralles-Durán A, Miyazaki A, Muñoz-Torres E, Oguiza JA, Ohm RA, Olmedo M, Orejas M, Ortiz-Castellanos L, Pisabarro AG, Rodríguez-Romero J, Ruiz-Herrera J, Ruiz-Vázquez R, Sanz C, Schackwitz W, Shahriari M, Shelest E, Silva-Franco F, Soanes D, Syed K, Tagua VG, Talbot NJ, Thon MR, Tice H, de Vries RP, Wiebenga A, Yadav JS, Braun EL, Baker SE, Garre V, Schmutz J, Horwitz BA, Torres-Martínez S, Idnurm A, Herrera-Estrella A, Gabaldón T, Grigoriev IV. Expansion of

signal transduction pathways in fungi by extensive genome duplication. Curr Biol 26:1–8.

Desiro A, Faccio A, Kaech A, Bidartondo MI, Bonfante P. 2014. *Endogone*, one of the oldest plant-associated fungi, host unique *Mollicutes*-related endobacteria. New Phytol 205:1464–1472, doi:10.1111/nph.13136

Dickie IA, Alexander I, Lennon S, Öpik M, Selosse MA, van der Heijden MGA, Martin FM. 2015. Evolving insights to understanding mycorrhizas. New Phytol 205:1369–1374, doi:10.1111/nph.13290

Doggett JS, Wong B. 2014. Mucormycosis. In: Loriaux L, ed. Endocrine emergencies. New York: Humana Press. p 57–63.

Dreschler C. 1935. Some conidial phycomycetes destructive to terricolous amoebae. Mycologia 27:6–40, doi:10.2307/3754021

———. 1936. New conidial phycomycetes destructive to terricolous amoebae. Mycologia 28:363–389, doi:10.2307/3754001

Dujon B, Shermann D, Fischer G, Durrens P, et al. 2004. Genome evolution in yeasts. Nature 430:35–44, doi:10.1038/nature02579

Duplessis S, Cuoma CA, Lin YC, Aerts A, et al. 2011. Obligate biotrophy features unraveled by the genomic analysis of rust fungi. Proc Natl Acad Sci USA 108:9166–9171, doi:10.1073/pnas.1019315108

Ebersberger I, de Matos Simoes R, Kupczok A, Gube M, Kothe E, Voigt K, Haeseler von A. 2012. A consistent phylogenetic backbone for the fungi. Mol Biol Evol 29:1319–1334, doi:10.1093/molbev/msr285

Eddy SR. 2011. Accelerated profile HMM searches. PLoS Comput Biol 7:e1002195, doi:10.1371/journal.pcbi.1002195

Field KJ, Pressel S, Duckett JG, Rimington WR, Bidartondo MI. 2015b. Symbiotic options for the conquest of land. Trends Ecol Evol 30:477–486, doi:10.1016/j.tree.2015.05.007

———, Rimington WR, Bidartondo MI, Allinson KE, Beerling DJ, Cameron DD, Duckett JG, Leake JR, Pressel S. 2015a. First evidence of mutualism between ancient plant lineages (Haplomitriopsida liverworts) and Mucoromycotina fungi and its response to simulated Palaeozoic changes in atmospheric CO_2. New Phytol 205:743–756, doi:10.1111/nph.13024

Fisher KE, Roberson RW. 2016. Hyphal tip cytoplasmic organization in four zygomycetous fungi. Mycologia 108:533–542, doi:10.3852/15-226

Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, Martínez AT, Otillar R, Spatafora JW, Yadav JS, Aerts A, Benoit I, Boyd A, Carlson A, Copeland A, Coutinho PM, de Vries RP, Ferreira P, Findley K, Foster B, Gaskell J, Glotzer D, Górecki P, Heitman J, Hesse C, Hori C, Igarashi K, Jurgens JA, Kallen N, Kersten P, Kohler A, Kües U, Kumar TK, Kuo A, LaButti K, Larrondo LF, Lindquist E, Ling A, Lombard V, Lucas S, Lundell T, Martin R, McLaughlin DJ, Morgenstern I, Morin E, Murat C, Nagy LG, Nolan M, Ohm RA, Patyshakuliyeva A, Rokas A, Ruiz-Dueñas FJ, Sabat G, Salamov A, Samejima M, Schmutz J, Slot JC, St John F, Stenlid J, Sun H, Sun S, Syed K, Tsang A, Wiebenga A, Young D, Pisabarro A, Eastwood DC, Martin F, Cullen D, Grigoriev IV, Hibbett DS. 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336:1715–1719, doi:10.1126/science.1221748

Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S, Rehman B, Elkins T, Engels R, Wang S, Nielsen CB, Butler J, Endrizzi M, Qui D, Ianakiev P, Bell-Pedersen D, Nelson MA, Werner-Washburne M, Selitrennikoff CP, Kinsey JA, Braun EL, Zelter A, Schulte U, Kothe GO, Jedd G, Mewes W, Staben C, Marcotte E, Greenberg D, Roy A, Foley K, Naylor J, Stange-Thomann N, Barrett R, Gnerre S, Kamal M, Kamvysselis M, Mauceli E, Bielke C, Rudd S, Frishman D, Krystofova S, Rasmussen C, Metzenberg RL, Perkins DD, Kroken S, Cogoni C, Macino G, Catcheside D, Li W, Pratt RJ, Osmani SA, DeSouza CP, Glass L, Orbach MJ, Berglund JA, Voelker R, Yarden O, Plamann M, Seiler S, Dunlap J, Radford A, Aramayo R, Natvig DO, Alex LA, Mannhaupt G, Ebbole DJ, Freitag M, Paulsen I, Sachs MS, Lander ES, Nusbaum C, Birren B. 2003. The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422:859–868, doi:10.1038/nature01554

Gerdemann J, Trappe JM. 1974. The Endogonaceae in the Pacific Northwest. Mycol Mem 5:1–76.

Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG. Life with 6000 genes. Science 274:546–567, doi:10.1126/science.274.5287.546

Gryganskyi AP, Humber RA, Smith ME, Miadlikowska J, Miadlikovska J, Wu S, Voigt K, Walther G, Anishchenko IM, Vilgalys R. 2012. Molecular phylogeny of the Entomophthoromycota. Mol Phylogenet Evol 65:682–694, doi:10.1016/j.ympev.2012.07.026

———, Muszewska A. 2014. Whole genome sequencing and the Zygomycota. Fungal Genom Biol 4:e116, doi:10.4172/2165-8056.1000e116

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Kõljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schüssler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N. 2007. A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547, doi:10.1016/j.mycres.2007.03.004

Higashiyama K, Fujikawa S, Park EY. 2002. Production of arachidonic acid by *Mortierella* fungi. Biotechnol Bioprocess Eng 7:252–262, doi:10.1007/BF02932833

Hoff JA, Klopfenstein NB, McDonald GI. 2004. Fungal endophytes in woody roots of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*). For Pathol 34:255–271, doi:10.1111/j.1439-0329.2004.00367.x

Hoffmann K, Voigt K, Kirk PM. 2011. Mortierellomycotina subphyl. nov., based on multi-gene genealogies. Mycotaxon 115:353–363, doi:10.5248/115.353

———, Pawłowska J, Walther G. 2013. The family structure of the Mucorales: a synoptic revision based on comprehensive multigene-genealogies. Persoonia 30:57–76, doi:10.3767/003158513X666259

Humber RA. 2012. Entomophthoromycota: a new phylum and reclassification for entomophthoroid fungi. Mycotaxon 120:477–492, doi:10.5248/120.477

Idnurm A, Walton FJ, Floyd A, Heitman J. 2008. Identification of the sex genes in an early diverged fungus. Nature 451:193–196, doi:10.1038/nature06453

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüssler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822, doi:10.1038/nature05110

———, Pelin A, Bonen L, Ahrendt S, Sain D, Corradi N, Stajich JE. 2013. Shared signatures of parasitism and phylogenomics unite Cryptomycota and microsporidia. Curr Biol 23:1548–1553, doi:10.1016/j.cub.2013.06.057

Jeffries P, Young T. 1979. Ultrastructure of septa in *Dimargaris cristalligena* RK Benjamin. J Gen Microbiol 111: 303–311, doi:10.1099/00221287-111-2-303

Jennessen J, Schnürer J, Olsson J, Samson RA, Dijksterhuis J. 2008. Morphological characteristics of sporangiospores of the tempe fungus *Rhizopus oligosporus* differentiate it from other taxa of the *R. microsporus* group. Mycol Res 112:547–563, doi:10.1016/j.mycres.2007.11.006

Joneson S, Stajich JE, Shiu SH, Rosenblum EB. 2011. Genomic transition to pathogenicity in chytrid fungi. PLoS Pathog 7:e1002338.

Kamper J, Kahmann R, Bolker M, Ma LJ, Brefort T, Saville BJ, Banuett F, Kronstad JW, Gold SE, Müller O, Perlin MH, Wösten HA, de Vries R, Ruiz-Herrera J, Reynaga-Peña CG, Snetselaar K, McCann M, Pérez-Martín J, Feldbrügge M, Basse CW, Steinberg G, Ibeas JI, Holloman W, Guzman P, Farman M, Stajich JE, Sentandreu R, González-Prieto JM, Kennell JC, Molina L, Schirawski J, Mendoza-Mendoza A, Greilinger D, Münch K, Rössel N, Scherer M, Vranes M, Ladendorf O, Vincon V, Fuchs U, Sandrock B, Meng S, Ho EC, Cahill MJ, Boyce KJ, Klose J, Klosterman SJ, Deelstra HJ, Ortiz-Castellanos L, Li W, Sanchez-Alonso P, Schreier PH, Häuser-Hahn I, Vaupel M, Koopmann E, Friedrich G, Voss H, Schlüter T, Margolis J, Platt D, Swimmer C, Gnirke A, Chen F, Vysotskaia V, Mannhaupt G, Güldener U, Münsterkötter M, Haase D, Oesterheld M, Mewes HW, Mauceli EW, DeCaprio D, Wade CM, Butler J, Young S, Jaffe DB, Calvo S, Nusbaum C, Galagan J, Birren BW. 2006. Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. Nature 444:97–101, doi:10.1038/nature05248

King N, Westbrook MJ, Young SL, Kuo A, Abedin M, Chapman J, Fairclough S, Hellsten U, Isogai Y, Letunic I, Marr M, Pincus D, Putnam N, Rokas A, Wright KJ, Zuzow R, Dirks W, Good M, Goodstein D, Lemons D, Li W, Lyons JB, Morris A, Nichols S, Richter DJ, Salamov A, Sequencing JG, Bork P, Lim WA, Manning G, Miller WT, McGinnis W, Shapiro H, Tjian R, Grigoriev IV, Rokhsar D. The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. Nature 451: 783–788.

Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N, Clum A, Colpaert J, Copeland A, Costa MD, Doré J, Floudas D, Gay G, Girlanda M, Henrissat B, Herrmann S, Hess J, Högberg N, Johansson T, Khouja HR, LaButti K, Lahrmann U, Levasseur A, Lindquist EA, Lipzen A, Marmeisse R, Martino E, Murat C, Ngan CY, Nehls U, Plett JM, Pringle A, Ohm RA, Perotto S, Peter M, Riley R, Rineau F, Ruytinx J, Salamov A, Shah F, Sun H, Tarkka M, Tritt A, Veneault-Fourrey C, Zuccaro A; Mycorrhizal Genomics Initiative Consortium, Tunlid A, Grigoriev IV, Hibbett DS, Martin F. 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat Genet 47:410–415, doi:10.1038/ng.3223

Lee SC, Ni M, Li W, Shertz C, Heitman J. 2010. The evolution of sex: a perspective from the fungal kingdom. Microbiol Mol Biol Rev 74:298–340, doi:10.1128/MMBR.00005-10

Lichtwardt RW. 1986. The Trichomycetes: fungal associates of arthropods. New York: Springer-Verlag. 343 p.

Liu Y, Hodson MC, Hall BD. 2006. Loss of flagellum happened only once in the fungal lineage: phylogenetic structure of Kingdom Fungi inferred from RNA polymerase II subunit genes. BMC Evol Biol 6:74, doi:10.1186/1471-2148-6-74

———, Steenkamp ET, Brinkmann H, Forget L, Philippe H, Lang BF. 2009. Phylogenomic analyses predict sistergroup relationship of nucleariids and fungi and paraphyly of zygomycetes with significant support. BMC Evol Biol 9:272, doi:10.1186/1471-2148-9-272

Loftus BJ, Fung E, Roncaglia P, Rowley D, et al. 2005. The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. Science 307:1321–1324, doi:10.1126/science.1103773

Ma LJ, Ibrahim AS, Skory C, Grabherr MG, Burger G, Butler M, Elias M, Idnurm A, Lang BF, Sone T, Abe A, Calvo SE, Corrochano LM, Engels R, Fu J, Hansberg W, Kim JM, Kodira CD, Koehrsen MJ, Liu B, Miranda-Saavedra

D, O'Leary S, Ortiz-Castellanos L, Poulter R, Rodriguez-Romero J, Ruiz-Herrera J, Shen YQ, Zeng Q, Galagan J, Birren BW, Cuomo CA, Wickes BL. 2009. Genomic analysis of the basal lineage fungus *Rhizopus oryzae* reveals a whole-genome duplication. PLoS Genet 5:e1000549, doi:10.1371/journal.pgen.1000549

McKerracher LJ, Heath IB, 1985. The structure and cycle of thenucleus-associated organelle in two species of *Basidiobolus*. Mycologia 77:412–417, doi:10.2307/3793197

McLaughlin DJ, Healy RA, Celio GJ, Roberson RW, Kumar TKA. 2015. Evolution of zygomycetous spindle pole bodies: evidence from *Coemansia reversa* mitosis. Am J Bot 102:707–717, doi:10.3732/ajb.1400477

McNeill J, Barrie FF, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GF, Wiersema JH, Turland N, eds. 2012. International code of nomenclature for algae, fungi, and plants (Melbourne code). [Regnum Vegetabile no. 154.] Königstein: Koeltz Scientific Books. 208 p.

Mirarab S, Reaz R, Bayzid MS, Zimmermann T. 2014. ASTRAL: Genome-scale coalescent-based species tree estimation. Bioinformatics 30:i541–i548, doi:10.1093/bioinformatics/btu462

Moreau F. 1954 ["1953"]. Les Champignons. Physiologie, morphologie, développment et systématique. Vol. 2. Paris: Lechevalier. 1179 p.

Morton JB, Benny GL. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomacae. Mycotaxon 37:471–491.

Nadimi M, Beaudet D, Forget L, Hijri M, Lang BF. 2012. Group I intron-mediated trans-splicing in mitochondria of *Gigaspora rosea* and a robust phylogenetic affiliation of arbuscular mycorrhizal fungi in Mortierellales. Mol Biol Evol 29:2199–2012, doi:10.1093/molbev/mss088

Nagy LG, Ohm RA, Kovács GM, Floudas D, Riley R, Gácser A, Sipiczki M, Davis JM, Doty SL, de Hoog GS, Lang BF, Spatafora JW, Martin FM, Grigoriev IV, Hibbett DS. 2014. Latent homology and convergent regulatory evolution underlies the repeated emergence of yeasts. Nat Commun 5:4471, doi:10.1038/ncomms5471

———, Petkovits T, Kovács GM, Voigt K, Vágvölgyi C, Papp T. 2011. Where is the unseen fungal diversity hidden? A study of *Mortierella* reveals a large contribution of reference collections to the identification of fungal environmental sequences. New Phytol 191:789–794, doi:10.1111/j.1469-8137.2011.03707.x

Notredame C, Higgins DG, Heringa J. 2000. T-Coffee: a novel method for fast and accurate multiple sequence alignment. J Molec Biol 302:205–217, doi:10.1006/jmbi.2000.4042

Papanikolaou S, Panayotou MG. 2007. Lipid production by oleaginous Mucorales cultivated on renewable carbon sources. Eur J Lipid Sci Tech 109:1060–1070, doi:10.1002/ejlt.200700169

Pelin A, Pombert JF, Salvioli A, Bonen L, Bonfante P, Corradi N. 2012. The mitochondrial genome of the arbuscular mycorrhizal fungus *Gigaspora margarita* reveals two unsuspected trans-splicing events of group I introns. New Phytol 194:836–845, doi:10.1111/j.1469-8137.2012.04072.x

Redecker D, Schüßler A. 2014. Glomeromycota. In: McLaughlin DJ, Spatafora JW, eds. Mycota VII. Part A. Systematics and Evolution. New York: Springer-Verlag. p 251–269.

Riley R, Charron P, Idnurm A, Farinelli L, Dalpé Y, Martin F, Corradi N. 2013. Extreme diversification of the mating type -high-mobility group (MATA-HMG) gene family in a plant-associated arbuscular mycorrhizal fungus. New Phytol 201:254–268, doi:10.1111/nph.12462

Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu WT, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T. 2013. Insights into the phylogeny and coding potential of microbial dark matter. Nature 499:431–437, doi:10.1038/nature12352

Roberson RW, Saucedo E, Maclean D, Propster J, Unger B, Oneil TA, Parvanehgohar K, Cavanaugh C, Lowry D. 2011. The hyphal tip structure of *Basidiobolus* sp.: a zygomycete fungus of uncertain phylogeny. Fungal Biol 115:485–492, doi:10.1016/j.funbio.2011.02.012

Ropars J, Toro KS, Noel J, Pelin A, Charron P, Farinelli L, Marton T, Krüger, Fuchs J, Brachmann A, Corradi N. 2016. Evidence for the sexual origin of heterokaryosis in arbuscular mycorrhizal fungi. Nat Microbiol 1: art. no.16033.

Saikawa M. 1989. Ultrastructure of the septum in *Ballocephala verrucospora* (Entomophthorales, Zygomycetes). Can J Bot 67:2484–2488, doi:10.1139/b89-318

Salichos L, Stamatakis A, Rokas A. 2014. Novel information theory-based measures for quantifying incongruence among phylogenetic trees. Mol Biol Evol 31:1261–1271, doi:10.1093/molbev/msu061

Sato Y, Narisawa K, Tsuruta K, Umezu M. 2010. Detection of Betaproteobacteria inside the mycelium of the fungus *Mortierella elongata*. Microbes Environ 25:321–324, doi:10.1264/jsme2.ME10134

Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421, doi:10.1017/S0953756201005196

Sekimoto S, Rochon D, Long JE, Dee JM, Berbee ML. 2011. A multigene phylogeny of *Olpidium* and its implications for early fungal evolution. BMC Evol Biol 11:331, doi:10.1186/1471-2148-11-331

Selosse MA, Le Tacon F. 1998. The land flora: a phototroph-fungus partnership? Trends Ecol Evol 13:15–20, doi:10.1016/S0169-5347(97)01230-5

Shakya M, Gottel N, Castro H, Yang ZK, Gunter L, LabbÃ J, Muchero W, Bonito G, Vilgalys R, Tuskan G, Podar M, Schadt CW. 2013. A multifactor analysis of fungal and bacterial community structure in the root microbiome of mature *Populus deltoides* trees. PLoS One 8:e76382, doi:10.1371/journal.pone.0076382

Smith ME, Gryganskyi A, Bonito G, Nouhra E. 2013. Phylogenetic analysis of the genus *Modicella* reveals an independent evolutionary origin of sporocarp-forming fungi in

the Mortierellales. Fungal Genet Biol 61:61–68, doi:10.1016/j.fgb.2013.10.001

Stajich JE, Berbee ML, Blackwell M, Hibbett DS, James TY, Spatafora JW, Taylor JW. 2009. The fungi. Curr Biol 19:R840–R845, doi:10.1016/j.cub.2009.07.004

———, Wilke SK, Ahrén D, Au CH, Birren BW, Borodovsky M, Burns C, Canbäck B, Casselton LA, Cheng CK, Deng J, Dietrich FS, Fargo DC, Farman ML, Gathman AC, Goldberg J, Guigó R, Hoegger PJ, Hooker JB, Huggins A, James TY, Kamada T, Kilaru S, Kodira C, Kües U, Kupfer D, Kwan HS, Lomsadze A, Li W, Lilly WW, Ma LJ, Mackey AJ, Manning G, Martin F, Muraguchi H, Natvig DO, Palmerini H, Ramesh MA, Rehmeyer CJ, Roe BA, Shenoy N, Stanke M, Ter-Hovhannisyan V, Tunlid A, Velagapudi R, Vision TJ, Zeng Q, Zolan ME, Pukkila PJ. 2010. Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). Proc Natl Acad Sci USA 107:11889–11894, doi:10.1073/pnas.1003391107

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690, doi:10.1093/bioinformatics/btl446

Strullu-Derrien C, Kenrick P, Pressel S, Duckett JG, Rioult J-P, Strullu D-G. 2014. Fungal associations in *Horneophyton ligneri* from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: novel insights into ancestral plant-fungus symbioses. New Phytol 203:964–979, doi:10.1111/nph.12805

Suga H, Chen Z, de Mendoza A, Sebé-Pedrós A, Brown MW, Kramer E, Carr M, Kerner P, Vervoort M, Sánchez-Pons N, Torruella G, Derelle R, Manning G, Lang BF, Russ C, Haas BJ, Roger AJ, Nusbaum C, Ruiz-Trillo I. 2013. The *Capsaspora* genome reveals a complex unicellular prehistory of animals. Nat Comm 4:2325, doi:10.1038/ncomms3325

Summerbell RC. 2005. Root endophyte and mycorrhizosphere fungi of black spruce. Stud Mycol 53:121–145, doi:10.3114/sim.53.1.121

Taylor TN, Hass H, Kerp H, Krings M, Hanlin RT. 2005. Perithecial ascomycetes from the 400 million year old Rhynie chert: an example of ancestral polymorphism. Mycologia 97:269–285, doi:10.3852/mycologia.97.1.269

———, Krings M, Taylor E. 2014. Fossil Fungi. Amsterdam: Academic Press, Elsevier. 382 p.

———, Remy W, Hass H, Kerp H. 1995. Fossil arbuscular mycorrhizae from the Early Devonian. Mycologia 87:560–573, doi:10.2307/3760776

Tedersoo L, Smith ME. 2013. Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. Fungal Biol Rev 27:83–99, doi:10.1016/j.fbr.2013.09.001

Terhonen E, Keriö S, Sun H, Asiegbu FO. 2014. Endophytic fungi of Norway spruce roots in boreal pristine mire, drained peatland and mineral soil and their inhibitory effect on *Heterobasidion parviporum* in vitro. Fungal Ecol 9:17–26, doi:10.1016/j.funeco.2014.01.003

Than C, Ruths D, Nakhleh L. 2008. PhyloNet: a software package for analyzing and reconstructing reticulate evolutionary relationships. BMC Bioinformatics 9:322, doi:10.1186/1471-2105-9-322

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V, Gilbert LB, Handa Y, Herr JR, Hijri M, Koul R, Kawaguchi M, Krajinski F, Lammers PJ, Masclaux FG, Murat C, Morin E, Ndikumana S, Pagni M, Petitpierre D, Requena N, Rosikiewicz P, Riley R, Saito K, San Clemente H, Shapiro H, van Tuinen D, Bécard G, Bonfante P, Paszkowski U, Shachar-Hill YY, Tuskan GA, Young JP, Sanders IR, Henrissat B, Rensing SA, Grigoriev IV, Corradi N, Roux C, Martin F. 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. Proc Natl Acad Sci USA 110:20117–20122, doi:10.1073/pnas.1313452110

Torres-Cortés G, Ghignone S. 2015. Mosaic genome of endobacteria in arbuscular mycorrhizal fungi: transkingdom gene transfer in an ancient mycoplasma-fungus association. Proc Natl Acad Sci USA 112:7785–7790, doi:10.1073/pnas.1501540112

Tretter ED, Johnson EM, Benny GL, Lichtwardt RW, Wang Y, Kandel P, Novak SJ, Smith JF, White MM. 2014. An eight-gene molecular phylogeny of the Kickxellomycotina, including the first phylogenetic placement of Asellariales. Mycologia 106:912–935, doi:10.3852/13-253

Wagner L, Stielow B, Hoffmann K. 2013. A comprehensive molecular phylogeny of the Mortierellales (Mortierellomycotina) based on nuclear ribosomal DNA. Persoonia 30:77–93, doi:10.3767/003158513X666268

Wang D, Wu R, Xu Y, Li M. 2013. Draft genome sequence of *Rhizopus chinensis* CCTCCM201021, used for brewing traditional Chinese alcoholic beverages. Genome Announc 1:e0019512, doi:10.1128/genomeA.00195-12

Wang L, Chen W, Feng Y, Ren Y, Gu Z, Chen H, Wang H, Thomas MJ, Zhang B, Berquin IM, Li Y, Wu J, Zhang H, Song Y, Liu X, Norris JS, Wang S, Du P, Shen J, Wang N, Yang Y, Wang W, Feng L, Ratledge C, Zhang H, Chen YQ. 2011. Genome characterization of the oleaginous fungus *Mortierella alpina*. PLoS One 6:e28319, doi:10.1371/journal.pone.0028319

White MM, James TY, O'Donnell K, Cafaro MJ, Tanabe Y, Sugiyama J. 2006. Phylogeny of the Zygomycota based on nuclear ribosomal sequence data. Mycologia 98:872–884, doi:10.3852/mycologia.98.6.872

Wiens JJ, Morrill MC. 2011. Missing data in phylogenetic analysis: reconciling results from simulations and empirical data. Syst Biol 60:719–731, doi:10.1093/sysbio/syr025

Wood V, Gwilliam R, Rajandream MA, Lyne M, Lyne R, Stewart A, Sgouros J, Peat N, Hayles J, Baker S, Basham D, Bowman S, Brooks K, Brown D, Brown S, Chillingworth T, Churcher C, Collins M, Connor R, Cronin A, Davis P, Feltwell T, Fraser A, Gentles S, Goble A, Hamlin N, Harris D, Hidalgo J, Hodgson G, Holroyd S, Hornsby T, Howarth S, Huckle EJ, Hunt S, Jagels K, James K, Jones L, Jones M, Leather S, McDonald S, McLean J, Mooney P, Moule S, Mungall K, Murphy L, Niblett D, Odell C, Oliver K, O'Neil S, Pearson D, Quail MA, Rabbinowitsch E, Rutherford K, Rutter S, Saunders D, Seeger K, Sharp S, Skelton J, Simmonds M, Squares R, Squares S, Stevens

K, Taylor K, Taylor RG, Tivey A, Walsh S, Warren T, Whitehead S, Woodward J, Volckaert G, Aert R, Robben J, Grymonprez B, Weltjens I, Vanstreels E, Rieger M, Schäfer M, Müller-Auer S, Gabel C, Fuchs M, Düsterhöft A, Fritzc C, Holzer E, Moestl D, Hilbert H, Borzym K, Langer I, Beck A, Lehrach H, Reinhardt R, Pohl TM, Eger P, Zimmermann W, Wedler H, Wambutt R, Purnelle B, Goffeau A, Cadieu E, Dréano S, Gloux S, Lelaure V, Mottier S, Galibert F, Aves SJ, Xiang Z, Hunt C, Moore K, Hurst SM, Lucas M, Rochet M, Gaillardin C, Tallada VA, Garzon A, Thode G, Daga RR, Cruzado L, Jimenez J, Sánchez M, del Rey F, Benito J, Domínguez A, Revuelta JL, Moreno S, Armstrong J, Forsburg SL, Cerutti L, Lowe T, McCombie WR, Paulsen I, Potashkin J, Shpakovski GV, Ussery D, Barrell BG, Nurse P. 2002. The genome sequence of *Schizosaccharomyces pombe*. Nature 415:871–880, doi:10.1038/nature724

Yang J, Wang L, Ji X, Feng Y, Li X, Zou C, Xu J, Ren Y, Mi Q, Wu J, Liu S, Liu Y, Huang X, Wang H, Niu X, Li J, Liang L, Luo Y, Ji K, Zhou W, Yu Z, Li G, Liu Y, Li L, Qiao M, Feng L, Zhang KQ. 2011. Genomic and proteomic analyses of the fungus *Arthrobotrys oligospora* provide insights into nematode-trap formation. PLoS Pathog 7: e1002179, doi:10.1371/journal.ppat.1002179

Youssef NH, Couger MB, Struchtemeyer CG, Liggenstoffer AS, Prade RA, Najar FZ, Atiyeh HK, Wilkins MR, Elshahed MS. 2013. The genome of the anaerobic fungus *Orpinomyces* sp. strain C1A reveals the unique evolutionary history of a remarkable plant biomass degrader. Appl Environ Microbiol 79:4620–4634, doi:10.1128/AEM.00821-13

Published in final edited form as:
Curr Biol. 2009 September 29; 19(18): R840–R845. doi:10.1016/j.cub.2009.07.004.

Primer -- The Fungi

Jason E. Stajich[1,2], **Mary L. Berbee**[3], **Meredith Blackwell**[4], **David S. Hibbett**[5], **Timothy Y. James**[6], **Joseph W. Spatafora**[7], and **John W. Taylor**[1]

Jason E. Stajich: jason.stajich@ucr.edu; John W. Taylor: jtaylor@ nature.berkeley.edu

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102 USA.

[2]Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA.

[3]Department of Botany, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.

[4]Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803 USA.

[5]Biology Department, Clark University, Worcester, MA 01610 USA.

[6]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA.

[7]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331 USA.

The Kingdom Fungi, home to molds, mushrooms, lichens, rusts, smuts and yeasts, comprises eukaryotes with remarkably diverse life histories that make essential contributions to the biosphere, human industry, medicine and research. With the aim of enticing biologists to include fungi in their research, we note that many fungi have haploid genetics, and that those in cultivation are essentially immortal, two features that make it easier to associate traits with genotype, even for complex traits, than with *Drosophila* or *Arabidopsis*. The typical fungal genome size of 30–40 Mb is small by eukaryotic standards, which is why fungi have led the way as models for eukaryote genome sequencing with over 100 assembled genome sequences available [1,2]. For some fungi, DNA transformations, gene knockouts and knockdowns are routine. Species of Ascomycota and Basidiomycota show simple, multicellular development with differentiated tissues. In many species these tissues are large enough to support studies of transcription and translation in the lab and even in nature.

About a billion years ago, give or take 500 million years [3], a population of aquatic, unicellular eukaryotes making sporangia containing zoospores each with a single posterior flagellum split into two lineages: one eventually gave rise to animals, the other to fungi. Here we shall summarize the major diversifications of the Fungi by introducing each major fungal branch in the order that it is thought to have diverged (Figure 1) and presenting salient facts about fungal modes of nutrition, reproduction, communication and interaction with other life. Our views are strongly influenced by the Fungal Tree of Life Project [4–6]. Readers interested in learning more about fungi are encouraged to consult any of a number of comprehensive texts [7,8].

The exact order of divergence in deep regions of the eukaryotic tree is controversial. On the lineage that leads to the Fungi there are thought to be two other groups; the first to diverge are the nucleariid amoebae [9], and the next the Microsporidia. Microsporidia are either the sister group to the Fungi, or lie within the Fungi, (Figure 1), and they should be included in studies of fungi. They are unculturable, obligate parasites of animals, including humans. They have extremely reduced eukaryotic genomes — with a genome size of ~2.6 Mb and

~2000 genes [10,11] — remnant mitochondria, and unique morphologies related to parasitism, including a very frightening polar tube used to initiate infection [10].

Rozella

Staying in the Kingdom Fungi, we next arrive at a divergence leading to *Rozella allomycis* [4] (Figure 2A), an intracellular parasite of the Blastocladiomycota fungus *Allomyces*. *Rozella* has a small body without a cell wall, which branches within the host and makes two types of sporangia: zoosporangia, which produce zoospores with posterior flagella that swim from the parent to find new hosts, and resistant sporangia, around which a thick cell wall develops to ensure persistence long after the host has died and decayed [12]. Nothing is known about mating and meiosis in *Rozella*. Curiously, and like the Microsporidia, the lineage leading to *Rozella* diverged before that leading to its host, raising the worry that phylogenetic artifacts place parasite lineages at the base of phylogenies.

Chytridiomycota

The next divergence leads to the Phylum Chytridiomycota [13], which constitute < 1% of described fungi and, like *Rozella*, are presumed to retain key characters of the last common ancestor of Fungi and Animals [10]. These include a unicellular body bounded by cell wall, which matures into to a sporangium (Figure 2B), within which develop many posteriorly-uniflagellate zoospores (Figure 2C). The zoospores are cleaved from the sporangial cytoplasm by fusion of vesicles produced by a Golgi apparatus, and they swim to a fresh substrate, retract the flagellum, and secret a cell wall to encyst. The cyst germinates to start the life cycle anew. Although some Chytridiomycota have developed filamentous growth (hyphae), most have determinate development, and those living outside a substrate produce small, anucleate hyphae (rhizoids) that penetrate the food source. Species that live inside a host typically lack rhizoids, as does *Rozella*.

Chytriomyces hyalinus (Figure 2B) is the best studied Chytridiomycota species in terms of the morphology of sexual reproduction, however no mating types are known. In this species, two individuals fuse at their rhizoids to form a thick-walled resistant spore [14]. This fungus is a saprobe, but other Chytridiomycota, such as *Batrachochytrium* [15], are parasites, associated with amphibian decline, or like *Neocallimastix*, mutualists found in the stomachs of ruminate mammals [16]. Another group member is one of the few fungi judged to be a weapon of terror, the agent causing potato black wart, *Synchytrium endobioticum*, infamous for making resting spores that can persist in soil for decades [17]. Chytridiomycota have been thought to be haploid with zygotic meiosis, but DNA sequences of individual loci [18], as well as of the entire genome sequences of two *Batrachochytrium dendrobatidis* individuals (our unpublished data), raise the possibility that, like animals, these fungi can be diploid with gametic meiosis.

Blastocladiomycota

Back on the main fungal lineage, the next divergence leads to the Blastocladiomycota [13], the second phylum of Fungi with single, posterior flagella and home to *Allomyces*, the host for *Rozella allomycis*. Blastocladiomycota, once considered members of the Chytridiomycota, also account for < 1% of described Fungi. Indeterminate, hyphal growth is better developed in Blastocladiomycota than in Chytridiomycota, although the hyphae often sprout rhizoids. Blastocladiomycota are unusual in alternating their haploid and diploid generations [19]. Gametes in Blastocladiomycota resemble zoospores and, in *Allomyces*, female gametes produce a sesquiterpene pheromone, sirenin, that attracts male gametes [20]. In these organsisms, meiosis occurs in thick-walled, resistant sporangia, but, as in Chytridiomycota, mating types are unknown. Blastocladiomycota may be saprobic or

parasitic on plants or animals; the best-studied animal parasite, *Coelomomyces*, kills mosquito larvae and copopods as it alternates generations [21].

Travelling back to the main branch, again, we encounter one of the major shifts in fungal form, the loss of the flagellum [3,4]. This loss is associated with two other major changes: from this point in evolution forward, all stages of fungal life cycles have cell walls, and the microtubule organizing centers of nuclear division no longer are centrioles. Released from the constraint of organizing both flagella and spindles, the microtubule organizing centers associated with spindles, known as spindle pole bodies, have diversified morphologically and probably functionally, as is likely to become apparent when genomes of fungi with and without flagella are compared.

The next five major clades on our march through the fungi, subphyla Mucoromycotina, Entomophthoromycotina, Zoopagomycotina, and Kickxellomycotina, and the phylum Glomeromycota, formerly constituted the phylum Zygomycota [22], and together account for < 1% of described fungi. These taxa are organized into three clades, Mucoromycotina, Entomophthoromycotina + Zoopagomycotina + Kickxellomycotina and Glomeromycota, whose composition and relationships are not strongly supported.

Mucoromycotina

Mucoromycotina, the best studied of this group, will be familiar to all who have found their fresh berries rendered inedible by enveloping wefts of white mycelium. These fungi are saprobes, commonly growing on damaged fruit but also on mammal dung. Among them are two genera of model fungi, for example *Rhizopus* and *Phycomyces*. These fungi grow primarily as hyphae, or as yeasts where oxygen is scarce and carbon dioxide is abundant [23]. As in all the hyphal fungi encountered so far, septa are rare, apart from adventitious septa defining reproductive structures. Mitotic spores are formed in sporangia in a process very similar to zoospore formation within chytrid sporangia, but without flagella and with cell walls [24].

These fungi are haploid with zygotic meiosis. Sexual spores (zygospores) result when differentiated gametangia form and fuse, in a process involving pheromones derived from the carotenoid pathway [25]. Mating compatibility is regulated by one mating locus with two alleles that encode a high mobility group (HMG) domain transcription factor related to product of the human sex-determining gene *SRY* [26]. Zygospores show little variation among members of the subphylum, but the diversity of mitospore morphology and dispersal is staggering. For example, *Pilobolus* launches its sporangium by water pressure (recently captured by high-speed videography [27]), *Gilbertella* presents its mitospores to insect vectors in a drop of liquid held between halves of the sporangial wall and *Phycomyces* perches the sporangium on a 10 cm stalk that, as it elongates, responds to light and can sense and avoid obstructions without the need for physical contact [28,29]. The tremendous potential for developmental studies in these fungi has been given a boost by genome sequencing of several Mucoromycotina, among them *Phycomyces*, *Rhizopus* and *Mucor* [30].

Entomophthoromycotina, Zoopagomycotina and Kickxellomycotina

Entomophthoromycotina, Zoopagomycotina and Kickxellomycotina form a single clade of Fungi that, like Mucoromycotina, are hyphal, produce thick-walled, sexual zygospores [22] and are haploid with zygotic meiosis. The best-studied of these clades is Entomophthoromycotina, aptly named parasites of insects that manipulate host behavior to promote the transmission of their mitospores. *Entomophthora muscae*, for example, induces its fly host, just before death, to attach itself to elevated vegetation while enlarging its

abdomen to increase sexual attractiveness, all the better to lure males for spore transmission during pointless copulation [31,32]. The large, multispored sporangia typical of Mucoromycotina are not the rule in this subphylum. Instead, what appear to be multispored sporangia are often single, multinucleate spores, termed conidia.

Zoopagomycotina comprise fungi that are parasites on animals or other fungi and that form haustoria, hyphae that are specialized to promote nutrient transfer from host to fungus (Figure 2E). One likely member of Zoopagomycotina, *Zoophagus*, traps rotifers, amoebae or nematodes by attracting the tiny animals to feed on short, lateral hyphae that are covered with adhesives, so called 'lethal lollipops' [33]. Kickxellomycotina comprise saprobes, mycoparasites of Mucoromycotina and animal parasites and, as with Entomophthoromycotina and Zoopagomycotina, they show reduced reliance on multispored sporangia and increased reliance on conidia (Figure 2E). With each mitosis in their hyphae, Kickxellomycotina produce septa containing central pores that, with age, become plugged (Figure 2D). The development of regular septa may have helped initiate the shift from spores cleaved from inside sporangia by fusion of vesicles to conidia formed by hyphal septation. This shift in the method of spore formation might have been a key evolutionary event, because conidia are well developed not only in Kickxellomycotina and Zoopagomycotina, but also in the two dominant groups of fungi, Ascomycota and Basidiomycota.

Glomeromycota [34], another species-poor group, is one of most ecologically-important groups of fungi, because of its mutualisms with the roots of ~90% of plant species, known as arbuscular mycorrhizae [35]. Arbuscular mycorrhizae, which are seen in below-ground parts of the earliest plant fossils, facilitate nutrient acquisition by plants in exchange for photosynthate; they are vital to plant fitness, and may drive the composition of plant communities [36]. Arbuscular mycorrhizal fungi are hyphal and produce highly branched haustoria that promote nutrient exchange with host root cells. They also produce asexual, thick-walled multinucleate spores defined by adventitious septa. The fragmentary knowledge about most aspects of Glomeromycota biology belies their importance, because they cannot be cultivated apart from the host plant. For example, controversy clouds their ploidy, their genome size, and whether or not they reproduce sexually [37]. Evidence for recombination has been provided, but whether mating and meiosis are involved is unknown [38]. There might be no more important contribution to mycology than discovering how to axenically cultivate arbuscular mycorrhizae fungi.

Dikarya

We now arrive at the Dikarya, a subkingdom embracing the two largest fungal phyla, Ascomycota and Basidiomycota, home to ~98% of described fungi. The name, Dikarya, emphasizes an amazing feature of mating in these fungi: nuclear fusion does not follow directly from gamete fusion, so that hyphae with two nuclei (a dikaryon), one from each parent, constitute a significant (in Ascomycota), or the most significant (in Basidiomycota), part of the life cycle. The role of the dikaryon in adaptation will be revisited when we get to Basidiomycota, but one advantage applies to all Dikarya: a dramatic increase in the diversity of recombined progeny. In earlier diverging phyla, most matings lead to one zygote and one meiotic event. In Dikarya, one mating can lead to zygotes and independent meioses that number in the tens of thousands (as in a *Neurospora* colony) or even hundreds of trillions (in long-lived Basidiomycota with large or perennial fruiting bodies, such as the puffballs of *Calvatia*, false truffles of *Rhizopogon*, or shelf fungi of *Ganoderma*).

Morphologically, species with hyphae or unicellular yeasts, or both, are common throughout Dikarya. In hyphae, mitosis is followed by septum formation to produce regular septa, as

opposed to the adventitious septa found in most earlier-branching groups. These regular septa form centripetally and have central pores, most often with a means of regulating the passage of cytoplasm and organelles, including nuclei, between hyphal segments. Filters involve membranes in Basidiomycota (Figure 2G) or modified microbodies (Woronin bodies) [39] in most Ascomycota (Figure 2H). The advent of regular septa is also correlated with the evolution of macroscopic, multicellular fungi where different hyphal segments evolve to perform different functions, for example, the stalk, cap and gills of an *Agaricus* mushroom (Basidiomycota) or the stalk, cup and ascus layer of a morel (Ascomycota). Remarkably, it appears that multicellularity with differentiated tissues evolved independently in each phylum.

Ascomycota

Ascomycota is the larger taxon of Dikarya, with ~64% of described fungi, including species in four genera that helped researchers win Nobel Prizes (*Penicillium*, *Neurospora*, *Saccharomyces* and *Schizosaccharomyces*). Although each of these fungi is best known from laboratory studies, Ascomycota in nature earn their livings in all possible ways, as saprobes, as mutualists (forming lichens with algae or ectomycorrhizae with woody plants in Pinaceae, Fagales, Dipterocarpaceae, Fabaceae and Ericaceae), and as parasites. Pathogenic Ascomycota pose as great a threat to agriculture as any group of organisms [40], and parasitic Ascomycota adapted to animals account for almost all the severe, systemic human mycoses as well as athlete's foot and similar fungal skin diseases [41].

These fungi are typically haploid with one mating locus occuring as two alleles. The alleles are so diverged that they are termed idiomorphs and they code for homeodomain, alpha box and HMG-domain transcription factors [42]. Potential partners communicate by oligopeptide pheromones. In hyphal species, mating leads to a short dikaryotic stage that produces a multitude of zygotes and meiocytes (asci) as previously mentioned. Sporangia with internal mitospores are not found in Ascomycota. Instead conidia are the means of asexual reproduction. Within asci, however, meiotic spores (ascospores) form as membranous vesicles fuse to delimit uninucleate portions of cytoplasm (Figure 2F) in a process reminiscent of sporangiospore formation in early-diverging fungi. The hypothesis that internal spore formation by cytoplasmic cleavage seen in mitotic sporangia of Chytridiomycota or Zygomycota homologous to meiotic ascospore formation in Ascomycota would be worth testing with transcriptional genomic approaches. In most Ascomycota, turgor pressure generated in the mature ascus forcibly ejects the ascospores.

There are three deep clades of Ascomycota: Taphrinomycotina, Saccharomycotina and Pezizomycotina. The subphylum Pezizomycotina is home to almost all Ascomycota that protect their asci with multicellular structures, ranging from microscopic fruiting bodies to 25 cm tall morels. The subphylum Saccharomycotina contains the industrial yeasts, parasitic *Candida* species and, at the base of the clade, filamentous forms. No members of this subphylum, however, protect their asci with a fruiting body. The third subphylum, Taphrinomycotina [43], actually diverged before the other two. It contains species that have both yeasts and hyphae (*Taphrina*), species with just yeasts (*Schizosaccharomyces*, *Pneumocystis*), and one remarkable filamentous fungus, *Neolecta*, which makes a macroscopic fruitbody to support its asci [44]. Both filaments and yeasts are found in Taphrinomycotina (and Mucoromycotina), suggesting that both morphologies are ancestral in Ascomycota and that hyphae were lost early in the evolution of Saccharomycotina. Multicellular species with differentiated tissues are seen in Taphrinomycotina and Pezizomycotina, so this trait may have evolved early in the Ascomycota, only to be lost in the Saccharomycotina and all extant Taphrinomycotina, except *Neolecta*, or it may have evolved independently in *Neolecta* and Pezizomycotina.

Saccharomycotina [45] harbors a fungus that is famous and atypical, the baking and brewing yeast, *Saccharomyces cerevisiae*. It is primarily unicellular, although capable of polarized growth resembling hyphae. Natural isolates are diploid and meiosis leads to naked asci with ascospores that are not forcibly ejected. Most often, sibling ascospores mate to reestablish the diploid. If haploid colonies establish from single ascospores, they rapidly switch mating types, allowing them to essentially self-fertilize and become diploid. Rare mating with other genotypes is sufficient to maintain an outbred population [46]. Yeast genomes are small, introns were lost early in Saccharomycotina evolution [47], and these fungi do not appear to contain genes involved in RNA imterference (RNAi)-like gene regulation. In short, *S. cerevisiae* is an excellent model for the basic features of eukaryotes and for experimentation, but a poor model for other fungi. Although genomes of many Saccharomycotina have been sequenced, those of the very basal, filamentous taxa with forcible ascospore discharge, such as *Dipodascopsis*, have not. A *Dipodascopsis* genome sequence would stimulate studies of genome reduction and the loss of morphological complexity.

In Pezizomycotina [48], the largest and most diverse group of Ascomycota, hyphae are the rule. Mating of haploid partners results in short-lived, dikaryotic hyphae in which karyogamy and meiosis occur to produce asci and ascospores. The ancestral, widespread fruiting body in Pezizomycotina is a multicellular cup (apothecium) filled with asci (Figure 2I) and forcibly discharged ascospores. Apothecia have evolved into more enclosed fruiting bodies by narrowing the broad cup's surface to a pore — apparently independently in Sordariomycetes (*Neurospora, Ophiostoma*), Dothidiomycetes (*Cochliobolus*), Chaetothyriomycetidae (*Capronia*) and others — or by closing the cup completely — independently in Eurotiomycetes (*Emericella/Aspergillus*), Erysiphales (powdery mildews), Pezizales (truffles) and others. In most cases, closed fruiting bodies correlate with loss of forcible ascospore discharge, features that could only evolve after development of an alternative dispersal mechanism, for example, *Tuber* ascospores are dispersed by mammals attracted to truffles by fungal pheromones that mimic mammalian reproductive sterols [49].

Many Pezizomycotina, like truffles or the *Penicillium* species responsible for cheeses (Camembert, Brie and Roquefort), are socially-celebrated fungi. Alas, the socially-despised species are probably better known: *Ophiostoma*, worldwide devastator of elms; *Cryphonectria*, killer of four billion chestnuts in Eastern North America; *Fusarium*, principal pathogen of wheat, rice and banana and instrument of economic collapse in rural communities [50]; or the agents of the potentially fatal human mycoses histoplasmosis, blastomycosis, paracoccidioidomycosis and coccidioidomycosis. (*Coccidioides* species are also on the US government list of select terrorist agents).

Basidiomycota

Basidiomycota account for 34% of described fungi and comprise three subphyla, Pucciniomycotina, Ustilaginomycotina and Agaricomycotina, groups that are best known as containing the rusts, smuts and mushrooms, respectively. In all three groups, the growth form can be a yeast, a hypha or dimorphic. There can be one or two mating loci (one coding for a homeodomain transcription factor, the other for pheromone and receptor) each with from two to many alleles [51]. Mating is by fusion of yeast cells or hyphae with the involvement of oligopeptide mating pheromones similar to those seen in Ascomycota. Cell fusion produces a dikaryon that can grow for days, years, or even centuries before karyogamy and meiosis occur [52]. Recent research delving into dikaryons has shown that proportions of the two nuclei in a colony can vary as the environment changes [53] and that dikaryons are quicker to adapt to changed environments than their constituent haploids [54].

In dikaryotic hyphae, karyogamy and meiosis take place in terminal meiocytes (basidia). The meiotic spores (basidiospores) are not formed within the meiocyte, but develop on stalks that emerge from the surface of the basidium (Figure 2J). In all three subphyla, basidiospores are launched from the basidium by the shifting mass of a water drop (also a subject of high speed videography [55]). However, this ingenious process has often been lost wherever other means of spore discharge and dispersal have evolved. Pucciniomycotina (with the possible exception of *Septobasidium* [56]) and Ustilaginomycotina lack the multicellularity and differentiation of tissues seen in Agaricomycotina, indicating that multicellularity with differentiation of tissues developed independently in Ascomycota and Basidiomycota. Among the best-known Basidiomycota are wheat rust (*Puccinia graminis*), maize smut (*Ustilago maydis*), and any of ~21,000 described mushrooms (i.e., Agaricomycetes, most of which are mushrooms or close relatives [57]). Among the best model systems for genetics, development, and sexual reproduction are *U. maydis* [58] and the mushroom *Coprinopsis cinerea* [59].

Pucciniomycotina [60] probably diverged first among subphyla of Basidiomycota and shares some ancestral traits with Ascomycota, including regular septa with simple pores and mating loci within typically just two alleles. Most Pucciniomycotina species are obligate parasites of plants (rusts), but there are also parasites of insects (*Septobasidium*) and even parasites of fungi (including a remarkable fungus, *Helicobasidium*, which is parasitic on rust fungi as a haploid and on plant roots as a dikaryon) [61]. Pucciniomycotina can grow as hyphae, yeasts, or both, and the yeasts are often saprobic. The dikaryotic phase can be dominant, and basidia and basidiospores develop without protecting fruiting bodies. Many species can manipulate host behavior, *Microbotryum*, for example, reproduces in the anthers of its dioecious host and, if the plant is female, causes its flowers to switch to male [62].

The majority of Ustilaginomycotina [63] are parasitic on plants (smuts), almost exclusively on just two angiosperm families, grasses and sedges. The model organism in this group is *Ustilago maydis*, which grows as a saprobic yeast when haploid, and after mating as a dikaryotic, parasitic mycelium [58]. Mating is controlled by two loci, in contrast to the one-locus systems in the previously described; one of the loci has two alleles, but the other has many. Partners must have different alleles at both loci to mate, an arrangement that restricts inbreeding to 25% of siblings. Smuts are amazingly sneaky parasites, often lying in wait as endophytes before commandeering the plant's developing ovaries for their own reproduction. One Ustilaginomycotina, *Malassezia*, is among the few Basidiomycota parasitic on humans, albeit mildly; it causes dandruff.

The final clade, Agaricomycotina [64], is home to the most iconic of fungi: mushrooms and their allies. Different species of Agaricomycotina can grow as yeasts, as hyphae or as both. Species can have two mating loci and each locus can have many alleles, both restricting inbreeding and promoting outbreeding. In spite of this elegant control of mating, there are many self-fertile species. Multicellular fruiting bodies are the norm in Agaricomycotina and they come in seemingly endless variation. If basidiospores are forcibly launched, fruiting body form is evolved to increase the surface area for basidia, whether gills or tubes of a mushroom, branches of a coral fungus, or cerebriform folds of a jelly fungus. Where alternative methods of spore dispersal have evolved, fruitbody forms can only be described as bizarre: tiny bird-nest-shaped splash cups containing tiny 'eggs'; phallic columns topped by foul smelling ooze that attracts flies; small, pear-shaped bellows that puff spores, either perched on the soil or raised on columns or hygroscopic arches; soccer ball sized fungal tumble weeds filled with trillions of spores that gradually disperse; and small mortars that launch tiny cannonballs when turgid layers of the fruitbody separate catastrophically [65]. Agaricomycotina are socially important as food (*Agaricus* mushrooms), as agents of wood decay (dry rot fungi now starring in the biofuels field), as the human pathogen now causing

an outbreak of potentially-fatal cryptococcosis in Canada [66], and as ectomycorrhizae with most of the woody plants listed for Ascomycota [35].

Numbers of Fungi

We have been coy about the numbers of fungi, referring only to percentages of described fungi throughout. No one knows how many fungal species exist, although as many as 100,000 have been described and as many as 1.5 million have been estimated to exist in nature [67]. Population genetic studies of described species invariably find that one morphological species is actually several phylogenetic species [68], and metagenomic studies of alpine soils [69] or cultivation studies from beetles find new or greatly expanded clades [70]; 1.5 million species may be an underestimate. We can be far more concrete about the number of sequenced fungal genomes [1,71], which is at more than 100 for different species and at 70 for individuals of just two sibling species of yeast [72]. Comparative genomics at all levels is now the norm in fungi and has become an essential tool to help frame testable hypotheses in all fields of biology. The next decade of mycological research is going to be even more amazing than the last because next-generation sequencing will enable individual researchers to bring genomics to almost any fungus. Our challenge will be to maximize possible comparisons by making it possible for all of the data soon to be harvested in individual labs available to the community.

Acknowledgments

This work was supported by grants NIH NIAID R01AI070891 and NSF DEB 0516511 to JWT. JES was supported by a postdoctoral fellowship from the Miller Institute for Basic Research in Science. We thank B. Waaland for cultivating and preparing the fungi photographed in figures 2C and 2D.

References

1. Stajich JE. Fungal Genome Links. 2009 http://fungalgenomes.org/wiki/Fungal_Genome_Links.
2. Liolios K, Mavromatis K, Tavernarakis N, Kyrpides NC. The Genomes On Line Database (GOLD) in 2007: status of genomic and metagenomic projects and their associated metadata. Nucleic Acids Res 2008;36:D475–D479. [PubMed: 17981842]
3. Taylor JW, Berbee ML. Dating divergences in the Fungal Tree of Life: review and new analyses. Mycologia 2006;98:838–849. [PubMed: 17486961]
4. James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, et al. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 2006;443:818–822. [PubMed: 17051209]
5. Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Luecking R, et al. A higher-level phylogenetic classification of the Fungi. Mycological Research 2007;111:509–547. [PubMed: 17572334]
6. Multiple Authors. A phylogeny for the Kingdom Fungi: Deep hyphae issue. Mycologia 2006;98:829–1103. [PubMed: 17486960]
7. Alexopoulos, CJ.; Mims, CW.; Blackwell, M. Introductory Mycology. 4th Edition. New York: John Wiley and Sons; 1996.
8. Carlile, MJ.; Watkinson, SC.; Gooday, G. The Fungi. 2nd Edition. New York: Academic Press; 2001.
9. Steenkamp ET, Wright J, Baldauf SL. The protistan origins of animals and fungi. Mol Biol Evol 2006;23:93–106. [PubMed: 16151185]
10. Keeling PJ, Fast NM. Microsporidia: Biology and evolution of highly reduced intracellular parasites. Annu Rev Microbiol 2002;56:93–116. [PubMed: 12142484]
11. Williams BAP, Hirt RP, Lucocq JM, Embley TM. A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. Nature 2002;418:865–869. [PubMed: 12192407]

12. Held AA. Development of *Rozella* in *Allomyces* - a single zoospore produced numerous zoosporangia and resistant sporangia. Can J Bot 1980;58:959–979.
13. James TY, Letcher PM, Longcore JE, Mozley-Standridge SE, Porter D, Powell MJ, Griffith GW, Vilgalys R. A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia 2006;98:860–871. [PubMed: 17486963]
14. Miller CE, Dylewski DP. Syngamy and resting body development in *Chytriomyces hyalinus* (Chytridiales). Am J Bot 1981;68:342–349.
15. Longcore JE, Pessier AP, Nichols DK. *Batrachochytrium dendrobatidis* gen et sp nov, a chytrid pathogenic to amphibians. Mycologia 1999;91:219–227.
16. Gordon GLR, Phillips MW. The role of anaerobic gut fungi in ruminants. Nutr Res Rev 1998;11:133–168. [PubMed: 19087463]
17. van den Boogert PHJF, van Gent-Pelzer MPE, Bonants PJM, De Boer SH, Wander JGN, Levesque CA, van Leeuwen GCM, Baayen RP. Development of PCR-based detection methods for the quarantine phytopathogen *Synchytrium endobioticum*, causal agent of potato wart disease. Eur J Plant Pathol 2005;113:47–57.
18. Liu YJ, Hodson MC, Hall BD. Loss of the flagellum happened only once in the fungal lineage: phylogenetic structure of kingdom Fungi inferred from RNA polymerase II subunit genes. BMC Evol Biol 2006;6:74. [PubMed: 17010206]
19. Emerson R, Wilson CM. The significance of meiosis in *Allomyces*. Science 1949;110:86–88. [PubMed: 17837663]
20. Pommerville J. Analysis of gamete and zygote motility in *Allomyces*. Exp Cell Res 1978;113:161–172. [PubMed: 565293]
21. Whisler HC, Zebold SL, Shemanchuk JA. Life-history of *Coelomomyces psorophorae*. Proc Natl Acad Sci U S A 1975;72:693–696. [PubMed: 235761]
22. White MM, James TY, O'Donnell K, Cafaro MJ, Tanabe Y, Sugiyama J. Phylogeny of the Zygomycota based on nuclear ribosomal sequence data. Mycologia 2006;98:872–884. [PubMed: 17486964]
23. Haidle CW, Storck R. Control of dimorphism in *Mucor rouxii*. J Bacteriol 1966;92:1236–1244. [PubMed: 4288798]
24. Bracker CE. The ultrastructure and development of sporangia in *Gilbertella persicaria*. Mycologia 1968;60:1016–1067. [PubMed: 5700455]
25. Schachtschabel D, David A, Menzel K, Schimek C, Woestemeyer J, Boland W. Cooperative Biosynthesis of Trisporoids by the (+) and (−) Mating Types of the Zygomycete *Blakeslea trispora*. Chembiochem 2008;9:3004–3012. [PubMed: 19035372]
26. Idnurm A, Walton FJ, Floyd A, Heitman J. Identification of the sex genes in an early diverged fungus. Nature 2008;451:193–196. [PubMed: 18185588]
27. Yafetto L, Carroll L, Cui Y, Davis DJ, Fischer MW, Henterly AC, Kessler JD, Kilroy HA, Shidler JB, Stolze-Rybczynski JL, et al. The fastest flights in nature: high-speed spore discharge mechanisms among fungi. PLOS One 2008;3:e3237. [PubMed: 18797504]
28. Bergman K, Burke PV, Cerda-Olmedo E, David CN, Delbruck M, Foster KW, Goodell EW, Heisenberg M, Meissner G, Zalokar M, et al. Phycomyces. Bacteriol Rev 1969;33:99–157. [PubMed: 4889151]
29. Idnurm A, Rodriguez-Romero J, Corrochano LM, Sanz C, Iturriaga EA, Eslava AP, Heitman J. The *Phycomyces* madA gene encodes a blue-light photoreceptor for phototropism and other light responses. Proc Natl Acad Sci U S A 2006;103:4546–4551. [PubMed: 16537433]
30. URLs FG. 2009 http://genome.jgi-psf.org/Phycomyces/; http://www.broad.mit.edu/annotation/genome/rhizopus_oryzae/; http://mucorgen.um.es/.
31. Maitland DP. A parasitic fungus infecting yellow dungflies manipulates host perching behavior. Philos Trans R Soc Lond B Biol Sci 1994;258:187–193.
32. Roy HE, Steinkraus DC, Eilenberg J, Hajek AE, Pell JK. Bizarre interactions and endgames: Entomopathogenic fungi and their arthropod hosts. Annu Rev Entomol 2006;51:331–357. [PubMed: 16332215]
33. Whisler HC, Travland LB. Rotifer trap of *Zoophagous*. Arch Microbiol 1974;101:95–107.

34. Redecker D, Raab P. Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. Mycologia 2006;98:885–895. [PubMed: 17486965]
35. Smith, SE.; Read, DJ. Mycorrhizal Symbiosis. 3rd ed.. New York: Academic Press; 2008.
36. Bever JD. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytologist 2003;157:465–473.
37. Pawlowska TE. Genetic processes in arbuscular mycorrhizal fungi. Fems Microbiol Lett 2005;251:185–192. [PubMed: 16140474]
38. Croll D, Sanders IR. Recombination in *Glomus intraradices*, a supposed ancient asexual arbuscular mycorrhizal fungus. BMC Evol Biol 2009;9:13. [PubMed: 19146661]
39. Jedd G, Chua NH. A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. Nat Cell Biol 2000;2:226–231. [PubMed: 10783241]
40. Agrios, GN. Plant Pathology. 5th ed.. New York: Academic Press; 2005.
41. Heitman, J.; Filler, SG.; Mitchell, AP., editors. Molecular Principles of Fungal Pathogenesis. Washington, DC: ASM Press; 2006.
42. Butler, G. The evolution of MAT: the Ascomycetes. In: Heitman, J.; Kronstad, JW.; Taylor, JW.; Casselton, LA., editors. Sex in fungi: Molecular determination and evolutionary implications. Washington, DC: ASM Press; 2007.
43. Sugiyama J, Hosaka K, Suh S-O. Early diverging Ascomycota: phylogenetic divergence and related evolutionary enigmas. Mycologia 2006;98:996–1005. [PubMed: 17486975]
44. Landvik S, Schumacher TK, Eriksson OE, Moss ST. Morphology and ultrastructure of *Neolecta* species. Mycological Research 2003;107:1021–1031. [PubMed: 14563128]
45. Suh S-O, Blackwell M, Kurtzman CP, Lachance M-A. Phylogenetics of Saccharomycetales, the ascomycete yeasts. Mycologia 2006;98:1006–1017. [PubMed: 17486976]
46. Tsai IJ, Bensasson D, Burt A, Koufopanou V. Population genomics of the wild yeast *Saccharomyces paradoxus*: Quantifying the life cycle. Proc Natl Acad Sci U S A 2008;105:4957–4962. [PubMed: 18344325]
47. Stajich JE, Dietrich FS, Roy SW. Comparative genomic analysis of fungal genomes reveals intron-rich ancestors. Genome Biol 2007;8:R223. [PubMed: 17949488]
48. Spatafora JW, Sung G-H, Johnson D, Hesse C, O'Rourke B, Serdani M, Spotts R, Lutzoni F, Hofstetter V, Miadlikowska J, et al. A five-gene phylogeny of Pezizomycotina. Mycologia 2006;98:1018–1028. [PubMed: 17486977]
49. Claus R, Hoppen HO, Karg H. The secret of truffles - a steroidal pheromone. Experientia 1981;37:1178–1179.
50. Windels CE. Economic and social impacts of *Fusarium* head blight: Changing farms and rural communities in the Northern Great Plains. Phytopathology 2000;90:17–21. [PubMed: 18944567]
51. Fraser, JA.; Hsueh, Y-P.; Findley, KM.; Heitman, J. Evoltuion of the mating-type locus: the Basidiomycetes. In: Heitman, J.; Kronstad, JW.; Taylor, JW.; Casselton, LA., editors. Sex in fungi: Molecular determination and evolutionary implications. Washington, DC: ASM Press; 2007.
52. Smith ML, Bruhn JN, Anderson JB. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 1992;356:428–432.
53. James TY, Stenlid J, Olson A, Johannesson H. Evolutionary significance of imbalanced nuclear ratios within heterokaryons of the basidiomycete fungus *Heterobasidion parviporum*. Evolution 2008;62:2279–2296. [PubMed: 18637961]
54. Clark TA, Anderson JB. Dikaryons of the basidiomycete fungus *Schizophyllum commune*: Evolution in long-term culture. Genetics 2004;167:1663–1675. [PubMed: 15342506]
55. Pringle A, Patek SN, Fischer M, Stolze J, Money NP. The captured launch of a ballistospore. Mycologia 2005;97:866–871. [PubMed: 16457355]
56. Couch, JN. The genus *Septobasidium*. Chapel Hill, North Carolina: University of North Carolina Press; 1938.
57. Kirk, PM.; Cannon, PF.; Minter, DW.; Stalpers, JA., editors. Dictionary of Fungi. 10th ed. Wallingford: CABI; 2008. 10th Edition

58. Steinberg G, Perez-Martin J. *Ustilago maydis*, a new fungal model system for cell biology. Trends Cell Biol 2008;18:61–67. [PubMed: 18243705]
59. Kües U. Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol R 2000;64:316–353.
60. Aime MC, Matheny PB, Henk DA, Frieders EM, Nilsson RH, Piepenbring M, McLaughlin DJ, Szabo LJ, Begerow D, Sampaio JP, et al. An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. Mycologia 2006;98:896–905. [PubMed: 17486966]
61. Lutz M, Bauer R, Begerow D, Oberwinkler F. Tuberculina-*Thanatophytum/Rhizoctonia crocorum-Helicobasidium*: a unique mycoparasitic-phytoparasitic life strategy. Mycological Research 2004;108:227–238. [PubMed: 15185975]
62. Giraud T, Yockteng R, Lopez-Villavicencio M, Refregier G, Hood ME. Mating system of the anther smut fungus *Microbotryum violaceum*: Selfing under heterothallism. Eukaryot Cell 2008;7:765–775. [PubMed: 18281603]
63. Begerow D, Stoll M, Bauer R. A phylogenetic hypothesis of Ustilaginomycotina based on multiple gene analyses and morphological data. Mycologia 2006;98:906–916. [PubMed: 17486967]
64. Hibbett DS. A phylogenetic overview of the Agaricomycotina. Mycologia 2006;98:917–925. [PubMed: 17486968]
65. Hibbett DS. After the gold rush, or before the flood? Evolutionary morphology of mushroom-forming fungi (Agaricomycetes) in the early 21st century. Mycol Res 2007;111:1001–1018. [PubMed: 17964768]
66. Kidd SE, Hagen F, Tscharke RL, Huynh M, Bartlett KH, Fyfe M, MacDougall L, Boekhout T, Kwon-Chung KJ, Meyer W. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc Natl Acad Sci U S A 2004;101:17258–17263. [PubMed: 15572442]
67. Hawksworth DL. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological Research 2001;105:1422–1432.
68. Taylor JW, Turner E, Townsend JP, Dettman JR, Jacobson D. Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. Philos Trans R Soc Lond B Biol Sci 2006;361:1947–1963. [PubMed: 17062413]
69. Schadt CW, Martin AP, Lipson DA, Schmidt SK. Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 2003;301:1359–1361. [PubMed: 12958355]
70. Suh SO, McHugh JV, Blackwell M. Expansion of the *Candida tanzawaensis* yeast clade: 16 novel *Candida* species from basidiocarp-feeding beetles. International Journal of Systematic and Evolutionary Microbiology 2004;54:2409–2429. [PubMed: 15545491]
71. Liolios K, Mavromatis K, Tavernarakis N, Kyrpides NC. Nucleic Acids Res 36, D475-479. 2008 The Genomes On Line Database (GOLD) in 2007: status of genomic and metagenomic projects and their associated metadata. Nucleic Acids Research 2008;36:D475–D479. http://www.genomesonline.org/. [PubMed: 17981842]
72. Liti G, Carter DM, Moses AM, Warringer J, Parts L, James SA, Davey RP, Roberts IN, Burt A, Koufopanou V, et al. Population genomics of domestic and wild yeasts. Nature 2009;458:337–341. [PubMed: 19212322]
73. Sanderson MJ. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 2003;19:301–302. [PubMed: 12538260]

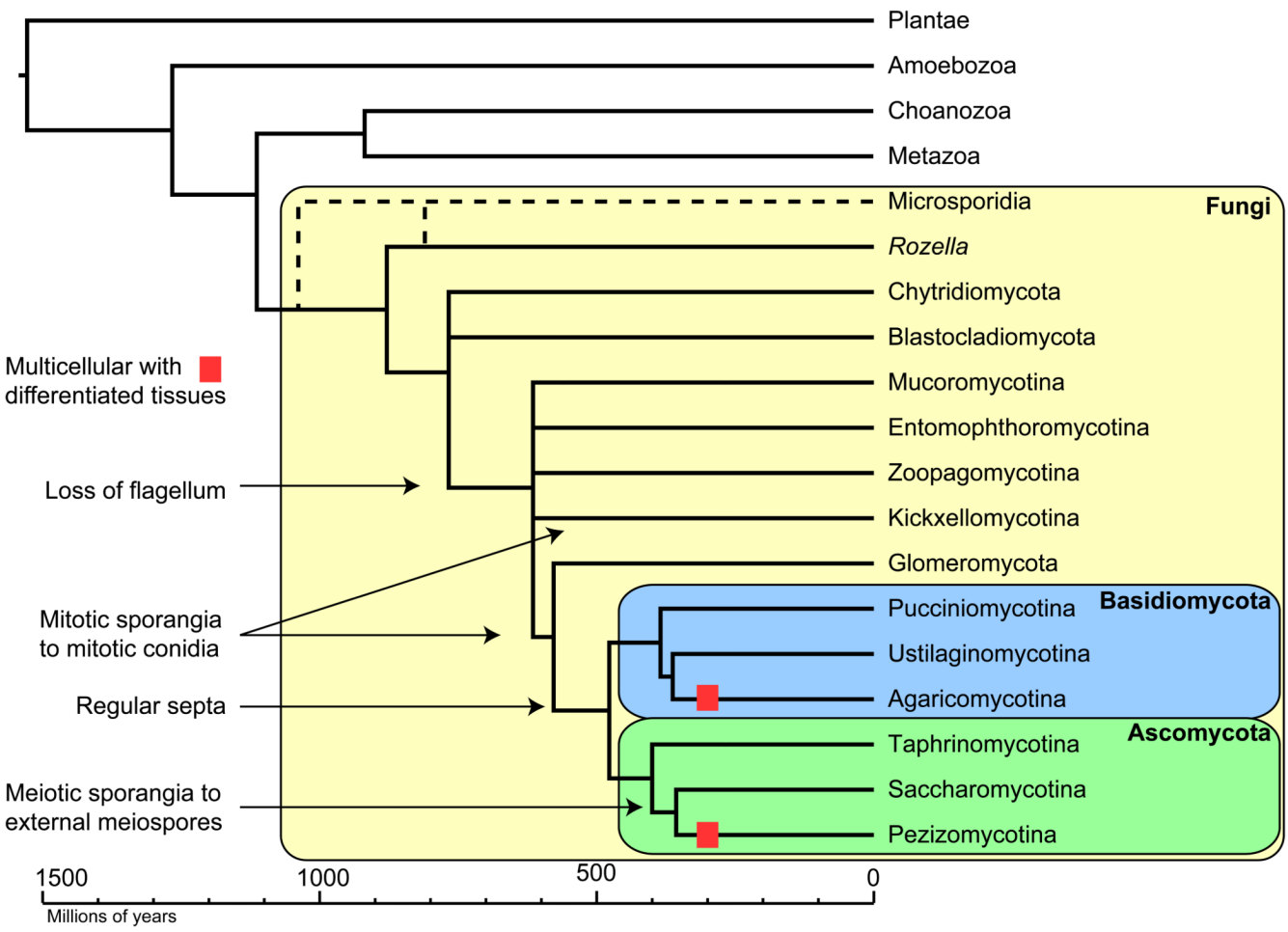


Figure 1.

The fungi. Phylogenetic tree, based on [4], showing relationships of many of the fungal lineages fit to geologic time using the program r8s [73] and considering *Paleopyrenomycites* to be a member of the Ascomycota [3]. Arrows depict changes in morphology including the major loss of the flagellum, transition of mitotic sporangia to mitotic conidia, invention of regular septa, and meiotic sporangia to external meiospores. The blocks indicate branches where most members have multicellular differentiated tissues. The phylogenetic position of the Microsporidia is not confidently resolved as indicated by the dotted line.

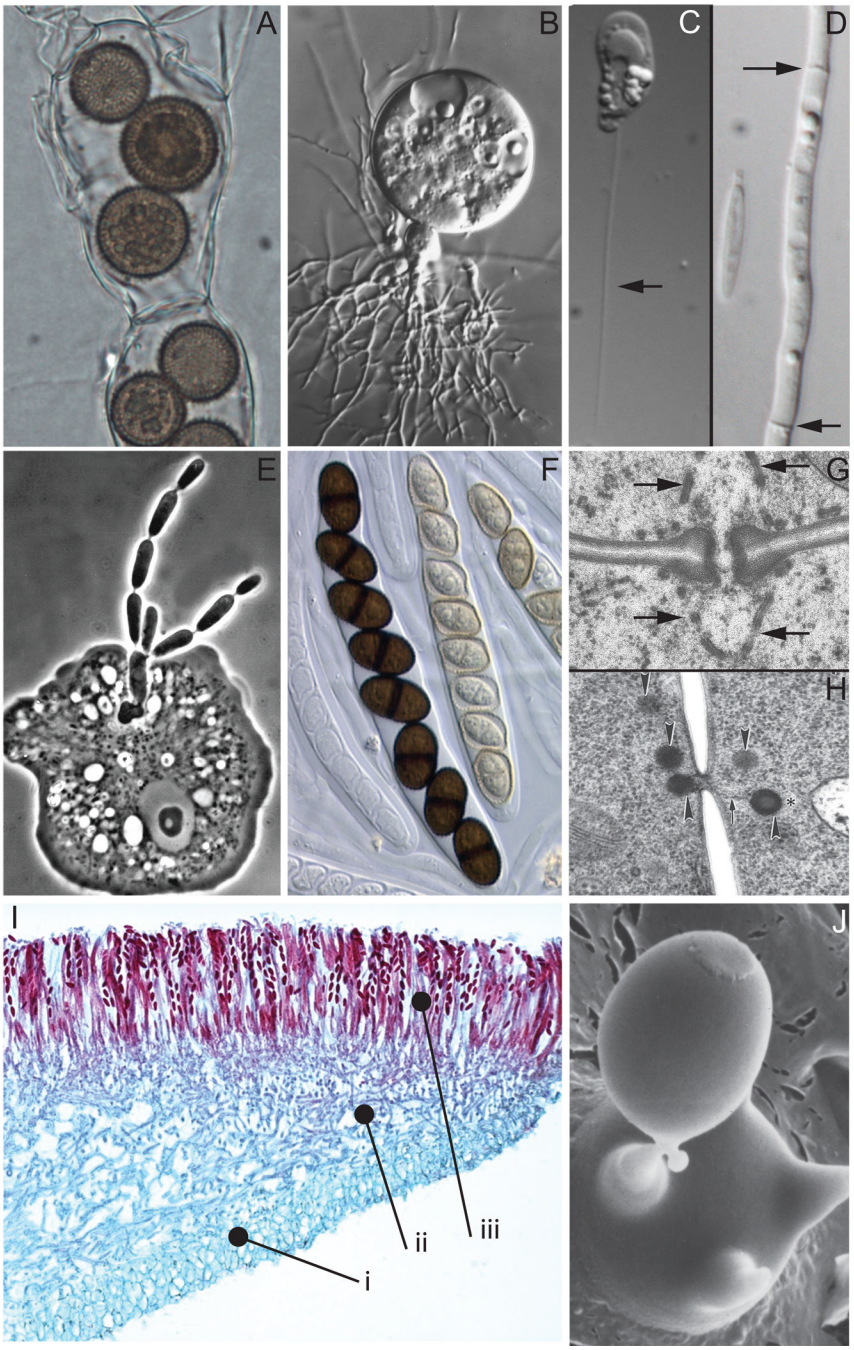


Figure 2.
Cellular structures of unicellular and multicellular fungi
A. *Rozella allomycis* resistant sporangia formed inside hyphae of the host *Allomyces sp.* (photomicrograph from T.Y. James).
B. *Chytriomyces hyalinus* (Chytridiomycota) sporangium showing the anucleate hyphae (rhizoids) essential for feeding the growing, spherical sporangium.
C. *Blastocladiella simplex* (Blastocladiomycota) zoospore with flagellum (arrow).
D. *Coemansia sp.* (Kickellomycotina) hypha with regular septa (arrows).
E. *Amoebophilus simplex* (Zoopagomycotina) on its amoeba host. Note the haustorium below the primary attack spore that initiated the infection. The primary attack spore and

haustorium become the body from which chains of spores develop (photomicrograph from G.L. Barron)

F. *Valsaria rubricosa* (Pezizomycotina) asci (meiocytes) at various stages of maturity, indicated by the increasing melanization of the ascospores. (photomicrograph from S.M. Huhndorf)

G. *Auriscalpium vulgare* (Agaricomycotina) hyphal septum with associated membranes (arrows) that regulate the flow of cytoplasm and organelles through the central pore. (TEM with permission from Celio GJ, Padamsee M, Dentinger BTM, Josephsen KA, Jenkinson TS, McLaughlin EG, McLaughlin DJ. Septal pore apparatus and nuclear division of *Auriscalpium vulgare*. Mycologia 2007; 99:644–654).

H. *Aspergillus nidulans* (Pezizomycotina) hyphal septum with Woronin bodies that can plug the pore when hyphae are damaged. (TEM with permission from Momany, M., Richardson EA, Van Sickle C, Jedd G. Mapping Woronin body position in *Aspergillus nidulans*. Mycologia 2002; 94:260–266.)

I. *Sclerotinia sclerotiorum* (Pezizomycotina) fruiting body showing the capacity of fungi to make a multicellular structure with differentiated tissues: Pseudoparenchymatous cortex (i), hyphal medulla (ii) and meiocytes (asci) and supporting hyphae in the hymenium (iii). (photomicrograph from J. Rollins)

J. *Coprinopsis cinerea* (Agaricomycotina) basidium with a mature basidiospore developing on one of four sterigma that emerge from the basidium. This partially frozen-hydrated specimen shows Buller’s drop of liquid developing at the base of the basidiospore, which is essential to spore discharge. (SEM with permission from McLaughlin DJ, Beckett A, Yoon KS. Ultrastructure and evolution of ballistosporic basidiospores. Bot J Linnean Society 1985; 91:253–271)

Primer

The Fungi

Jason E. Stajich[1,2], Mary L. Berbee[3], Meredith Blackwell[4], David S. Hibbett[5], Timothy Y. James[6], Joseph W. Spatafora[7], and John W. Taylor[1]

The Kingdom Fungi, home to molds, mushrooms, lichens, rusts, smuts and yeasts, comprises eukaryotes with remarkably diverse life histories that make essential contributions to the biosphere, human industry, medicine and research. With the aim of enticing biologists to include fungi in their research, we note that many fungi have haploid genetics, and that those in cultivation are essentially immortal, two features that make it easier to associate traits with genotype, even for complex traits, than with *Drosophila* or *Arabidopsis.* The typical fungal genome size of 30–40 Mb is small by eukaryotic standards, which is why fungi have led the way as models for eukaryote genome sequencing with over 100 assembled genome sequences available [1,2]. For some fungi, DNA transformations, gene knockouts and knockdowns are routine. Species of Ascomycota and Basidiomycota show simple, multicellular development with differentiated tissues. In many species these tissues are large enough to support studies of transcription and translation in the lab and even in nature.

About a billion years ago, give or take 500 million years [3], a population of aquatic, unicellular eukaryotes making sporangia containing zoospores each with a single posterior flagellum split into two lineages: one eventually gave rise to animals, the other to fungi. Here we shall summarize the major diversifications of the Fungi by introducing each major fungal branch in the order that it is thought to have diverged (Figure 1) and presenting salient facts about fungal modes of nutrition, reproduction, communication and interaction with other life. Our views are strongly influenced by the Fungal Tree of Life Project [4–6]. Readers interested in learning more about fungi are encouraged to consult any of a number of comprehensive texts [7,8].

The exact order of divergence in deep regions of the eukaryotic tree is controversial. On the lineage that leads to the Fungi there are thought to be two other groups; the first to diverge are the nucleariid amoebae, and the next the Microsporidia. Microsporidia are either the sister group to the Fungi, or lie within the Fungi (Figure 1), and they should be included in studies of fungi. They are unculturable, obligate parasites of animals, including humans. They have extremely reduced eukaryotic genomes — with a genome size of ~2.6 Mb and ~2000 genes [9] — remnant mitochondria, and unique morphologies related to parasitism, including a very frightening polar tube used to initiate infection [9].

Staying in the Kingdom Fungi, we next arrive at a divergence leading to *Rozella allomycis* [4] (Figure 2A), an intracellular parasite of the Blastocladiomycota fungus *Allomyces*. *Rozella* has a small body without a cell wall, which branches within the host and makes two types of sporangia: zoosporangia, which produce zoospores with posterior flagella that swim from the parent to find new hosts, and resistant sporangia, around which a thick cell wall develops to ensure persistence long after the host has died and decayed. Nothing is known about mating and meiosis in *Rozella*. Curiously, and like the Microsporidia, the lineage leading to *Rozella* diverged before that leading to its host, raising the worry that phylogenetic artifacts place parasite lineages at the base of phylogenies.

The next divergence leads to the Phylum Chytridiomycota, which constitute <1% of described fungi and, like *Rozella*, are presumed to retain key characters of the last common ancestor of Fungi and Animals [10]. These include a unicellular body bounded by a cell wall, which matures into a sporangium (Figure 2B), within which develop many posteriorly-uniflagellate zoospores (Figure 2C). The zoospores are cleaved from the sporangial cytoplasm by fusion of vesicles produced by a Golgi apparatus, and they swim to a fresh substrate, retract the flagellum, and secret a cell wall to encyst. The cyst germinates to start the life cycle anew. Although some Chytridiomycota have developed filamentous growth (hyphae), most have determinate development, and those living outside a substrate produce small, anucleate hyphae (rhizoids) that penetrate the food source. Species that live inside a host typically lack rhizoids, as does *Rozella*.

Chytriomyces hyalinus (Figure 2B) is the best studied Chytridiomycota species in terms of the morphology of sexual reproduction; however, no mating types are known. In this species, two individuals fuse at their rhizoids to form a thick-walled resistant spore. This fungus is a saprobe, but other Chytridiomycota, such as *Batrachochytrium*, are parasites, associated with amphibian decline, or like *Neocallimastix*, mutualists found in the stomachs of ruminate mammals. Another group member is one of the few fungi judged to be a weapon of terror, the agent causing potato black wart, *Synchytrium endobioticum*, infamous for making resting spores that can persist in soil for decades. Chytridiomycota have been thought to be haploid with zygotic meiosis, but DNA sequences of individual loci, as well as of the entire genome sequences of two *Batrachochytrium dendrobatidis* individuals (our unpublished data), raise the possibility that, like animals, these fungi can be diploid with gametic meiosis.

Back on the main fungal lineage, the next divergence leads to the Blastocladiomycota [10], the second phylum of Fungi with single, posterior flagella and home to *Allomyces*, the host for *Rozella allomycis*. Blastocladiomycota, once considered members of the Chytridiomycota, also account for <1% of described Fungi. Indeterminate, hyphal growth is better developed in Blastocladiomycota than in Chytridiomycota, although the hyphae often sprout rhizoids. Blastocladiomycota are unusual in alternating their haploid and diploid generations. Gametes in Blastocladiomycota resemble zoospores and, in *Allomyces*, female gametes produce a sesquiterpene pheromone, sirenin, that attracts male gametes. In these organsisms, meiosis occurs in thick-walled, resistant sporangia, but, as in Chytridiomycota, mating types are

unknown. Blastocladiomycota may be saprobic or parasitic on plants or animals; the best-studied animal parasite, *Coelomomyces*, kills mosquito larvae and copopods as it alternates generations.

Travelling back to the main branch, again, we encounter one of the major shifts in fungal form, the loss of the flagellum [3,4]. This loss is associated with two other major changes: from this point in evolution forward, all stages of fungal life cycles have cell walls, and the microtubule organizing centers of nuclear division no longer are centrioles. Released from the constraint of organizing both flagella and spindles, the microtubule organizing centers associated with spindles, known as spindle pole bodies, have diversified morphologically and probably functionally, as is likely to become apparent when genomes of fungi with and without flagella are compared.

The next five major clades on our march through the fungi, subphyla Mucoromycotina, Entomophthoromycotina, Zoopagomycotina, and Kickxellomycotina, and the phylum Glomeromycota, formerly constituted the phylum Zygomycota [11], and together account for <1% of described fungi. These taxa are organized into three clades, Mucoromycotina, Entomophthoromycotina + Zoopagomycotina + Kickxellomycotina and Glomeromycota, whose composition and relationships are not strongly supported.

Mucoromycotina, the best studied of this group, will be familiar to all who have found their fresh berries rendered inedible by enveloping wefts of white mycelium. These fungi are saprobes, commonly growing on damaged fruit but also on mammal dung. Among them are two genera of model fungi, for example *Rhizopus* and *Phycomyces*. These fungi grow primarily as hyphae, or as yeasts where oxygen is scarce and carbon dioxide is abundant. As in all the hyphal fungi encountered so far, septa are rare, apart from adventitious septa defining reproductive structures. Mitotic spores are formed in sporangia in a process very similar to zoospore formation within chytrid sporangia, but without flagella and with cell walls.

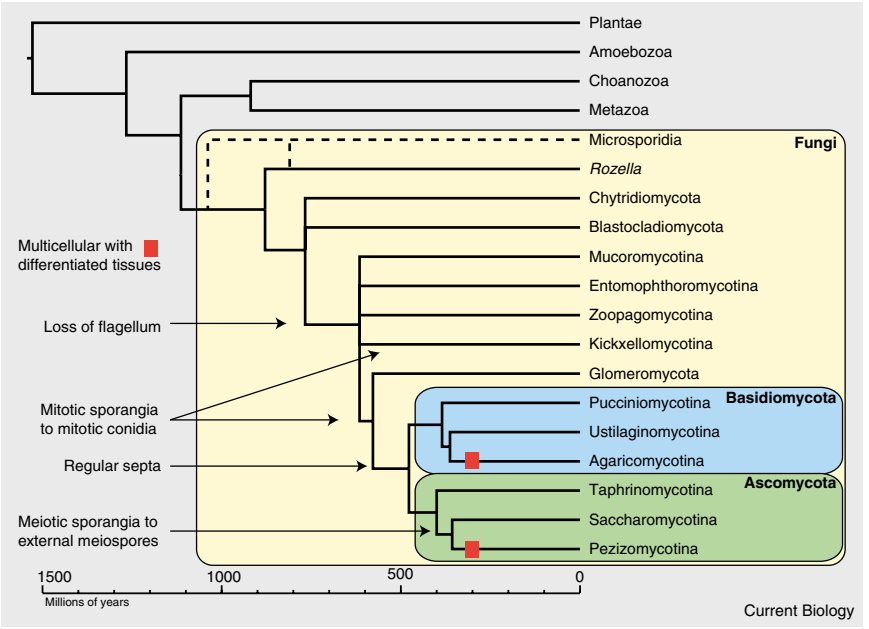


Figure 1. The Fungi.

Phylogenetic tree, based on [4], showing relationships of many of the fungal lineages fit to geologic time using the program r8s and considering *Paleopyrenomycites* to be a member of the Ascomycota [3]. Arrows depict changes in morphology including the major loss of the flagellum, transition of mitotic sporangia to mitotic conidia, invention of regular septa, and meiotic sporangia to external meiospores. The blocks indicate branches where most members have multicellular differentiated tissues. The phylogenetic position of the Microsporidia is not confidently resolved as indicated by the dotted line.

These fungi are haploid with zygotic meiosis. Sexual spores (zygospores) result when differentiated gametangia form and fuse, in a process involving pheromones derived from the carotenoid pathway. Mating compatibility is regulated by one mating locus with two alleles that encode a high mobility group (HMG) domain transcription factor related to the product of the human sex-determining gene *SRY*. Zygospores show little variation among members of the subphylum, but the diversity of mitospore morphology and dispersal is staggering. For example, *Pilobolus* launches its sporangium by water pressure (recently captured by high-speed videography [12]), *Gilbertella* presents its mitospores to insect vectors in a drop of liquid held between halves of the sporangial wall and *Phycomyces* perches the sporangium on a 10 cm stalk that, as it elongates, responds to light and can sense and avoid obstructions without the need for physical contact. The tremendous potential for developmental studies in these fungi has been given a boost by genome sequencing of several Mucoromycotina, among them *Phycomyces*, *Rhizopus* and *Mucor* [13].

Entomophthoromycotina, Zoopagomycotina and Kickxellomycotina form a single clade of Fungi that, like Mucoromycotina, are hyphal, produce thick-walled, sexual zygospores [11] and are haploid with zygotic meiosis. The best-studied of these clades is Entomophthoromycotina, aptly named parasites of insects that manipulate host behavior to promote the transmission of their mitospores. *Entomophthora muscae*, for example, induces its fly host, just before death, to attach itself to elevated vegetation while enlarging its abdomen to increase sexual attractiveness, all the better to lure males for spore transmission during pointless copulation. The large, multispored sporangia typical of Mucoromycotina are not the rule in this subphylum. Instead, what appear to be multispored sporangia are often single, multinucleate spores, termed conidia.

Zoopagomycotina comprise fungi that are parasites on animals or other fungi and that form haustoria, hyphae that are specialized to promote

nutrient transfer from host to fungus (Figure 2E). One likely member of Zoopagomycotina, *Zoophagus*, traps rotifers, amoebae or nematodes by attracting the tiny animals to feed on short, lateral hyphae that are covered with adhesives, so called 'lethal lollipops'. Kickxellomycotina comprise saprobes, mycoparasites of Mucoromycotina and animal parasites and, as with Entomophthoromycotina and Zoopagomycotina, they show reduced reliance on multispored sporangia and increased reliance on conidia (Figure 2E). With each mitosis in their hyphae, Kickxellomycotina produce septa containing central pores that, with age, become plugged (Figure 2D). The development of regular septa may have helped initiate the shift from spores cleaved from inside sporangia by fusion of vesicles to conidia formed by hyphal septation. This shift in the method of spore formation might have been a key evolutionary event, because conidia are well developed not only in Kickxellomycotina and Zoopagomycotina, but also in the two dominant groups of fungi, Ascomycota and Basidiomycota.

Glomeromycota [14], another species-poor group, is one of most ecologically-important groups of fungi, because of its mutualisms with the roots of ~90% of plant species, known as arbuscular mycorrhizae [15]. Arbuscular mycorrhizae, which are seen in below-ground parts of the earliest plant fossils, facilitate nutrient acquisition by plants in exchange for photosynthate; they are vital to plant fitness, and may drive the composition of plant communities. Arbuscular mycorrhizal fungi are hyphal and produce highly branched haustoria that promote nutrient exchange with host root cells. They also produce asexual, thick-walled multinucleate spores defined by adventitious septa. The fragmentary knowledge about most aspects of Glomeromycota biology belies their importance, because they cannot be cultivated apart from the host plant. For example, controversy clouds their ploidy, their genome size, and whether or not they reproduce sexually. Evidence for recombination has been provided, but whether mating and meiosis are involved is unknown. There might be no more important contribution to mycology than discovering how to axenically cultivate arbuscular mycorrhizae fungi.

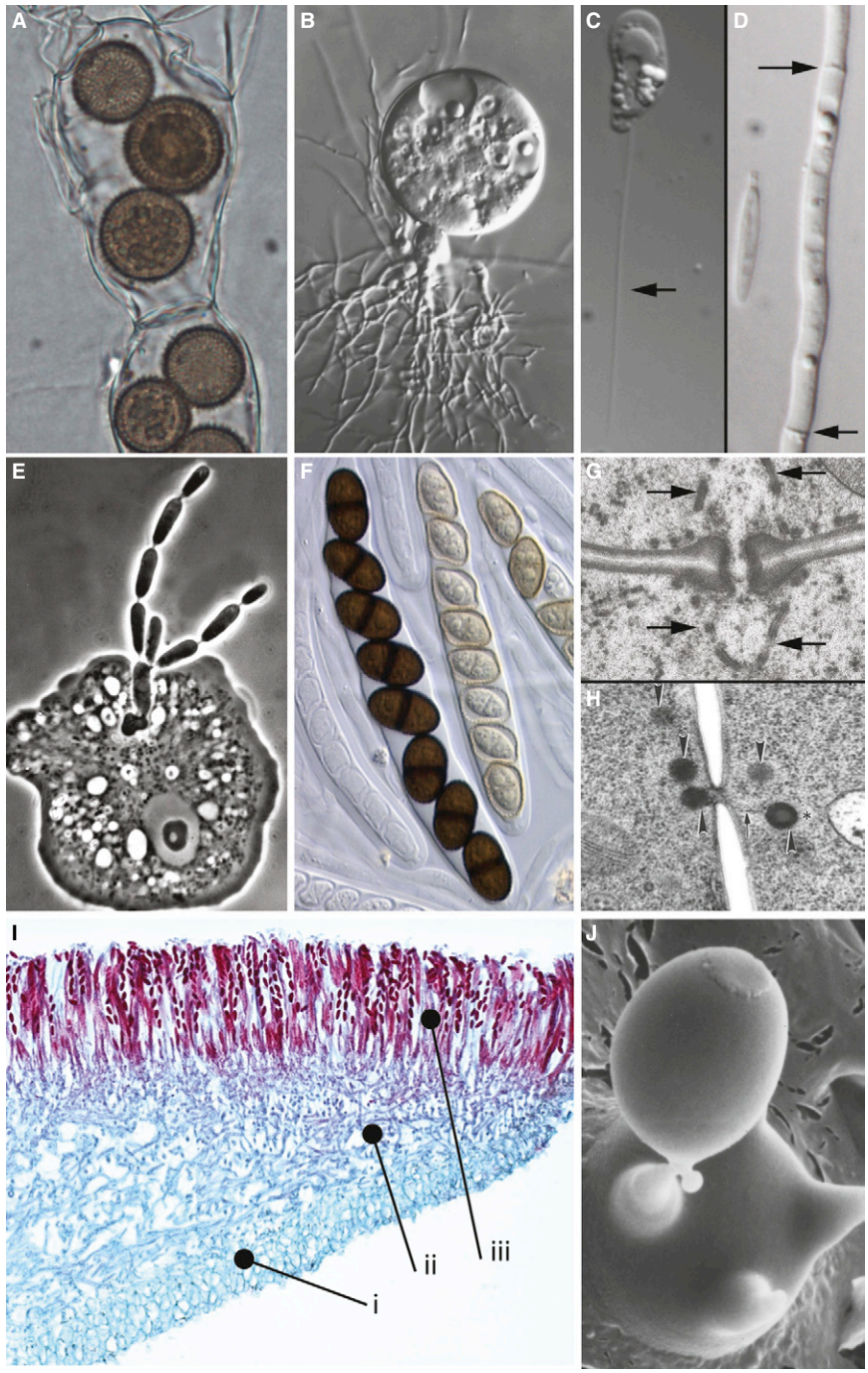
We now arrive at the Dikarya, a subkingdom embracing the two largest fungal phyla, Ascomycota and Basidiomycota, home to ~98% of described fungi. The name Dikarya emphasizes an amazing feature of mating in these fungi: nuclear fusion does not follow directly from gamete fusion, so that hyphae with two nuclei (a dikaryon), one from each parent, constitute a significant (in Ascomycota), or the most significant (in Basidiomycota), part of the life cycle. The role of the dikaryon in adaptation will be revisited when we get to Basidiomycota, but one advantage applies to all Dikarya: a dramatic increase in the diversity of recombined progeny. In earlier diverging phyla, most matings lead to one zygote and one meiotic event. In Dikarya, one mating can lead to zygotes and independent meioses that number in the tens of thousands (as in a *Neurospora* colony) or even hundreds of trillions (in long-lived Basidiomycota with large or perennial fruiting bodies, such as the puffballs of *Calvatia*, false truffles of *Rhizopogon*, or shelf fungi of *Ganoderma*).

Morphologically, species with hyphae or unicellular yeasts, or both, are common throughout Dikarya. In hyphae, mitosis is followed by septum formation to produce regular septa, as opposed to the adventitious septa found in most earlier-branching groups. These regular septa form centripetally and have central pores, most often with a means of regulating the passage of cytoplasm and organelles, including nuclei, between hyphal segments. Filters involve membranes in Basidiomycota (Figure 2G) or modified microbodies (Woronin bodies) in most Ascomycota (Figure 2H). The advent of regular septa is also correlated with the evolution of macroscopic, multicellular fungi where different hyphal segments evolve to perform different functions, for example, the stalk, cap and gills of an *Agaricus* mushroom (Basidiomycota) or the stalk, cup and ascus layer of a morel (Ascomycota). Remarkably, it appears that multicellularity with differentiated tissues evolved independently in each phylum.

Ascomycota is the larger taxon of Dikarya, with ~64% of described fungi, including species in four genera that helped researchers win Nobel Prizes (*Penicillium*, *Neurospora*, *Saccharomyces* and *Schizosaccharomyces*). Although each of these fungi is best known from laboratory studies, Ascomycota in nature earn their livings in all possible ways, as saprobes, as mutualists (forming lichens with algae or ectomycorrhizae with woody plants in Pinaceae, Fagales, Dipterocarpaceae, Fabaceae and Ericaceae), and as parasites. Pathogenic Ascomycota pose as great a threat to agriculture as any group of organisms, and parasitic Ascomycota adapted to animals account for almost all the severe, systemic human mycoses as well as athlete's foot and similar fungal skin diseases [16].

These fungi are typically haploid with one mating locus occuring as two alleles. The alleles are so diverged that they are termed idiomorphs and they code for homeodomain, alpha box and HMG-domain transcription factors. Potential partners communicate by oligopeptide pheromones. In hyphal species, mating leads to a short dikaryotic stage that produces a multitude of zygotes and meiocytes (asci) as previously mentioned. Sporangia with internal mitospores are not found in Ascomycota. Instead conidia are the means of asexual reproduction. Within asci, however, meiotic spores (ascospores) form as membranous vesicles fuse to delimit uninucleate portions of cytoplasm (Figure 2F) in a process reminiscent of sporangiospore formation in early-diverging fungi. The hypothesis that internal spore formation by cytoplasmic cleavage seen in mitotic sporangia of Chytridiomycota or Zygomycota homologous to meiotic ascospore formation in Ascomycota would be worth testing with transcriptional genomic approaches. In most Ascomycota, turgor pressure generated in the mature ascus forcibly ejects the ascospores.

There are three deep clades of Ascomycota: Taphrinomycotina, Saccharomycotina and Pezizomycotina. The subphylum Pezizomycotina is home to almost all Ascomycota that protect their asci with multicellular structures, ranging from microscopic fruiting bodies to 25 cm tall morels. The subphylum Saccharomycotina contains the industrial yeasts,



Current Biology

Figure 2. Cellular structures of unicellular and multicellular fungi.

(A) *Rozella allomycis* resistant sporangia formed inside hyphae of the host *Allomyces* sp. (Photomicrograph from T.Y. James.) (B) *Chytriomyces hyalinus* (Chytridiomycota) sporangium showing the anucleate hyphae (rhizoids) essential for feeding the growing, spherical sporangium. (C) *Blastocladiella simplex* (Blastocladiomycota) zoospore with flagellum (arrow). (D) *Coemansia* sp. (Kickellomycotina) hypha with regular septa (arrows). (E) *Amoebophilus simplex* (Zoopagomycotina) on its amoeba host. Note the haustorium below the primary attack spore that initiated the infection. The primary attack spore and haustorium become the body from which chains of spores develop. (Photomicrograph from G.L. Barron.) (F) *Valsaria rubricosa* (Pezizomycotina) asci (meiocytes) at various stages of maturity, indicated by the increasing melanization of the ascospores. (Photomicrograph from S.M. Huhndorf.) (G) *Auriscalpium vulgare* (Agaricomycotina) hyphal septum with associated membranes (arrows) that regulate the flow of cytoplasm and organelles through the central pore. (TEM reproduced with permission from Celio *et al*. 2007, Septal pore apparatus and nuclear division of *Auriscalpium vulgare*. Mycologia *99*, 644–654.) (H) *Aspergillus nidulans* (Pezizomycotina) hyphal septum with Woronin bodies that can plug the pore when hyphae are damaged. (TEM reproduced with permission from Momany *et al.* 2002, Mapping Woronin body position in *Aspergillus nidulans*. Mycologia *94*, 260–266.) (I) *Sclerotinia sclerotiorum* (Pezizomycotina) fruiting body showing the capacity of fungi to make a multicellular structure with differentiated tissues: Pseudoparenchymatous cortex (i), hyphal medulla (ii) and meiocytes (asci) and supporting hyphae in the hymenium (iii). (Photomicrograph from J. Rollins.) (J) *Coprinopsis cinerea* (Agaricomycotina) basidium with a mature basidiospore developing on one of four sterigma that emerge from the basidium. This partially frozen-hydrated specimen shows Buller's drop of liquid developing at the base of the basidiospore, which is essential to spore discharge. (SEM reproduced with permission from McLaughlin *et al.* 1985, Ultrastructure and evolution of ballistosporic basidiospores. Bot. J. Linn. Soc. *91*, 253–271.)

parasitic *Candida* species and, at the base of the clade, filamentous forms. No members of this subphylum, however, protect their asci with a fruiting body. The third subphylum, Taphrinomycotina [17], actually diverged before the other two. It contains species that have both yeasts and hyphae (*Taphrina*), species with just yeasts (*Schizosaccharomyces*, *Pneumocystis*), and one remarkable filamentous fungus, *Neolecta*, which makes a macroscopic fruitbody to support its asci. Both filaments and yeasts are found in Taphrinomycotina (and Mucoromycotina), suggesting that both morphologies are ancestral in Ascomycota and that hyphae were lost early in the evolution of Saccharomycotina. Multicellular species with differentiated tissues are seen in Taphrinomycotina and Pezizomycotina, so this trait may have evolved early in the Ascomycota, only to be lost in the Saccharomycotina and all extant Taphrinomycotina, except *Neolecta*, or it may have evolved independently in *Neolecta* and Pezizomycotina.

Saccharomycotina [18] harbors a fungus that is famous and atypical, the baking and brewing yeast, *Saccharomyces cerevisiae*. It is primarily unicellular, although capable of polarized growth resembling hyphae. Natural isolates are diploid and meiosis leads to naked asci with ascospores that are not

forcibly ejected. Most often, sibling ascospores mate to reestablish the diploid. If haploid colonies establish from single ascospores, they rapidly switch mating types, allowing them to essentially self-fertilize and become diploid. Rare mating with other genotypes is sufficient to maintain an outbred population. Yeast genomes are small, introns were lost early in Saccharomycotina evolution, and these fungi do not appear to contain genes involved in RNA interference (RNAi)-like gene regulation. In short, *S. cerevisiae* is an excellent model for the basic features of eukaryotes and for experimentation, but a poor model for other fungi. Although genomes of many Saccharomycotina have been sequenced, those of the very basal, filamentous taxa with forcible ascospore discharge, such as *Dipodascopsis*, have not. A *Dipodascopsis* genome sequence would stimulate studies of genome reduction and the loss of morphological complexity.

In Pezizomycotina [19], the largest and most diverse group of Ascomycota, hyphae are the rule. Mating of haploid partners results in short-lived, dikaryotic hyphae in which karyogamy and meiosis occur to produce asci and ascospores. The ancestral, widespread fruiting body in Pezizomycotina is a multicellular cup (apothecium) filled with asci (Figure 2I) and forcibly discharged ascospores. Apothecia have evolved into more enclosed fruiting bodies by narrowing the broad cup's surface to a pore — apparently independently in Sordariomycetes (*Neurospora*, *Ophiostoma*), Dothidiomycetes (*Cochliobolus*), Chaetothyriomycetidae (*Capronia*) and others — or by closing the cup completely — independently in Eurotiomycetes (*Emericella/Aspergillus*), Erysiphales (powdery mildews), Pezizales (truffles) and others. In most cases, closed fruiting bodies correlate with loss of forcible ascospore discharge, features that could only evolve after development of an alternative dispersal mechanism, for example, *Tuber* ascospores are dispersed by mammals attracted to truffles by fungal pheromones that mimic mammalian reproductive sterols.

Many Pezizomycotina, like truffles or the *Penicillium* species responsible for cheeses (Camembert, Brie and Roquefort), are socially-celebrated fungi. Alas, the socially-despised species are probably better known: *Ophiostoma,* worldwide devastator of elms; *Cryphonectria,* killer of four billion chestnuts in Eastern North America; *Fusarium*, principal pathogen of wheat, rice and banana and instrument of economic collapse in rural communities; or the agents of the potentially fatal human mycoses histoplasmosis, blastomycosis, paracoccidioidomycosis and coccidioidomycosis. (*Coccidioides* species are also on the US government list of select terrorist agents.)

Basidiomycota account for 34% of described fungi and comprise three subphyla, Pucciniomycotina, Ustilaginomycotina and Agaricomycotina, groups that are best known as containing the rusts, smuts and mushrooms, respectively. In all three groups, the growth form can be a yeast, a hypha or dimorphic. There can be one or two mating loci (one coding for a homeodomain transcription factor, the other for pheromone and receptor) each with from two to many alleles. Mating is by fusion of yeast cells or hyphae with the involvement of oligopeptide mating pheromones similar to those seen in Ascomycota. Cell fusion produces a dikaryon that can grow for days, years, or even centuries before karyogamy and meiosis occur. Recent research delving into dikaryons has shown that proportions of the two nuclei in a colony can vary as the environment changes [20] and that dikaryons are quicker to adapt to changed environments than their constituent haploids [21].

In dikaryotic hyphae, karyogamy and meiosis take place in terminal meiocytes (basidia). The meiotic spores (basidiospores) are not formed within the meiocyte, but develop on stalks that emerge from the surface of the basidium (Figure 2J). In all three subphyla, basidiospores are launched from the basidium by the shifting mass of a water drop (also a subject of high speed videography [22]). However, this ingenious process has often been lost wherever other means of spore discharge and dispersal have evolved. Pucciniomycotina (with the possible exception of *Septobasidium*) and Ustilaginomycotina lack the multicellularity and differentiation of tissues seen in Agaricomycotina, indicating that multicellularity with differentiation of tissues developed independently in Ascomycota and Basidiomycota. Among the best-known Basidiomycota are wheat rust (*Puccinia graminis*), maize smut (*Ustilago maydis*), and any of ~8,000 described mushrooms. Among the best model systems for genetics, development, and sexual reproduction are *U. maydis* and the mushroom *Coprinopsis cinerea*.

Pucciniomycotina [23] probably diverged first among subphyla of Basidiomycota and shares some ancestral traits with Ascomycota, including regular septa with simple pores and mating loci with typically just two alleles. Most Pucciniomycotina species are obligate parasites of plants (rusts), but there are also parasites of insects (*Septobasidium*) and even parasites of fungi (including a remarkable fungus, *Helicobasidium,* which is parasitic on rust fungi as a haploid and on plant roots as a dikaryon). Pucciniomycotina can grow as hyphae, yeasts, or both, and the yeasts are often saprobic. The dikaryotic phase can be dominant, and basidia and basidiospores develop without protecting fruiting bodies. Many species can manipulate host behavior; *Microbotryum*, for example, reproduces in the anthers of its dioecious host and, if the plant is female, causes its flowers to switch to male.

The majority of Ustilaginomycotina [24] are parasitic on plants (smuts), almost exclusively on just two angiosperm families, grasses and sedges. The model organism in this group is *Ustilago maydis*, which grows as a saprobic yeast when haploid, and after mating as a dikaryotic, parasitic mycelium. Mating is controlled by two loci, in contrast to the one-locus system in the previously described fungal phyla; one of the loci has two alleles, but the other has many. Partners must have different alleles at both loci to mate, an arrangement that restricts inbreeding to 25% of siblings. Smuts are amazingly sneaky parasites, often lying in wait as endophytes before commandeering the plant's developing ovaries for their own reproduction. One Ustilaginomycotina, *Malassezia*, is among the few Basidiomycota

parasitic on humans, albeit mildly; it causes dandruff.

The final clade, Agaricomycotina [25], is home to the most iconic of fungi: mushrooms and their allies. Different species of Agaricomycotina can grow as yeasts, as hyphae or as both. Species can have two mating loci and each locus can have many alleles, both restricting inbreeding and promoting outbreeding. In spite of this elegant control of mating, there are many self-fertile species. Multicellular fruiting bodies are the norm in Agaricomycotina and they come in seemingly endless variation. If basidiospores are forcibly launched, fruiting body form is evolved to increase the surface area for basidia, whether gills or tubes of a mushroom, branches of a coral fungus, or cerebriform folds of a jelly fungus. Where alternative methods of spore dispersal have evolved, fruitbody forms can only be described as bizarre: tiny bird-nest-shaped splash cups containing tiny 'eggs'; phallic columns topped by foul smelling ooze that attracts flies; small, pear-shaped bellows that puff spores, either perched on the soil or raised on columns or hygroscopic arches; soccer ball sized fungal tumble weeds filled with trillions of spores that gradually disperse; and small mortars that launch tiny cannonballs when turgid layers of the fruitbody separate catastrophically. Agaricomycotina are socially important as food (*Agaricus* mushrooms), as agents of wood decay (dry rot fungi now starring in the biofuels field), as the human pathogen now causing an outbreak of potentially-fatal cryptococcosis in Canada, and as ectomycorrhizae with most of the woody plants listed for Ascomycota [15].

We have been coy about the numbers of fungi, referring only to percentages of described fungi throughout. No one knows how many fungal species exist, although as many as 100,000 have been described and as many as 1.5 million have been estimated to exist in nature. Population genetic studies of described species invariably find that one morphological species is actually several phylogenetic species, and metagenomic studies of alpine soils or cultivation studies from beetles find new or greatly expanded clades; 1.5 million species may be an underestimate. We can be far more concrete about the number of sequenced fungal genomes [1,2], which is at more than 100 for different species and at 70 for individuals of just two sibling species of yeast. Comparative genomics at all levels is now the norm in fungi and has become an essential tool to help frame testable hypotheses in all fields of biology. The next decade of mycological research is going to be even more amazing than the last because next-generation sequencing will enable individual researchers to bring genomics to almost any fungus. Our challenge will be to maximize possible comparisons by making it possible for all of the data soon to be harvested in individual labs available to the community.

Acknowledgments

This work was supported by grants NIH NIAID R01AI070891 and NSF DEB 0516511 to J.W.T. J.E.S. was supported by a postdoctoral fellowship from the Miller Institute for Basic Research in Science. We thank B. Waaland for cultivating and preparing the fungi photographed in Figures 2C and 2D.

References

1. Stajich, J.E. (2009). Fungal Genome Links: http://fungalgenomes.org/wiki/Fungal_Genome_Links.
2. Liolios, K., Mavromatis, K., Tavernarakis, N., and Kyrpides, N.C. (2008). The Genomes On Line Database (GOLD). Nucleic Acids Res. *36*, D475–D479.
3. Taylor, J.W., and Berbee, M.L. (2006). Dating divergences in the Fungal Tree of Life: review and new analyses. Mycologia *98*, 838–849.
4. James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., *et al.* (2006). Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature *443*, 818–822.
5. Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Luecking, R., *et al.* (2007). A higher-level phylogenetic classification of the Fungi. Mycological Res. *111*, 509–547.
6. Multiple authors. (2006). A phylogeny for the Kingdom Fungi: Deep hyphae issue. Mycologia *98*, 829–1103.
7. Alexopoulos, C.J., Mims, C.W., and Blackwell, M. (1996). Introductory Mycology, 4th Edition, (New York: John Wiley and Sons).
8. Carlile, M.J., Watkinson, S.C., and Gooday, G. (2001). The Fungi, 2nd Edition, (New York: Academic Press).
9. Keeling, P.J., and Fast, N.M. (2002). Microsporidia: Biology and evolution of highly reduced intracellular parasites. Annu. Rev. Microbiol. *56*, 93–116.
10. James, T.Y., Letcher, P.M., Longcore, J.E., Mozley-Standridge, S.E., Porter, D., Powell, M.J., Griffith, G.W., and Vilgalys, R. (2006). A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia *98*, 860–871.
11. White, M.M., James, T.Y., O'Donnell, K., Cafaro, M.J., Tanabe, Y., and Sugiyama, J. (2006). Phylogeny of the Zygomycota based on nuclear ribosomal sequence data. Mycologia *98*, 872–884.
12. Yafetto, L., Carroll, L., Cui, Y., Davis, D.J., Fischer, M.W., Henterly, A.C., Kessler, J.D., Kilroy, H.A., Shidler, J.B., Stolze-Rybczynski, J.L., *et al*. (2008). The fastest flights in nature: high-speed spore discharge mechanisms among fungi. PLOS One *3*, e3237.
13. Genome websites (2009). http://genome.jgi-psf.org/Phycomyces/; http://www.broad.mit.edu/annotation/genome/rhizopus_oryzae/; http://mucorgen.um.es/.
14. Redecker, D., and Raab, P. (2006). Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. Mycologia *98*, 885–895.
15. Smith, S.E., and Read, D.J. (2008). Mycorrhizal Symbiosis, 3rd ed., (New York: Academic Press).
16. Heitman, J., Filler, S.G., and Mitchell, A.P. eds. (2006). Molecular Principles of Fungal Pathogenesis (Washington DC: ASM Press).
17. Sugiyama, J., Hosaka, K., and Suh, S.-O. (2006). Early diverging Ascomycota: phylogenetic divergence and related evolutionary enigmas. Mycologia *98*, 996–1005.
18. Suh, S.-O., Blackwell, M., Kurtzman, C.P., and Lachance, M.-A. (2006). Phylogenetics of Saccharomycetales, the ascomycete yeasts. Mycologia *98*, 1006–1017.
19. Spatafora, J.W., Sung, G.-H., Johnson, D., Hesse, C., O'Rourke, B., Serdani, M., Spotts, R., Lutzoni, F., Hofstetter, V., Miadlikowska, J., *et al*. (2006). A five-gene phylogeny of Pezizomycotina. Mycologia *98*, 1018–1028.
20. James, T.Y., Stenlid, J., Olson, A., and Johannesson, H. (2008). Evolutionary significance of imbalanced nuclear ratios within heterokaryons of the basidiomycete fungus *Heterobasidion parviporum*. Evolution *62*, 2279–2296.
21. Clark, T.A., and Anderson, J.B. (2004). Dikaryons of the basidiomycete fungus *Schizophyllum commune*: Evolution in long-term culture. Genetics *167*, 1663–1675.
22. Pringle, A., Patek, S.N., Fischer, M., Stolze, J., and Money, N.P. (2005). The captured launch of a ballistospore. Mycologia *97*, 866–871.
23. Aime, M.C., Matheny, P.B., Henk, D.A., Frieders, E.M., Nilsson, R.H., Piepenbring, M., McLaughlin, D.J., Szabo, L.J., Begerow, D., Sampaio, J.P., *et al*. (2006). An overview of the higher-level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. Mycologia *98*, 896–905.
24. Begerow, D., Stoll, M., and Bauer, R. (2006). A phylogenetic hypothesis of Ustilaginomycotina based on multiple gene analyses and morphological data. Mycologia *98*, 906–916.
25. Hibbett, D.S. (2006). A phylogenetic overview of the Agaricomycotina. Mycologia *98*, 917–925.

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA. [2]Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA. [3]Department of Botany, University of British Columbia, Vancouver, BC V6T 1Z4, Canada. [4]Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA. [5]Biology Department, Clark University, Worcester, MA 01610 USA. [6]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA. [7]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.
E-mail: jason.stajich@ucr.edu, jtaylor@nature.berkeley.edu