

A new fungal phylum, the *Glomeromycota*: phylogeny and evolution*

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The ecologically and economically important arbuscular mycorrhizal (AM) fungi, crucial in the ecology and physiology of land plants, and the endocytobiotic fungus, *Geosiphon pyriformis*, are phylogenetically analysed by their small subunit (SSU) rRNA gene sequences. They can, from molecular, morphological and ecological characteristics, unequivocally be separated from all other major fungal groups in a monophyletic clade. Consequently they are removed from the polyphyletic *Zygomycota*, and placed into a new monophyletic phylum, the *Glomeromycota*.

The recognition of this monophyletic group, which probably diverged from the same common ancestor as the *Ascomycota* and *Basidiomycota*, gives these fungi their proper status, and provides a basis for a new and natural systematics of these fascinating, yet largely hidden organisms, with three new orders (*Archaeosporales*, *Paraglomerales*, *Diversisporales*) described herein. Additionally, several clades resolve at family level; their formal description is in progress.

INTRODUCTION

Importance of arbuscular mycorrhizal (AM) fungi

One of the most widely distributed, ecologically and economically important fungal groups are the arbuscular mycorrhizal (AM) fungi, currently included in the order *Glomales* (Morton & Benny 1990) or, orthographically more correctly, the *Glomerales*,¹ and placed in the phylum *Zygomycota*. All the AM fungi are, as far as is known, obligately symbiotic, asexual organisms. For the species with known biology, these fungi obtain their organic nutrients through an obligate symbiosis with vascular plants, the arbuscular mycorrhiza (AM). The described species of *Glomerales* for which biological knowledge is lacking (i.e. those known only from herbarium collections), are assumed, by analogy, to be similarly symbiotic.

For most land plants, mycorrhizas rather than the roots alone are the organs of uptake for the poorly-labile phosphate ion (Smith & Read 1997), and under certain circumstances also for other inorganic ions. The AM fungi explore the soil or other substrata through an extensive mycelium. Plants acquire inorganic nutrients through the fungus, whilst the fungus obtains carbohydrates from the plant. The nutrient exchange between the partners of such symbioses takes place *via* complex ‘intracellular’ symbiotic interfaces.

Members of more than 80% of extant vascular plant families form AM and ‘primitive’ plants such as hepatics and hornworts also produce AM-like symbioses (e.g. Schüßler 2000). Moreover, one apparently unique symbiosis represents a consortium of *Cyanobacteria* (blue-green algae) and an obligately symbiotic, *Glomerales*-related fungus, *Geosiphon pyriformis*. In this case the situation is reversed compared with AM fungi, as the fungus is the macrosymbiont (exhabitant) and the photosynthetic partner the microsymbiont or inhabitant (Schüßler *et al.* 1996, Schüßler & Kluge 2001). Several recent articles have drawn attention to the importance of the AM fungi. They, for example, influence plant biodiversity (van der Heijden *et al.* 1998), help to control pests (e.g. nematodes) and fungal pathogens (Azcon-Aguilar & Barea 1996), and affect the fitness of plants in polluted environments (Hildebrandt, Kaldorf & Bothe 1999). The AM fungi thus have a profound influence, directly or indirectly, on life on land. However, the phylogeny of this important fungal group and its placement within the *Fungi* was still unclear.

* Dedicated to Manfred Kluge (Technische Universität Darmstadt) on the occasion of his retirement.

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¹ Under the provisions of the International Code of Botanical Nomenclature (Greuter *et al.* 2000), the name of a family is formed from the genitive singular of a legitimate name of an included genus by replacing the genitive singular inflection with the termination *-aceae*. The genitive of *Glomus* is *Glomeris*, and hence the name of the family should be *Glomeraceae*. This is reflected in all higher taxon names, which should consequently be *Glomerales*, etc. The same argument must also be applied to *Paraglomus*. The *Code* allows for such errors to be corrected (Art. 61.4), which we do herein to avoid further incorrect naming.

Earlier classification of the AM fungi

When the first fungi in the genus *Glomus* were described, they were known only from clusters of spores (so-called sporocarps) found in the upper layers of soil (e.g. Tulasne & Tulasne 1844, Thaxter 1922). The history of their study was summarised by Butler (1939), by which time the vesicles and arbuscules, already clearly illustrated in the 19th century (Janse 1896), were recognised as being produced by a root colonising fungal symbiont. In the early 1950s, Barbara Mosse, at East Malling (UK), first showed experimentally that a fungus, later described as *Glomus mosseae*, was responsible for the mycorrhizal colonisation of strawberry roots (Mosse 1953).

Morphologically, the nearest similar group of fungi with known sexuality belongs to the genus *Endogone*, and by analogy the AM fungi were placed with them in a single family, the *Endogonaceae* (*Zygomycota*). This was from rather tenuous evidence, since the members of *Endogone* all produce zygosporangia, whereas the arbuscular mycorrhizal species do not. One reason for including them in *Endogone* was an observational error: a sporocarp was found with spores of both *Endogone* and *Glomus*, and, by their superficial similarity, it was presumed that one was an anamorph of the other (Thaxter 1922).

A comprehensive review of the group was carried out (Gerdemann & Trappe 1974), during which two new genera (*Acaulospora* and *Gigaspora*) were erected within the *Endogonaceae*. The fungi within this rather unnatural grouping were eventually formally accommodated in their own order, the *Endogonales*, though without further taxonomic clarification above genus level (Benjamin 1979). A cladistic analysis, mainly of morphological features, produced a 'species tree' with a new order, *Glomerales* containing two suborders and three families (Morton & Benny 1990). However, some of the conclusions of this work have been questioned. For example, it was suggested that the largest genus, *Glomus*, is non-monophyletic and probably reflects several genera (Walker 1992) or even families (Simon *et al.* 1993), and recently the monophyly of the AM fungi has itself been brought into doubt (Morton 2000).

Though the separation of this order, based on a shared mode of nutrition, was logical, the question of its position within the kingdom *Fungi* was not considered. With the error of the 'mixed sporocarp' resolved (Gerdemann & Trappe 1974) few, if any, morphological, anatomical, cytological, or sexual characters substantiated its inclusion in the *Zygomycota*, and the molecular evidence tells a different story.

Classification of the AM fungi within the Fungi

The kingdom *Fungi* has been circumscribed by the use of morphological, biochemical and molecular studies, but major phylogenetic groupings at the more 'primitive' end of the clade, including the *Zygomycota*, have not yet been defined with a natural systematics. The two best-characterised taxa, the ascomycetes and the basidiomycetes, share a common ancestor (they are monophyletic); they are sometimes considered to be phyla (*Ascomycota* and *Basidiomycota*), and sometimes subphyla (*Ascomycotina* and *Basidiomycotina* in the phylum *Dikaryomycota*). These fungi have been studied in

great depth, and are relatively clear and unambiguous in their lineage (e.g. Berbee & Taylor 2000, van de Peer *et al.* 2000).

On the contrary, the large phylum *Zygomycota* as defined is polyphyletic, and cannot yet be sustained in a truly phylogenetic classification (Benny, Humber & Morton 2000, O'Donnell *et al.* 2001, Tanabe *et al.* 2000, Tehler *et al.* 2000). Even its existence from a morphological point of view is of doubtful validity; the majority of the organisms assigned to it are not known to have a sexual stage, i.e. they lack fusion of gametangia and the subsequent formation of zygosporangia (Benjamin 1979, Benny 1995). The result, therefore, of the inclusion of superficially similar morphological entities in the zygomycetes, is an unsustainable and a disparate artificial group of sometimes-convergent lineages. Cavalier-Smith (1998) described with a brief Latin diagnosis the *Glomeromycetes*, orthographically corrected here to *Glomeromycetes*, as a new class within the *Zygomycota*, containing the *Glomerales* (AM fungi) and *Endogonales*, ignoring already known evidence regarding the relationships between these orders.

It is shown here, based on comprehensive SSU rRNA analyses, that the AM fungi can be separated in a monophyletic clade, which is not related to any zygomycetous group but probably shares common ancestry with the *Ascomycota-Basidiomycota* clade. Therefore, we can now close one of the general gaps regarding knowledge about fungal taxonomy and phylogeny, by recognizing a new, fungal phylum based on natural relationships for the arbuscular mycorrhizal and related fungi, the *Glomeromycota*. The class *Glomeromycetes* is circumscribed here as for the phylum, containing more than 150 described species, some of which are undoubtedly synonyms (Walker & Vestberg 1998, Walker & Trappe 1993).

MATERIALS AND METHODS

Single spore DNA isolation, PCR primers and conditions, cloning, and sequencing are described in detail in Schwarzott & Schüßler (2000). The small subunit (SSU) rRNA gene was phylogenetically analysed as previously described (Schüßler *et al.* 2001, Schwarzott *et al.* 2001). Two aligned data sets were analysed: one, comprising all fungal higher taxa (259 sequences), consisted of 1346 sites; the second smaller data set, comprising the newly proposed *Glomeromycota* and the *Ascomycota-Basidiomycota* clades as outgroups (82 sequences), of 1699 sites. Alignment was done manually, using ALIGN 4.0 (freeware: <http://domix0.tripod.com>). Consensus trees of both data sets were constructed from a 1000-fold bootstrapped neighbour-joining (NJ) analysis (based on Kimura's two-parameter distances) and a 1000-fold bootstrapped parsimony analyses (using gaps and polymorphic sites as missing data). PHYLIP 3.573 (Felsenstein 1989) was used for all computations. The phylogenetic distances shown in the trees (Figs 1–2) are derived from non-bootstrapped NJ analyses. There was no difference in tree topology between parsimony and NJ trees and only minor differences in bootstrap support at the levels shown in this study. The alignment is deposited at the EMBL database (<http://www3.ebi.ac.uk/Services/align/listali.html>) under accession number ALIGN_000208. Further details on the cultures, software used, and the sequence

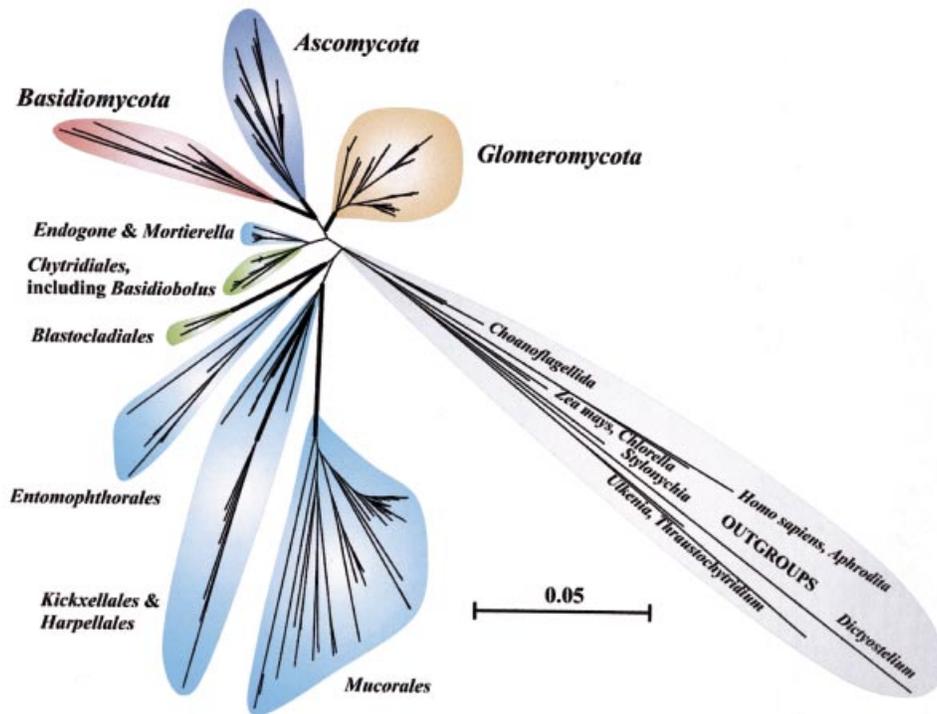


Fig. 1. Phylogeny of fungi based on SSU rRNA sequences. Thick lines delineate clades supported by bootstrap values above 90%. The *Zygomycota* and the *Chytridiomycota* do not form monophyletic clades and therefore are shown as the respective taxa representing the clade.

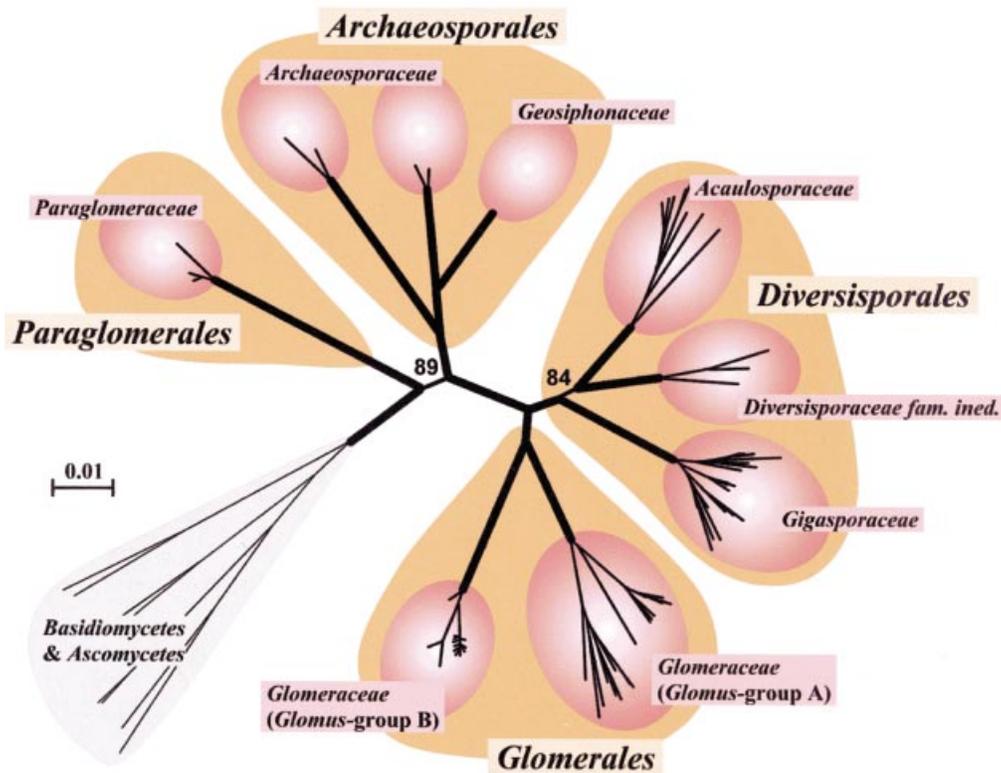


Fig. 2. Proposed generalised taxonomic structure of the AM and related fungi (*Glomeromycota*), based on SSU rRNA gene sequences. Thick lines delineate bootstrap support above 95%, lower values are given on the branches. The four-order structure for the *Glomeromycota* is shown, with many of the ‘classical’ AM fungi remaining in the order *Glomerales*. The proposed family ranking is shown by ovals and renders the recently erected genus *Archaeospora* paraphyletic. Note that in addition to the *Diversisporaceae* fam. ined. (formerly included in the *Glomeraceae*) two further ‘*Glomus*’-clades (*Glomus*-Group A and B) are resolved at family-rank.

alignments with secondary structure information, are available at <http://amf-phylogeny.com/>.

Near full-length SSU rRNA gene sequences of 69 AM fungal isolates belonging to 49 described and several undescribed species were analysed. Accession numbers assigned, and further information, are shown in Table 1. Further sequences used in the analyses follow.

Outgroup taxa used in the small data set: *Aspergillus fumigatus* M55626; *Boletus satanas* M94337; *Candida albicans* X53497; *Kluyveromyces lactis* X51830; *Leucostoma persoonii* M83259; *Neurospora crassa* X04971; *Penicillium notatum* M55628; *Russula compacta* U59093.

Outgroup taxa used in the large data set: *Aphrodita aculeata* Z83749; *Chlorella ellipsoidea* X63520; *Dermocystidium salmonis* U21337; *Diaphanoeca grandis* L10824; *Dictyostelium discoideum* X00134; *Homo sapiens* M10098; *Ichthyophonus hoferi* U25637; *Stylonychia pustulata* X03947; *Thraustochytrium kinnei* L34668; *Ulkenia profunda* L34054; *Zea mays* K02202.

Additional fungal taxa used in the large data set: *Absidia blakesleeana* AF157117; *A. coerulea* AF113405, AF113406; *A. corymbifera* AF113407, AF113408; *A. glauca* AF113409, AF157118; *A. repens* AF113410; *Actinomyces elegans* AF157119; *Allomyces macrogynus* U23935; *Amylomyces rouxii* AF157120; *Apophysomyces elegans* AF113411, AF113412; *Athelia bombacina* M55638; *Aureobasidium pullulans* M55639; *Backusella ctenidia* AF157122; *Basidiobolus haptosporus* AF113413; *Basidiobolus ranarum* AF113414, D29946; *Blakeslea trispora* AF157124; *Blastocladiella emersonii* X54264; *Blastomyces dermatitidis* M55624; *Blumeria graminis* L26253; *Capniomyces stellatus* AF007531; *Capronia pilosella* U42473; *Chaetocladium brefeldii* AF157125; *Chaetocladium jonesii* AF157126; *Chlamydoabsidia padenii* AF113415; *Choanephora cucurbitarum* AF157127; *Chytridium confervae* M59758; *Circinella umbellata* AF157128; *Circinomyces circinelloides* AF157129; *Coccidioides immitis* M55627; *Coemansia braziliensis* AF007532; *C. reversa* AF007533; *Cokeromyces recurvatus* AF113416; *Colletotrichum gloeosporioides* M55640; *Conidiobolus coronatus* AF113417, AF113418; *Conidiobolus incongruus* AF113419; *C. lamprauges* AF113420; *C. thromboides* AF052401; *Coprinus cinereus* M92991; *Cronartium ribicola* M94338; *Cryptococcus neoformans* L05428; *Cunninghamella bertholletiae* AF113421; *C. echinulata* AF157130; *C. elegans* AF113422; *C. polymorpha* AF113423; *Dichotomocladium elegans* AF157131; *Dicranophora fulva* AF157132; *Dipodascopsis uninucleata* U00969; *Dipsacomycetes acuminosporus* AF007534; *Dissophora decumbens* AF157133; *Echinosporangium transversale* AF113424; *Ellisomyces anomalus* AF157134; *Endogone pisiformis* X58724; *Entomophaga aulicae* U35394; *Entomophthora muscae* D29948; *E. schizophora* AF052402; *Eremascus albus* M83258; *Eryniopsis ptycopterae* AF052403; *Eurotium rubrum* U00970; *Fennellomyces linderi* AF157135; *Furculomyces boomerangus* AF007535; *Galactomyces geotrichum* U00974; *Genistelloides hibernus* AF007536; *Gilbertella persicaria* AF157136; *Gongronella butleri* AF157137; *Halteromyces radiatus* AF157138; *Helicostylum elegans* AF157139; *Herpotrichia juniperi* U42483; *Hesseltinella vesiculosa* AF157140; *Hyphomycetes assamensis* AF157141; *Hypomyces chryso-spermus* M89993; *Kickxella alabastrina* AF007537; *Kirkomyces cordense* AF157142; *Lecanora dispersa* L37734; *Leucosporidium scottii* X53499; *Leucostoma persoonii* M83259; *Linderina pennisporea* AF007538; *Martensomyces pterosporus* AF007539; *Microsporus* var. *chinensis* AF113437; *Morchella elata* L37537; *Mortierella chlamydospora* AF157143; *M. multidivariata* AF157144; *M. polycephala* X89436; *M. verticillata* AF157145; *M. wolfii* AF113425; *Mucor amphibiorum* AF113426; *M. circinelloides* f. *lusitanicus* AF113427; *M. hiemalis* f. *hiemalis* AF113428; *M. indicus* AF113429; *M. mucedo* X89434; *M. racemosus* AF113430; *M. ramosissimus* AF113431; *M. recurvus* var.

indicus AF157146; *Mycotypha africana* AF157147; *M. microspora* AF157148; *Neocallimastix frontalis* M62704; *N. joyonii* M62705; *Neocallimastix* sp. M59761; *Neurospora crassa* X04971; *Ophiostoma ulmi* M83261; *Pandora neoaphidis* AF052405; *Parasitella parasitica* AF157149; *Peridermium harknessii* M94339; *Peziza badia* L37539; *Phascolomyces articulatus* AF157150; *Phycomyces blakesleeana* AF157151; *Pilaira anomala* AF157152; *Pilobolus umbonatus* AF157153; *Piromyces communis* M62706; *Pleospora rudis* U00975; *Pneumocystis carinii* X12708; *Podospora anserina* X54864; *Poitrasia circinans* AF157155; *Porpidia crustulata* L37735; *Protomycocladus faisalabadensis* AF157156; *Radiomyces spectabilis* AF157157; *Rhizomucor miehei* AF113432, AF192506; *R. pusillus* AF113433, AF113434; *R. racemosus* X54863; *R. variabilis* AF113435; *R. azygosporus* AF113436; *R. microsporus* var. *microsporus* AF113438; *R. microsporus* var. *oligosporus* AF157158; *R. microsporus* var. *rhizopodiformis* AF113439; *R. oryzae* AF113440; *R. stolonifer* AF113441; *Saccharomyces cerevisiae* J01353; *Saksenaea vasiformis* AF113442; *Schizophyllum commune* X54865; *Schizosaccharomyces pombe* X54866; *Sclerotinia sclerotiorum* X69850; *Smittium culisetae* AF007540, D29950; *Sphaeromonas communis* M62707; *Spinellus fusiger* AF157159; *Spiromyces spiralis* AF007543; *S. minutus* AF007542; *Spizellomyces acuminatus* M59759; *Spongipellis unicolor* M59760; *Sporodiniella umbellata* AF157160; *Strongwellsea castrans* AF052406; *Syzygites megalocarpus* AF157162; *Talaromyces flavus* M83262; *Taphrina deformans* U00971; *Thamnidium elegans* AF157163; *Thamnostylum piriforme* AF157164; *Thanatephorus praticola* M92990; *Thermomucor indicae* AF157165; *Tilletia caries* U00972; *Tremella globospora* U00976; *Ustilago hordii* U00973; *Utharomyces epallocaulus* AF157168; *Xerocomus chrysenteron* M94340; *Zoophagus insidians* AB016009; *Zoophthora radicans* (syn. z. *culisetae*) D61381; *Z. radicans* AF052404; *Zychaea mexicana* AF157169; *Zygorhynchus heterogamus* AF157170.

RESULTS

The AM fungal near full-length sequences used in this study and information about vouchers and culture identities as well as taxonomic implications are shown in Table 1.

The analyses of the large data set, including about 100 zygomycotan sequences, resulted in a clear (bootstrap values above 90%) separation of the AM fungi from all other groups of fungi. *Zygomycota* and *Chytridiomycota* are not supported as monophyletic clades. All phylogenetic computations, along with evidence published in the past, demonstrated conclusively that the AM fungi (and *Geosiphon pyriformis*) belong to a monophyletic clade that probably represents a sister group to the clade comprising the *Basidiomycota* and *Ascomycota* (Fig. 1). The AM fungi, the *Ascomycota*, and the *Basidiomycota*, form three clades with high bootstrap support showing comparable phylogenetic distances and therefore represent comparable ranks with respect to a natural phylogeny and taxonomy of these groups. Therefore, we adopt the same rank used for the well-studied *Ascomycota* and *Basidiomycota* for the AM fungi and describe a new phylum for this fungal group.

Glomeromycota C. Walker & Schuessler, phylum nov.

Fungi plerumque hypogaei, nonnumquam epigaei, victu symbiotico obligato; formant endomycorrhizas arbusculares in radicibus et symbioses endomycorrhizas arbuscularibus similes cum plantis vivis, vel endocytobioses cum alteris organismis photosyntheticis (e.g. cyanobacteriis). Hyphae somaticae coenocyticae sunt. Reproductio non-sexualis e sporis magnis cum parietibus crassis peragitur. Tubuli germinativi parietem ipsam percutunt, vel e structura propria

Table 1. AM fungal near-full-length sequences used in the phylogenetic analyses.

Species ^a	Isolate-code(s) and/or voucher-no./culture-no. ^b	Culturing type, origin, originator ^c	Supplier of sequenced culture (if known); notes	Taxonomic affiliation ^d
¹ <i>Gl. caledonium</i>	BEG15	M, Denmark, Jakobsen.	INRA.	<i>Glomus</i> Group A (<i>Glomerales</i>)
² <i>Gl. caledonium</i>	W3294/ Att263-15; BEG20	N, England, Hayman.	Walker; ‘Rothamsted Culture’	
³ <i>Gl. coronatum</i>	W3153/Att143-5; COG1	S, Australia, McGee	Walker	
⁴ <i>Gl. fasciculatum</i>	BEG53	S, Canada, Furlan	INRA	
⁵ <i>Gl. fragilistratum</i>	W3238/Att112-6	M, Denmark, Jakobsen	Walker; ex-holotype	
⁶ <i>Gl. geosporum</i>	BEG11; W992/Att191-1	S, England, Dodd	Walker & INRA	
⁷ <i>Gl. mosseae</i>	W3528/Att867-10	S, Australia, Abbott	Walker	
⁸ <i>Gl. mosseae</i>	BEG12	M, England, Mosse	INRA; ‘Rothamsted Yellow Vacuolate’	
⁹ <i>Gl. mosseae</i>	FL156B	N, USA, Schenck	INVAM; formerly WV156	<i>Glomus</i> Group B (<i>Glomerales</i>)
¹⁰ <i>Gl. verruculosum</i>	W3295/Att298-6	S, Poland, Blaszkowski	Walker; ex-holotype	
¹¹ <i>Gl. sp. morph1</i>	WUM3; W2940/Att15-5	M, Australia, Abbott	Walker	
¹² <i>Gl. sp. morph2</i>	WUM3; W2939/Att15-5	M, Australia, Abbott	Walker	
¹³ <i>Gl. sp.</i>	UY110.6.10; W3347/Att565-7	SC, England, Merryweather	Walker	
¹⁴ <i>Gl. coremioides</i>	“Biorize”	N, Ivory Coast, Blal	Société Biorize, Dijon, France	
¹⁵ <i>Gl. intraradices</i>	DAOM197198	N, Canada, Parent	Piché	
¹⁶ <i>Gl. manihotis</i>	W3224/Att575-9	F, Colombia, Howeler	Walker; ex-holotype (CIAT-C-1-1)	
¹⁷ <i>Gl. manihotis</i>	FL879-3; W3181/Att575-25	F, Colombia, Howeler	INVAM; ex-holotype (CIAT-C-1-1)	
¹⁸ <i>Gl. manihotis/clarum</i> ^e	BR147B-8; W3163/Att72-1	N, Brazil, Ming Lin	Bioplanta Inc., Brazil	
¹⁹ <i>Gl. proliferum</i>	DAOM226389/MUCL41827	F, Guadeloupe, Risède	ex-holotype	
²⁰ <i>Gl. sinuosum</i>	MD126	N, USA, unknown	INVAM; formerly <i>Sclerocystis sinuosa</i>	
²¹ <i>Gl. vesiculiferum</i>	None	N, Canada, Chabot	unknown	<i>Glomus</i> Group B (<i>Glomerales</i>)
²² <i>Gl. claroideum</i>	BEG31; W1843/Att79-3	M, Finland, Vestberg	Walker	
²³ <i>Gl. claroideum</i>	BEG23	M, Czech Republic, Gryndler	INRA	
²⁴ <i>Gl. claroideum</i>	BEG14	N, Denmark, Rosendahl	INRA	
²⁵ <i>Gl. etunicatum</i>	UT316	N, unknown, Wood	INVAM	
²⁶ <i>Gl. lamellosum</i>	W3161/Att672-13	S, Iceland, Vestberg	Walker	
²⁷ <i>Gl. lamellosum</i>	W3160/Att244-13	S, Canada, Vestberg	Walker; ex-holotype	
²⁸ <i>Gl. luteum</i>	SA101-3; W3090/Att676-0	N, Canada, Talukdar	INVAM; formerly also ‘ <i>Gl. clarum</i> NT4’	
²⁹ <i>Gl. manihotis/clarum</i> ^e	BR212	N, Brazil, Sturmer	INVAM (isolate lost)	
³⁰ <i>Gl. viscosum</i>	BEG27; W3207/Att179-8	ST, uncertain, Giovannetti	Walker; ex-holotype	
³¹ <i>Gl. sp.</i>	UY110.6.9; W3349/Att565-11	SC, England, Merryweather	Walker	
³² <i>Gl. sp. ‘clustered’</i>	W3234/Att13-7	SC, India, Walker	Walker	
³³ <i>Gl. spurcum</i>	W3239/Att246-4	M, USA, Pfeiffer	Walker; ex-holotype	<i>Diversisporaceae</i> fam. ined. (<i>Diversisporales</i>)
³⁴ <i>Gl. etunicatum</i>	W2423/Att382-16	M, Scotland, Walker	Walker	
³⁵ <i>Gl. versiforme</i>	BEG47	M, USA, Daniels	INRA & Torino; ex-holotype	
³⁶ <i>Ac. laevis</i> (?)	WUM46; W3107/Att896-8	M, Australia, Abbott	Abbott	<i>Acaulosporaceae</i> (<i>Diversisporales</i>)
³⁷ <i>Ac. longula</i>	W3302/Att698-3	M, Venezuela, Cuenca	Walker	
³⁸ <i>Ac. rugosa</i>	WV949	N, unknown, Dant	INVAM; formerly WV935	
³⁹ <i>Ac. foveata</i>	BEG33; W2393/Att209-37	M, England, Walker	Gianinazzi-Pearson	
⁴⁰ <i>Ac. spinosa</i>	WV860	N, unknown, unknown	INVAM	
⁴¹ <i>Ac. undulata</i> (?)	WUM18; W2941/Att869-3	M, Australia, Abbott	Abbott	
⁴² <i>Ac. sp.</i>	W3424/Att729-0	ST, Venezuela, Cuenca	Cuenca	
⁴³ <i>E. colombiana</i>	FL356	N, unknown, unknown	INVAM; formerly WV877	
⁴⁴ <i>E. ‘contigua’</i>	WV201	N, unknown, Morton	INVAM; formerly WV796	
⁴⁵ <i>Gi. albida</i>	FL927	N, unknown, Perez	INVAM; formerly WV1034	
⁴⁶ <i>Gi. candida</i>	BEG17; W3292/Att26-19	S, Taiwan, Wen-Neng Chou	Gianinazzi-Pearson	
⁴⁷ <i>Gi. gigantea</i>	WV932	N, unknown, unknown	INVAM	
⁴⁸ <i>Gi. margarita</i>	DAOM194757	N, USA, Menge	unknown	
⁴⁹ <i>Gi. aff. margarita</i>	W2992/Field collected	DS, Argentina, Cabello	Cabello	
⁵⁰ <i>S. aurigloba</i>	WUM53; W3121/Att860-10	M, Australia, Abbott	Abbott	
⁵¹ <i>S. calospora</i>	BEG32; W3290/Att333-17	S, Scotland, Walker	Gianinazzi-Pearson	
⁵² <i>S. castanea</i>	BEG1	M, France, Gianinazzi-Pearson	INRA; ex-holotype	
⁵³ <i>S. cerradensis</i>	MAFF520056	S, Japan, Saito	unknown	
⁵⁴ <i>S. fulgida</i>	W2993/Field collected	DS, Argentina, Cabello	Cabello	
⁵⁵ <i>S. gilmorei</i>	W3085/Att590-1	S, USA, Walker; soil from I. Ho	Walker	
⁵⁶ <i>S. heterogama</i>	BEG35; W3214/Att334-16	N, unknown, unknown	Walker; ‘Rothamsted culture’	
⁵⁷ <i>S. heterogama</i>	BR154-5	N, Brazil, Ming Lin	INVAM	
⁵⁸ <i>S. heterogama</i>	WV858B	N, USA, Morton	INVAM; formerly WV929	

Table 1 (cont.)

Species ^a	Isolate-code(s) and/or voucher-no./culture-no. ^b	Culturing type, origin, originator ^c	Supplier of sequenced culture (if known); notes	Taxonomic affiliation ^d
⁵⁹ <i>S. nodosa</i>	BEG4; W3213/Att209-33	M, England, Walker	Gianinazzi-Pearson	
⁶⁰ <i>S. nodosa</i>	BEG4; W3485/Att209-44	M, England, Walker	Walker	
⁶¹ <i>S. pellucida</i>	WV873	N, unknown, Morton	INVAM	
⁶² <i>S. projecturata</i>	W3254/Att697-0	ST, Indonesia, Kramadibrata	Walker; ex-holotype	
⁶³ <i>S. spinosissima</i>	W3009/Att664-1	ST, Venezuela, Cuenca	Walker	
⁶⁴ <i>S. weresubiae</i>	W2988/Field collected	DS, Argentina, Cabello	Cabello	
⁶⁵ <i>P. brasilianum</i>	W3086/Att260-4; BR105	M, Brazil, Spain	Walker; ex-holotype	<i>Paraglomeraceae</i>
⁶⁶ <i>P. occultum</i>	IA702-3	N, USA, Klopfenstein	INVAM	(<i>Paraglomerales</i>)
⁶⁷ <i>P. occultum</i>	HA771	N, Hawaii, Koske	INVAM	
⁶⁸ <i>Ar. leptoticha</i>	NC176	N, unknown, unknown	INVAM	fam. ined.
⁶⁹ <i>Ar. leptoticha</i>	MAFF520055	S, Japan, Murakoshi & Siato	unknown	(<i>Archaeosporales</i>)
⁷⁰ <i>Ar. trappei</i>	NB112	N, Namibia, Klopatek	INVAM	<i>Archaeosporaceae</i>
⁷¹ <i>Ar. trappei</i>	W3179/Att186-1	ST, Austria, Schweiger	Walker	(<i>Archaeosporales</i>)
⁷² <i>Ge. pyriformis</i>	W3619/GEO1	S, Germany, Mollenhauer	Schüßler	<i>Geosiphonaceae</i> (<i>Archaeosporales</i>)

^a Sequence accession numbers (new near-full-length sequences published in the present study are printed in bold; more than one accession-no. means that a consensus sequence of those was used for analyses): 1: Y17653, AJ301854; 2: Y17635, AJ301853; 3: AJ276086; 4: Y17640; 5: AJ276085; 6: Y17643, AJ132664, AJ245637; 7: **AJ306438**; 8: U31995, U96139; 9: Z14007; 10: AJ301858; 11: AJ301864; 12: AJ301865; 13: AJ301857; 14: AJ249715; 15: X58725, AJ301859; 16: Y17648; 17: Y17638, U36590; 18: AJ276084; 19: AF213462; 20: AJ133706; 21: L20824; 22: Y17641, AJ276079; 23: AJ276080, Y17642; 24: AJ301851 & 52, AJ276075, Y17636; 25: Z14008, Y17639; 26: AJ276083; 27: AJ276087; 28: U36591, AJ276089, Y17645; 29: U36592; 30: Y17652; 31: AJ301856; 32: AJ301855; 33: AJ276077 & 78, Y17650 & 49; 34: Y17644, AJ276076, AJ301860 & 63; 35: Y17651, AJ132666, X86687, AJ276088; 36: **Y17633**; 37: **AJ306439**; 38: Z14005; 39: **AJ306442**; 40: Z14004; 41: **AJ306441**; 42: **AJ306440**; 43: Z14006; 44: Z14011; 45: Z14009; 46: **AJ276091**; 47: Z14010; 48: X58726; 49: **AJ276090**; 50: **AJ276092** & **93**; 51: **AJ306443**; 52: U31997, AF038590; 53: AB041344, AB041345; 54: **AJ306435**; 55: **AJ276094**; 56: **AJ306434**; 57: U36593; 58: Z14013; 59: **AJ306445** & **46**; 60: **AJ306437**; 61: Z14012; 62: AJ242729; 63: **AJ306436**; 64: **AJ306444**; 65: AJ301862; 66: AJ276081 & 82; 67: AJ006799; 68: AJ006466, AJ301861; 69: AB015052; 70: AJ006800; 71: Y17634; 72: X86686, AJ276074, AJ132665, Y15904 & 05, Y17831.

^b DAOM-no. (Department of Agriculture and Agri-Food Ottawa, National Mycological Herbarium, Canada) = herbarium voucher-no.; W-no./Att-no. (collection of Chris Walker, Great Britain) = voucher-no./culture-no.; MAFF-no. (Ministry of Agriculture, Forestry, and Fisheries, Japan) = culture identity-no.; BEG-no. = culture identity-no.; for INVAM culture identities see Morton, Bentivenga & Wheeler (1993).

^c N, no details; F, root fragments; M, multi-spore; S, single spore; SC, spore-cluster; ST, soil trap, DS, spores direct from field soil.

^d Diagnoses and revised descriptions:

Glomeromycetes Cavalier-Smith 1998. Description as for *Glomeromycota* (see above).

Glomerales Morton & Benny 1990. *Fungi* mostly hypogeous, sometimes epigeous, forming endomycorrhizas or mycorrhiza-like symbioses with spores, vesicles and/or arbuscules in plants. *Hyphae* of vegetative mycelium mostly non-septate, though forming septa on older hyphae as cytoplasm is withdrawn or to cut off resting spores. *Asexual* reproduction by chlamydospores (termed glomoid spores by Morton & Redecker 2001), mainly terminal, but sometimes intercalary. *Spores* solitary or formed in clusters, or in sporocarps. Differing from other arbuscular mycorrhizal fungi by the possession of the rRNA SSU gene sequence signature YTRRY/2-5/RYYARGTYGNCARCTTCTTAGAGGGACTATCGGTCGTYTACCGRTGG, corresponding to homologous position 1353 of the *Saccharomyces cerevisiae* SSU rRNA sequence J01353, with the underlined nucleotides being specific for the taxon.

Paraglomerales C. Walker & Schuessler **ord. nov.** *Fungi* hypogaei; formant endomycorrhizas arbusculares in radicibus cum plantis vivis. Vesiculae raras vel absentes. Sporae plerumque singulares formantur, tunica sine pigmentum, structura formationeque non distinctae ab *Glomere*. Sequentia typica acidi desoxyribonucleici monadis 'SSU' ribosomatum: GCGAAGCGTCATGGCCTTAACCGGCCGT (*Saccharomyces cerevisiae* sequentia J01353: positione numero 703).

Fungi hypogeous, forming endomycorrhizas with arbuscules and intraradical mycelium, rarely with vesicles. Producing glomoid spores lacking pigmentation. Differing from other arbuscular mycorrhizal fungi by the possession of the rRNA SSU gene sequence signature GCGAAGCGTCATGGCCTTAACCGGCCGT, corresponding to homologous position 703 of the *S. cerevisiae* SSU rRNA sequence J01353, with the underlined nucleotides being specific for the taxon. Note this diagnosis is based on only two species, and may be subject to modification as more species are discovered.

Diversisporales C. Walker & Schuessler **ord. nov.** *Fungi* hypogaei; formant endomycorrhizas arbusculares in radicibus cum plantis vivis. Vesiculae efformatae vel absentes. Cellulae auxiliares efformatae vel absentes. Sporae glomoidae vel gigasporoidae vel acaulosporoidae. Sequentia typica acidi desoxyribonucleici monadis 'SSU' ribosomatum: YVRRYW/1-5/NGYYGB (*Saccharomyces cerevisiae* sequentia J01353: positione numero 658); GTYARDYHMHYY/2-4/GRADRKKYGWCRAC (*S. cerevisiae* sequentia J01353: positione numero 1346).

Fungi hypogeous, forming endomycorrhizas with arbuscules, often lacking vesicles. With or without hypogeous auxiliary cells. Forming either complex spores produced within a sporiferous saccule (acaulosporoid spores of Morton & Redecker 2001), complex spores ('sporangioles?') developing from a bulbous base on the sporiferous hypha (termed here gigasporoid spores), or glomoid spores. Differing from other arbuscular mycorrhizal fungi by the possession of the rRNA SSU gene sequence signature YVRRYW/1-5/NGYYGB, corresponding to homologous position 658 of the *S. cerevisiae* SSU rRNA sequence J01353, and GTYARDYHMHYY/2-4/GRADRKKYGWCRAC, corresponding to homologous position 1346 of the *S. cerevisiae* SSU rRNA sequence J01353, with the underlined nucleotides being specific for the taxon.

Archaeosporales C. Walker & Schuessler **ord. nov.** *Fungi* hypogaei; formant endomycorrhizas arbusculares in radicibus cum plantis vivis, vel endocytobioses cum alteris organismis photosyntheticis (e.g. cyanobacteriis). Vesiculae efformatae vel absentes. Sporae monomorphae vel dimorphae, tunica sine pigmentum et non adfectio per solutione Melzeri purpureum. Sporae monomorphae acaulosporoidae vel glomoidae, sporae dimorphae acaulosporoidae et glomoidae. Sporeae glomoidae structura formationeque non distinctae ab *Glomere*. Sporae acaulosporoidae singulari pariete stratis pluribus, singulatum formatae hypha ramificanti ex hypha terminata in sacculo sporangifero. Sequentia typica acidi desoxyribonucleici monadis 'SSU' ribosomatum:

YCTATCYKYCTGGTGAKRCG (*Saccharomyces cerevisiae* sequentia J01353: positione numero 691).

Fungi hypogeous, forming endocytosymbioses with photoautotrophic prokaryotes, or producing mycorrhizas with arbuscules, with or without vesicles. Spores lacking pigmentation or reaction to Melzer's reagent. Glomoid spores formed singly or in loose clusters on or in the soil, acaulosporoid complex spores ('sporangioides?') formed singly in the soil. Dense spore clusters unknown. Differing from other arbuscular mycorrhizal fungi by the possession of the rRNA SSU gene sequence signature YCTATCYKYCTGGTGAKRCG, corresponding to homologous position 691 of the *S. cerevisiae* SSU rRNA sequence J01353, with the underlined nucleotides being specific for the taxon.

* *Gl. manihotis* and *Gl. clarum* are suggested by Morton (pers. comm.) to be synonymous, as indicated on the INVAM webpage http://invam.caf.wvu.edu/Myc_Info/Taxonomy/Glomeraceae/Glomus/manihotis/manihot.htm.

prae-germinativa crescunt, vel per hypham sporogeneam. Sporae plerumque singulares formantur, sed etiam in aggregationibus vel sporocarpis compactis.

Sola classis *Glomeromycetes* Cavalier-Smith 1998.

Typus: *Glomus* Tul. & C. Tul. 1844.

Fungi with coenocytic to sparsely septate mycelium, living mostly hypogeously, sometimes epigeously. Forming chlamydospores (in some genera) by blastic development of hyphal tip followed by thickening of structural wall components and occlusion by septum, spore-wall thickening, or deposition of an amorphous plug in the lumen of the subtending (sporogenous) hypha and spore. *Complex spores* (in some genera) with a rigid, chitinous structural wall component within a blastic terminal saccule, or by extension of a bulbous base, with or without flexible wall components. *Spores* produced singly, in loose clusters, in tight clusters (without a structured peridium), in sporocarps (with peridial development) or within the roots of plants.

Habitat: In soil, roots, or on the soil surface, vegetation, or decaying fragments of substrate. Forming close symbiotic relationships with photoautotrophic organisms. So far known to produce arbuscular or vesicular-arbuscular mycorrhizas, arbuscular mycorrhiza-like symbioses with non-vascular plants, or endocytosymbioses with cyanobacteria. Containing one class, *Glomeromycetes*, with the same characters.

Observations: In addition to the new phylum described here, the phylogenetic analyses clearly show that the *Glomeromycetes* further divide into three statistically highly supported main-branches, one represented by the 'classical' order *Glomerales* as understood by Morton & Benny (1990) and two by more ancestral lineages, *Paraglomerales* ord. nov. and *Archaeosporales* ord. nov. (Fig. 2, Table 1). Moreover, if seen from the phylogenetic standpoint, the *Glomerales* of Morton & Benny (1990) represent two orders, *Glomerales* and *Diversisporales* ord. nov., reflecting the large phylogenetic distances and the morphological differences between these clades (Fig. 2).

The phylogenetic trees show a very robust SSU rRNA phylogeny for the AM fungi with many clades supported by bootstrap values above 95%. Three of these clades represent the families *Paraglomeraceae*, orthographically corrected here to *Paraglomeraceae*, *Acaulosporaceae*, and *Gigasporaceae*. Since comparable genetic distances exist between several other clades, a family ranking for them is supported by our study. Therefore, the fungi presently remaining in the *Glomeraceae*, orthographically corrected here to *Glomeraceae*, and described in one genus, *Glomus*, represent at least three families: the recently proposed *Diversisporaceae* fam. ined. (Schwarzott *et al.* 2001; formerly included in *Glomeraceae*), *Glomus*-Group A, and *Glomus*-Group B. The recently described *Archaeosporaceae* as defined is paraphyletic, because the monogeneric family *Geosiphonaceae* is monophyletic with the *Archaeospora*

leptoticha – *Ar. gerdemannii* clade, forming a sister group to *Ar. trappei*, the type species of the *Archaeosporaceae*. The proposed new families formerly included in *Glomeraceae*, *Glomus*-Group A or *Glomus*-Group B and the *Diversisporaceae* fam. ined., will be formally described elsewhere including a re-examination of the type material of *Glomus microcarpum*, which will necessarily define the *Glomeraceae*. The proposed family structure within the *Glomeromycota* is indicated in Fig. 2 by the shaded ovals.

DISCUSSION

Co-evolution of AM fungi and plants

The origin and evolution of AM fungi is directly related to that of land plants, yet it is crucial to understand if the AM forming fungi share a common phylogenetic origin (i.e. are monophyletic), or if the present day AM symbioses are more or less independent results representing the convergent evolution of plants with different fungal lineages.

Simon *et al.* (1993) published the first SSU rRNA sequence-based phylogenetic tree of the AM fungi. Since then it has become increasingly obvious that the earliest land plants, which had no true roots, were already colonised by hyphal fungi. These formed vesicles and arbuscules strikingly similar to modern AM (Remy *et al.* 1994) and the occurrence of AM fungi has recently been dated back to at least 460 Myr (Redecker, Kodner & Graham 2000, Redecker, Morton & Bruns 2000). Absorption of water and nutrients (especially the poorly mobile element, P) from primeval 'soil' was a major barrier to the evolution of land plants. It was argued, therefore, that AM-like associations with symbiotic fungi were crucial elements in the advance of primitive plants from the water to the land (Malloch, Pirozynski & Raven 1980). Such a symbiotic organisation still exists as the AM-like symbioses between bryophyte thalli and 'typical' AM fungi (Schüßler 2000). The *Geosiphon pyriformis* endosymbiosis with cyanobacteria shows the possibility of even more ancestral types of symbioses between AM fungi and photoautotrophic organisms, perhaps reflecting an earlier evolutionary stage of an AM-like association (Gehrig *et al.* 1996, Schüßler & Kluge 2001), when non-vascular plants had not yet colonised land and cyanobacteria were prominent under the prevailing environmental conditions (Hoehler, Bebout & des Marais 2001). This hypothesis is, however, only one of several possibilities:

- (1) 'AM fungi' and plants already formed symbioses in the aquatic environment,
- (2) Plants emerged from the aquatic environment and 'AM fungi' later evolved the symbiosis,
- (3) AM precursor fungi emerged as saprobes or parasites, later becoming symbiotic with plants appearing on land,

(4) More primitive symbioses, similar to *Geosiphon pyriformis*, evolved before plants colonised land and the fungi later developed more complex relationships with plants.

Reconciling these different possibilities will require further fossils and more molecular data from extant organisms. Whichever option were correct, present knowledge indicates clearly that the formation of the fungus-autotroph symbiosis was a fundamental step in land colonisation by plants, supported considerably by the present study showing that the AM fungi represent a monophyletic group.

A natural taxonomy of AM fungi

It is now evident that the AM fungi originated more than 460 Myr ago (Berbee & Taylor 2000, Redecker, Kodner & Graham 2000) and they therefore represent an ancient phylogenetic clade within the fungi. However, there was still confusion about the monophyly of the AM fungi (Morton 2000). From the present analysis of the near full-length SSU rRNA gene sequences of 51 described and some undescribed species, there is now sufficient knowledge to show unquestionably that they belong to a distinct monophyletic group quite separate from other fungi. This comparison became possible only recently, when a large set of about 100 zygomycotan sequences from many different taxa became available in the databases (O'Donnell *et al.* 2001, O'Donnell, Cigelnik & Benny 1998, Tanabe *et al.* 2000).

Regardless of the method used, our phylogenetic analyses always produce the same results: the AM fungi (including *Geosiphon*) represent a statistically highly supported, monophyletic group, which probably shares a common ancestor with the *Ascomycota* and *Basidiomycota* (Fig. 1). They do not form a clade with any group of zygomycetes. On the basis of the phylogenetic distances, the *Glomerales s. lat.* represents a monophyletic taxon at an equivalent level to the phyla *Ascomycota* and *Basidiomycota*. We therefore propose a comparable formal ranking to accommodate the symbiotic AM fungi within a taxonomic system based on the natural phylogeny. Here we publish the molecular phylogenetic relationships and a formal description to establish the newly recognised fungal phylum, *Glomeromycota*, formerly circumscribed only as an order, *Glomerales*. Since all phylogenetic clades we refer to as taxa are highly bootstrap supported in different SSU rRNA analyses and we use a very broad species sampling approach, it is extremely unlikely that the sequencing of additional genes will change the phylogeny of the clades analysed. Nevertheless, such an approach would provide a robust test for the phylogeny shown by the SSU rRNA sequences, and the investigation of additional species might well lead to new clades as yet undiscovered.

The *Glomeromycota* divide into four statistically highly supported main clades (Fig. 2). In order to change the present taxonomic concept as little as possible we propose ordinal rank for these clades, the *Glomerales*, still representing many of the 'classical glomeralean' species as understood by Morton & Benny (1990), the *Diversisporales*, and the two 'ancestral' lineages, *Paraglomerales*, and *Archaeosporales*.

As to the family structure within those orders, the largest 'genus' within the AM fungi, *Glomus*, clearly is non-

monophyletic and represents at least three families. One of them is represented by the newly proposed family *Diversisporaceae* fam. ined. (Schwarzott, Walker & Schüßler 2001) which is monophyletic with the *Gigasporaceae* and *Acaulosporaceae*. The *Glomeraceae* will represent either *Glomus*-Group A or B, dependent on the yet unknown phylogeny of the type species, *Gl. microcarpum*, the other will require circumscribing as a new family. The implications of the recent taxonomic assertions of Morton & Redecker (2001), describing the *Archaeosporaceae* as a monogeneric family, are not congruent with the natural phylogeny based on SSU rRNA analyses, since *Geosiphon* (*Geosiphonaceae*) renders this clade, and therefore *Archaeospora* and the *Archaeosporaceae*, paraphyletic. Future emendation probably requires the erection of a new family for the *Ar. leptoticha* – *Ar. gerdemannii* clade or the inclusion of this clade within the *Geosiphonaceae*. The suborder concept within the former order *Glomerales* also does not reflect the natural phylogeny of these fungi (Schwarzott *et al.* 2001) and it remains open if one or more classes should be erected to represent the different main clades of AMF (see Fig. 2).

For some of the higher taxa indicated in Fig. 2, convincing morphological characters are still lacking, not surprising for asexual organisms such as these with a relatively simple morphology. Such characters might be recovered in future, based on the molecular phylogeny, which should form the base for an accepted new taxonomy for this important fungal group. A recircumscription of the genera and descriptions of the new families (ovals in Fig. 2) will be presented elsewhere, referring also to morphological data, which must be thoroughly re-evaluated in respect of their validity in a natural phylogeny-based taxonomy.

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