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Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming *Glomeromycetes*

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Abstract — Arbuscular mycorrhizal fungi forming spores on bulbous sporogenous cells were earlier organized in two genera of the *Gigasporaceae*: *Gigaspora* and *Scutellospora*. Molecular analyses indicated that *Scutellospora* is polyphyletic. The *Gigasporaceae* are revised on the basis of morphological spore characters and 18S and 25S rRNA gene sequences, and the 36 *Scutellospora* species are reorganised in three new families including five new genera: *Scutellosporaceae (Scutellospora)*, *Racocetraceae (Racocetra, Cetraspora)* and *Dentiscutataceae (Dentiscutata, Fuscutata, Quatunica)*. The family *Gigasporaceae* remains with a single genus *Gigaspora*. The molecular data indicate that the genus *Gigaspora* derived from within the former genus *Scutellospora*. The family *Scutellosporaceae* forms the most ancestral clade, while *Gigasporaceae* are phylogenetically not much distant from *Racocetraceae* and *Dentiscutataceae*. Keys are presented for the identification of all species of the reorganized former *Gigasporaceae*.

Key words — *Glomeromycota*, *Diversisporales*, germination shield, ontogeny, phylogeny

Introduction

Arbuscular mycorrhizal fungi (AMF), which form spores terminally on bulbous sporogenous cells, were described in the family *Gigasporaceae* (Morton & Benny 1990) of the order *Diversisporales*, *Glomeromycetes* (Cavalier-Smith 1998). In this family, with about 40 species, spores are relatively big, generally >200 µm and up to 700–1050 µm in diameter. Besides having this typical spore formation, species of the *Gigasporaceae* also differ morphologically from other

families of the *Glomeromycetes* by not forming intraradical vesicles but instead so-called auxiliary cells in the extraradical mycelium (Gerdemann & Trappe 1974, Morton & Benny 1990). Two genera have been taxonomically recognized based on spore wall characteristics and germination structures: *Gigaspora* and *Scutellospora* (Walker & Sanders 1986, Morton & Benny 1990). Spores of *Gigaspora* spp. differentate a single spore wall and multiple germ warts on the inner wall layer (= single wall group sensu Walker 1983), and *Scutellospora* spp. form two to four spore walls and a discrete germination compartment (the so-called germination shield) on the innermost wall. The spore wall structure, spore ontogeny and development of the mycorrhizal mycelia in *Scutellospora* were precisely described by Franke & Morton (1994), Morton (1995), de Souza & Declerck (2003), Declerck et al. (2004), de la Providencia et al. (2005) and de Souza et al. (2005).

The last publication unequivocally showed through molecular-based phylogenetic analyses that *Scutellospora* is polyphyletic, a finding that was also evident in other recent articles (e.g., Walker et al. 2004, Ahulu et al. 2006, Redecker et al. 2007). However, de Souza and co-workers found a disagreement between morphological based and molecular cladistic analyses that they could not resolve.

The objective of this study was to look at common morphological features of the spores of all species in the genus *Scutellospora* and to search for morphological conformity and differences, and to find common morphological features of spores for the species that were congruent with the rRNA-based molecular phylogenetic reconstruction. We concentrated our investigation on spore morphology because spores are the basis for species diagnosis in AMF. We had access to several collections of spore specimen of *Scutellospora* spp. Specimens with mycelium and in particular auxiliary cells in the root external mycelium were seldom available from species described of the *Gigasporaceae*, so that we did not consider these fungal parts in our investigations. According to Walker & Sanders (1986) the morphology of the auxiliary cells does not show much variability in *Scutellospora* anyway.

We analyzed type or isotype specimens of almost all described *Scutellospora* spp. and present germination shields of 31 species in colored photographs. We found that there are common morphological spore features in *Scutellospora* that are congruent with the molecular phylogenetic groups based on rRNA genes. The corresponding morphological and molecular groups are hereafter presented first. According to these groups, we then emend the family *Gigasporaceae*, reorganize the currently known species of *Scutellospora* taxonomically into three new families and six genera of the *Diversisporales,* and transfer 25 species of the *Scutellospora* genus into five new genera.

Material and methods

Specimen analyses

The *Scutellospora* specimens analyzed, their provenance and locations are listed in TABLE 1. In particular ex type, isotype and paratype material were examined, and we had access to several herbaria and private collections of arbuscular mycorrhizal fungi (e.g., OSC, FH, URM (Recife, Brazil), Embrapa Agrobiologia (Seropédica, Brazil), DCS-UFLA (Lavras, Brazil), International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM), and personal collections held by Sieverding, Oehl, de Souza, Trappe, Spain, Błaszkowski, Cuenca, and C. Castillo). Additionally, all original species descriptions and published species emendations were also considered.

During a visit at INVAM, F. Oehl analyzed stored vouchers of the following species: *S. arenicola*, *S. biornata*, *S. calospora*, *S. cerradensis*, *S. coralloidea*, *S. dipurpurescens*, *S. erythropus*, *S. fulgida*, *S. gregaria*, *S. heterogama*, *S. pellucida*, *S. persica*, *S. reticulata*, *S. rubra*, *S. scutata*, *S. verrucosa*, *S. weresubiae*. Spores of the following species were isolated from living culture accessions at INVAM and permanently fixed on microscope slides before analyzing: *S. calospora*, *S. coralloidea*, *S. dipurpurescens*, *S. erythropus*, *S. fulgida*, *S. heterogama*, *S. persica*, *S. verrucosa*.

Older specimens (mounted on microscopic slides before 1990) had generally been mounted either in lactophenol, while other specimens were fixed with polyvinyl alcohol-lactic acid-glycerol (PVLG) or in a mixture of PVLG + Melzer's reagent, which has become the major fixing media since 1990 (e.g. Brundrett et al. 1994). We fixed newly mounted spores from the collections using those two recent mountant media. When available, spores freshly isolated from soils were also mounted in PVLG or in PVLG + Melzer's reagent and sometimes also in a mixture of lactic acid to water at 1:1, in Melzer's reagent, and in water. Spore walls, germination structures and all other mycorrhizal structures were analysed using compound microscopes at 100–630× magnification. Most photographs presented in this paper were taken with a digital camera (Olympus model DP70-CU) on a Zeiss Axioplan compound microscope. A few photographs were taken by F. Oehl at INVAM or at OSC. The legends and scales on the photographs of the figures were inserted with Adobe Photoshop CS2 9.0.

Spore wall terminology

The terminology of the spore walls in our paper differs from the nomenclature for walls followed by Morton (1995) and Silva et al. (2006b) in *Scutellospora* (see also INVAM's homepage: www.invam.caf.wvu.edu). INVAM and Silva et al. (2006b) differentiate two to four walls: the spore wall (SW) and one to three germinal walls (GW1, GW2 and GW3), each with different layers. This nomenclature is misleading because in the *Gigasporaceae* spore germination always starts from the innermost layer of the innermost wall, from which we conclude that spores have only one germinal wall, and not several. Thus, we adopt the wall terminology we used for other AMF genera (e.g. Oehl & Sieverding 2004, Oehl et al. 2006, Spain et al. 2006, Palenzuela et al. 2008) for *Scutellospora* species: INVAM's 'spore wall' (SW) is termed here the 'outer spore wall' (OW) while INVAM's GW_n (where n = 1–3) we call 'middle wall(s)' (MW_n, where n=1–2) and 'inner wall' (IW). Our IW always is the germinal wall in all *Scutellospora* spores, as a germination shield is formed on the surface of this wall or between a thin outer layer and subsequent layers of the IW.

Table 1. Examined specimens from type and non-type material representing 36 *Scutellospora* spp.

Species name	Type material	Non type material (collection)
Scutellospora alborosea	Holotype IBACC 1-Herrera/Ferrer-HAC, isotypes IBACC 2-5-Herrera/ Ferrer-HAC;	
S. armeniaca	Type, ex type (Błaszkowski collection)	Specimens from Germany, France, Switzerland (Oehl)
S. arenicola	Holotype OSC #49'586; isotype 2374 at FH	Specimens deposited at FH and INVAM; specimens from Germany (Oehl)
S. aurigloba	Photographic type collection (Hall & Abbott 1979)	Specimens from Chile (Castillo), specimens from Brazil (Oehl)
S. biornata	Isotype #49'583, ex type (Sieverding collection), ex type (Spain collection)	Specimens at INVAM; specimens from Brazil (Oehl)
S. calospora	Type at FH	Specimens at INVAM; pure culture isolate from Bötzingen, Germany (Oehl)
S. castanea	Holotype OSC #83'344	Pure culture isolate BEG1; specimens from Germany and Switzerland (Oehl)
S. cerradensis	Holotype OSC #55'838; ex type (Spain collection)	Specimens at INVAM; specimens from Brazil and Benin (Oehl)
S. coralloidea	Holotype OSC #31'026, paratype OSC #31'025	Specimens at INVAM, and from Brazil (de Souza)
S. crenulata	Ex type (Gisela Cuenca collection, Oehl collection)	
S. dipapillosa	Holotype OSC #45'839; paratype OSC #45'848	Specimens from Colombia (Sieverding)
S. dipurpurescens	Holotype OSC #83'343, ex type INVAM	Specimens at INVAM; Specimens from Chile (Castillo), Germany and Switzerland (Oehl)
S. erythropus *	Holotype OSC #41'527	Specimens at INVAM; ex INVAM (Oehl)
S. fulgida	Holotype OSC #46'722; isotype 505 FH	Specimens at INVAM; specimens from Brazil (Oehl)
S. gilmorei	Holotype OSC #30'990; paratype OSC #31'018; paratype OSC #30'921	Specimens from Colombia (Sieverding)
S. gregaria	Holotype OSC #36'518	Specimens from India (Oehl), and from North America (DCS- UFLA and Embrapa Agrobiologia)
S. hawaiiensis	Type at H907 FH; paratype 807 at FH; isotype OSC; paratypes OSC #53'779 & OSC #53'781	
S. heterogama	Type of basionym *Endogone heterogama* at FH	

* 'erythropa' is a grammatical error for 'erythropus' which is a Greek noun in apposition and not an adjective. It therefore retains the same spelling regardless of the gender of the genus.

Table 1, concluded

S. heterogama sensu Franke & Morton	New type material deposited: Holotype & isotype at URM, isotype OSC #134'504 at OSC & isotype ZT Myc 642 at Z+ZT	Specimens at INVAM; Specimens from Benin, Brazil, Colombia (Oehl; de Souza; Sieverding)
S. minuta	Holotype IBACC 8-Herrera/Ferrer-HAC, IBACC 7; isotypes 9-11-Herrera/Ferrer-HAC	
S. nigra	Holotype OSC #37'516	Specimens from Brazil and Benin (Oehl)
S. nodosa	Holotype , ex type (Błaszkowski collection)	
S. pellucida	Holotype OSC #37'515	Specimens from Germany, Switzerland, Bolivia, Brazil, Benin, Costa Rica, Colombia (Oehl, Sieverding)
S. persica	Holotype OSC #45'837	Specimens at and from INVAM (Oehl); specimens from India (Oehl)
S. pernambucana	Holotype & isotypes at URM (79239-79241), isotypes OSC #134'503, isotypes ZT Myc 641at Z+ZT, ex type (Oehl collection)	
S. projecturata		
S. reticulata	Type OSC #41'174; paratype OSC #47'219	Specimens at INVAM, and from Brazil (Spain; Oehl, Embrapa Agrobiologia, strain CNPAB11)
S. rubra	Ex type material at INVAM	Specimens from Bolivia and Brazil (Oehl)
S. savannicola	Holotype IBACC 22-Herrera/Ferrer-HAC, isotypes IBACC 23-25-Herrera/Ferrer-HAC	Specimens from Benin, Bolivia (Oehl) and Colombia (Sieverding)
S. scutata	Ex type at University Göttingen (Diederichs); isotype at Göttingen University	Specimens at INVAM, specimens from Colombia (Sieverding) and Brazil (de Souza)
S. spinosissima	Ex type (Gisela Cuenca collection, Oehl collection)	
S. striata	Ex type (Gisela Cuenca collection, slides 1642-7 & 1641-3)	
S. tricalypta	Holotype IBACC 26-Herrera/Ferrer-HAC, isotypes IBACC 27-29-Herrera/Ferrer-HAC	
S. trirubiginopa		
S. verrucosa	Holotype OSC #45'838; paratype OSC #45'846	Specimens at and from INVAM; Brazil, Benin (Oehl)
S. weresubiae	Holotype OSC #46'723; paratype OSC #45'845; isotype 440 at FH	Specimens at INVAM

TABLE 2. Principal morphological spore characteristics in phylogenetic clades A, C1, C2, B1, B2 and B3 (see PLATES 2–3; FIGS. 10–11)

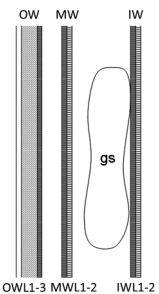
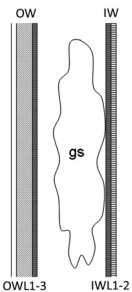
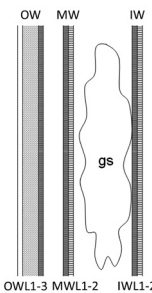
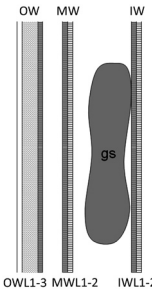
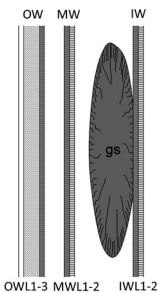
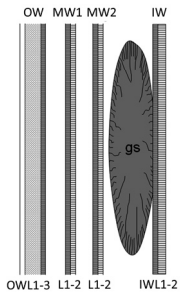
CLADE (modified from de Souza et al. 2005)	A	C1	C2	B1	B2	B3
SPORE WALLS						
Number of spore walls	3	2	3	3	3	4
Number of inner walls	2	1	2	2	2	3
Murograph;spore wall structure Abbreviations: gs (germination shield) OW (outer wall) MW (middle wall) IW (inner wall); walls with different layers: L1-3						
Walls staining in Melzer's reagent	SPECIES SPECIFIC: OW, MW, REGULARLY: IW	Generally OW staining, IW not staining	SPECIES SPECIFIC: OW, MW REGULARLY: IW	SPECIES SPECIFIC: OW, (MW) REGULARLY: IW	SPECIES SPECIFIC: OW, MW, REGULARLY: IW	OW, IW
Species specific outer wall ornamentation types (if present),	Spines, warts, knobs, warts on papillae	Spines, warts, pits only on warts	Spines, or finger-print-like processes in plan view	Spines, minute projections	Spines, sinuous lines, pits, reticula,	Unknown
Species specific outer wall double ornamentation types (if present)	Warts on dome-shaped papillae, spines and papillae	Central pits on warts	Unknown	Unknown	Spines in reticula, pits over sinuous lines, spines on 2 different wall layers	Unknown

Table 2, concluded

Clade	A	C1	C2	B1	B2	B3
Germination shield on outer surface of innermost wall						
Color	Hyaline to light yellow	Hyaline to light yellow	Hyaline to light yellow	Yellow-brown to brown	Yellow-brown to brown	Yellow-brown to brown
Shape and structure	(Mono) to bi-lobed, violin-shaped to cardioid or coiled, generally simple	Oval to ovoid, multiple-wavy-lobed; lobes rounded (curved)	Oval to ovoid, multiple-wavy lobed; lobes rounded (curved)	Violin-shaped to oval, simple -structured	Ellpisoid to oval, circular or heart-shaped, complex structured	Ellpisoid to cardioid, complex structured
Number of lobes or compartments	(One to) two lobes	Multiple small curved compartments, (species specific 4–12)	Multiple small curved compartments, (species specific 4–12)	Two lobes	Multiple compartments (Species-specific 8–30)	Many compartments (8–20)
Folds	Generally very few	Species-specific several to many	Species-specific several to many	Generally very few	Species-specific several to many	Several to many
Rounded germ tube initiations (gti)	Generally (1 or) 2	Species-specific few to several (4–12)	Species-specific few (4–12)	Generally 2	Several to many (8–30)	Several to many (8–20)
Species attributed	*S. arenicola* *S. aurigloba* *S. calospora* *S. crenulata* *S. dipapillosa* *S. dipurpurescens* *S. nodosa* *S. pernambucana* *S. projecturata* *S. tricalypta*	*S. alborosea** *S. castanea* *S. coralloidea* *S. fulgida* *S. gregaria* *S. minuta* *S. persica* *S. verrucosa* *S. weresubiae*	*S. armeniaca* *S. gilmorei* *S. pellucida* *S. spinosissima* *S. striata*	*S. heterogama* sensu Franke & Morton *S. rubra* *S. savannicola* *S. trirubiginopa*	*S. biornata* *S. cerradensis* *S. hawaiiensis* *S. heterogama* *S. nigra* *S. reticulata* *S. scutata*	*S. erythropus*
Genus name applied	*Scutellospora*	*Racocetra*	*Cetraspora*	*Fuscutata*	*Dentiscutata*	*Quatunica*
New family	*Scutellosporaceae*	*Racocetraceae*	*Racocetraceae*	*Dentiscutataceae*	*Dentiscutataceae*	*Dentiscutataceae*

* No germination shield observed in type material

Phylogenetic analyses

The phylogenetic relationship of species in the *Gigasporaceae* was reconstructed by independent analyses of 18S and 25S rRNA genes. Nearly complete sequences of 15 *Scutellospora* spp., seven *Gigaspora* spp. and one *Pacispora* were used to reconstruct the 18S rDNA-based phylogeny. *Pacisporaceae* sequences were chosen as outgroup because it is the closest family to *Gigasporaceae* (Walker et al. 2004). For the 25S rRNA genes partial sequences of 8 *Scutellospora* and 3 *Gigaspora* species were used. In this case, the *Acaulosporaceae* was selected as outgroup, as they were the closest group with sequences available (Silva et al. 2006a). The majority of the sequences were retrieved from public databases, with exception of some *Gigaspora* 18S rDNA sequences that were updated from previous work (de Souza et al. 2004). The sequences were aligned automatically using Clustal X (Thompson et al. 1997). The alignments were further improved by visual inspection and edited using SeaView (Galtier et al. 1996). The phylogeny was reconstructed by Distance, Maximum Parsimony, and Maximum Likelihood criteria using PAUP* version 4.0 Beta 10 (Swofford 2003). For distance (minimum evolution) and maximum likelihood criteria the trees were obtained using heuristic search with likelihood settings from best-fit model selected by Akaike Information Criterion in ModelTest 3.7 (Pousada & Crandall, 1998) and for Maximum Parsimony, the trees were obtained using two ways (1) considering gaps as fifth state or (2) as missing characters. The bootstrap analyses were carried out using 1000 replicates, plus 10 additional replicates per run, by heuristic search with sequences added randomly and branch swapping "tree-bisection and reconnection" as best tree search method. The *Gigaspora* sequences were updated from previous entries (see PLATE 2 for accession numbers) and the alignments generated in this study were deposited in EMBL-EBI nucleotide sequence database (http://www.ebi.ac.uk) under the alignment numbers ALIGN_001298 and ALIGN_001257.

Results

General morphological spore analyses

In order to identify corresponding morphological differences between the genetically different groups (groups A, B and C according to de Souza et al. 2005), we first listed all major spore characteristics of the *Scutellospora* spp. (TABLE 2). Spore size, spore and wall colors, wall ornamentations, numbers of walls, numbers of wall layers, staining features of wall layers and morphology of the germination shield alone were not suitable characters for grouping spores with the molecular clades. Only the number of spore walls combined with the germination shield morphology could be related to the genetically different groups. Additionally, we noticed that the positive staining reaction of the inner wall in Melzer's is somehow linked with the number of spore walls. According to these three major morphological characters, we sub-divided de Souza's groups B and C into sub-groups (B1, B2 and B3, and C1 and C2, respectively). Since germination shield morphology is so important for diagnosing the different groups, we describe their formation and their differences in the following.

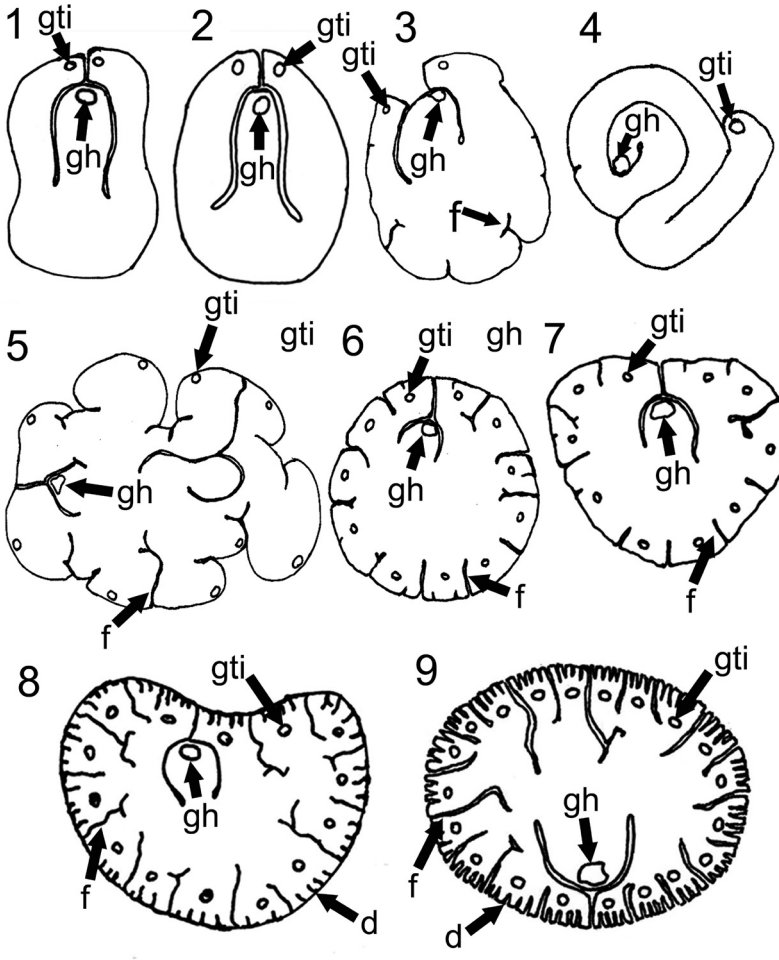


Plate 1— figs. 1–9: Characteristics of typical germination shields. All shields have i) a germination hole (gh) bridging in/on the innermost wall between the spore cytoplasm and the germination shield, ii) a variable number of germ tube initiations (gti) from where germination tubes may start growing, and iii) folds on the shield separating into different lobes and compartments, respectively. figs. 1–4. Bi-lobed shields: 1. Violin-shaped. 2. Oval to ovoid shaped. 3. Bi-lobed, with a few folds on the shield wall. 4. Mono-lobed, coiled. fig. 5. Open organized, wavy shield with multiple small compartments; wavy compartments separated by folds. Each compartment generally has one central gti from where the germ tubes emerge during germination. figs. 6–9. Compartmented shields with several to many (8–30) small compartments, separated by folds; each compartment having one gti in its center. Shield shapes circular, cardioid, reniform or ellipsoid. figs. 8–9. Highly complex structured germination shields; shield peripheries are conspicuously denticulate.

Morphology of germination shields

Common to all *Scutellospora* spore germination shields (also called "germ shield") is their formation on the inner wall facing the next outermost wall (MW or OW, see TABLE 2). Very seldom does the literature report two germ shields on the same spore. All germ shields initiate from a germination hole (gh) through the innermost IW layer or – more generally – through the innermost spore wall IW (PLATE 1). Walker & Sanders (1986) described the germ shield formation process as an extrusion, with the plasma membrane extending through the hole to form lobes on the IW surface or between a thin IW outer layer and subsequent adherent IW layer. The germ shield size ranges from ca. 35 × 70 µm to about 140 × 160 µm and up to 180 × 280 µm. The germ shield may be divided into one, two, several, or multiple (up to 30) small compartments (sensu Gerdemann & Trappe 1974) that are described below. Each compartment can contain a small, more or less circular (2–5 µm to 12–20 µm in diameter) germ tube initiation (gti, Walker & Sanders 1986, PLATE 1), from which a germination tube starts growing through the overlaying wall layers. Generally, however, only one or two (of 30 gti) actually form a germ tube during germination. Once grown through the walls, the germ tube may begin to branch on or near the spore surface although it often grows straight into the soil. Germ shields are rather more frequent (or at least more easily seen) on older rather than young spores, probably because the shield forms at the end of spore wall differentiation (Franke & Morton 1994, Morton 1995, de Souza et al. 2005). On some species germ shields are readily visible on young mature spores (e.g. *S. dipurpurescens*) whereas they are almost never visible on others (e.g. *S. pellucida*). It should also be noted that, in a very young or early stage of germ shield formation, gti are not visible, suggesting that they may form later in the shield formation process.

In principle, we differentiate the germ shields morphologically by color, spherical shape (including dentate structures), and number and shape of lobes or compartments, each with its own gti. In different species, germ shield size is generally related to spore size and is thus so variable that it is not a good diagnostic character for identifying species groups.

Germ shield color

Three of the genetically different groups, A, C1 and C2, form transparent hyaline to very light yellowish germ shields, e.g. *S. calospora, S. coralloidea* and *S. pellucida* (PLATES 4–6), whereas the genetic groups B1, B2 and B3 form distinctive yellow brown to dark brown pigmented germination shields, e.g. *S. heterogama*, *S. nigra* and *S. erythropus* (PLATES 7–9). The pigmentation of the germ shield is not correlated to the color of the spore walls; for example, the shield is brown-yellow to brown in white-spored *S. cerradensis* and *S. scutata*

but hyaline to light yellow in *S. coralloidea* whose spores are dark colored. Some hyaline germ shields, when mounted on slides or stored in liquids of different media for several years, may somewhat darken and appear brownish: e.g., a germ shield of *S. coralloidea* isolated from the type at OSC that had been stored in lactophenol for 35 years had a partly darkened germ shield wall (FIG. 20). Likewise, the inner spore wall including the germ shield of *S. tricalypta* was darkened (FIG. 14), 25 years after being fixed on a slide in Ferrant mountant by Ferrer & Herrera (1981).

Germ shield shape

In planar view, germ shields are violin-shaped, oval to ovoid, coiled, circular, cardioid to reniform, or ellipsoid (PLATE 1). In principle three morphologically different germ shield types are observed (see TABLE 2, PLATES 1 & 4–9). Species of the genetic groups A have simple shields that are bi-lobed (FIGS. 1–3) — with the inner borders between the folds often forming a lyre, as in *S. calospora* — or (rarely) mono-lobed (FIG. 4) and coiled, as in *S. projecturata*. Such shields have, near the end of each lobe, one gti. In some specimens, simple bi-lobed shields appeared to have three or four lobes but these 'additional' lobes were only prolongations of the two initial lobes, or infolds of the same lobes that became apparent when the shield could not be observed in completely planar view (e.g., FIGS. 3, 18 for *S. nodosa*). Group B1 germ shields are also rather simple, bi-lobed and lyre-like (e.g. *S. heterogama* and *S. rubra*, FIGS. 45–47); in group B1 as well, further infolds of the two initial lobes may sometimes cause the shield to appear to have three or four lobes (e.g. *S. savannicola* in FIG. 48) even though group B1 germ shields have only (1–)2 gti. Species assigned to genetic groups C1 and C2 have a distinctly different germ shield morphology and a clear lyre shape is not visible. Multiple wavy lobes form an open network of many small compartments with up to 12 compartments with respective gti visible (FIG. 5 + PLATES 5–6). Finally, genetic groups B2 and B3 have highly complex-structured, compact shields with from 8–10 to 30 compartments, each with each one gti (FIGS. 6–9). The shield periphery of these species generally looks conspicuously dentate (e.g. FIGS. 8–9 + PLATES 7, 9).

Spore wall numbers

The outer wall (OW) in all *Scutellospora* spp. has three to four layers and the inner wall (IW) generally is 2-to-3-layered. Differences between the genetic groups are found in the number (0–2) of middle walls (TABLE 2). Genetic group C1 species lack any MW. Group A, B1, B2 and C2 species have one single- or bi-layered middle wall (MW; called GW1 by Morton 1995). Only, *S. erythropus* (group B3) forms two middle walls (MW1 and MW2).

Staining reaction of spore walls

Scutellospora spp. with one or two middle walls (i.e. generally all species in morphological groups A, B1, B2, B3 and C2) stain purple to deep purple in Melzer's reagent on the hyaline inner wall. In these groups, Melzer's staining reactions are less frequent on the outer wall and rare on the middle walls. Remarkably, all *Scutellospora* spp. lacking a middle wall (morphological group C1) have a staining reaction on the outer wall – similar to all species in *Gigaspora* but with a different staining intensity from yellow to yellow-orange to orange-red to red brown — but generally lack a staining reaction on the inner wall (TABLE 2).

Molecular phylogeny

The 18S and 25S rDNA-based clades generated by the two independent molecular phylogenetic reconstructions of the family *Gigasporaceae* were congruent and indicate polyphyly and paraphyly in the current classification (PLATES 2–3). Although the alignments show slight differences in tree topologies and species sequence composition, the major clades are identical. Strikingly, the clades obtained support our morphological analyses based mainly upon germination shield characteristics.

The 25S rDNA trees utilized a smaller number of *Scutellospora* species sequences than the 18S rDNA trees. Despite this, the fully resolved 25S rDNA trees comprised all six morphological groups based on examination of spores from all species described in this genus. For that reason we use PLATE 3 to show the molecular support for placing monophyletic groups into new families and genera.

The molecular reconstruction confirmed that *Gigaspora* derives from within *Scutellospora* and not the other way around as hypothesized by Morton (1990) and Bentivenga & Morton (1995). Here, *Scutellospora* sequences cluster in three A, B and C clades (PLATES 2–3). All three clades received high bootstrap values, regardless of tree-building method used (PLATE 3). The proposed new families are based on these three clades. The phylogenetic trees further show sub-clades, which we name at this stage A, B1, B2, B3, C1, C2 (PLATES 2–3). Although some sub-clades represent only one or few species sequences, they are further supported by our morphological analyses.

Group A (formed by *S. calospora* and related species) does not share the same most recent common ancestor either with the other two *Scutellospora* clades (B and C) or with *Gigaspora* in the 18S and 25S trees (PLATES 2–3), indicating polyphyly. However, although the 25S rDNA-generated distance tree implies paraphyly, it also fully supports monophyly for the groups used by us to propose new families. All Group A 25S sequences possess an indel (insertion-deletion sequence) shared only with the outgroup *Acaulosporaceae*

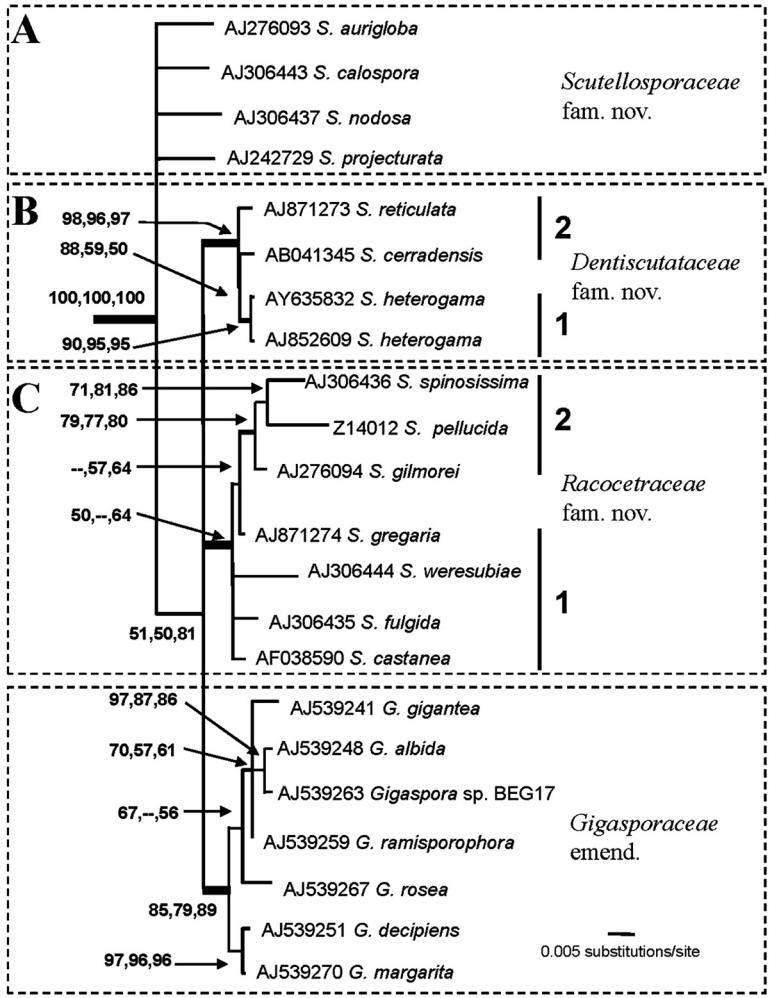


Plate 2— fig. 10. Phylogenetic reconstruction of the family *Gigasporaceae* based on nearly complete 18S rDNA sequences. The tree was generated using Maximum Likelihood criteria with 1000 bootstrap replicates using the GTR substitution model (Lanave et al.1984) + I + G with the following parameters: a = 2.0779; b: 10.9922; c: 3.3253; d: 0.7967; e: 18.3736; Nucleotide frequencies: pi(A) = 0.2740; pi(C) = 0.1857; pi(G) = 0.2541; pi(T) = 0.2862; number of substitutions types 6, rates gamma, shape 0.6903 and proportion of invariable sites 0.6860. The tree was rooted using *Pacispora* (AJ619940, AJ619951) as outgroup. Bootstrap values for topologies obtained under distance (minimum evolution), maximum parsimony and maximum likelihood criteria are shown respectively. The thickest branch lines indicate the monophyletic groups used to erect new families; the second thickest branch lines show the genera. The three major *Scutellospora* clades are indicated by capital letters A, B and C, as well as the sub-clades B1, B2, C1 and C2. Note: sequences of sub-clade B3 unavailable for these trees.

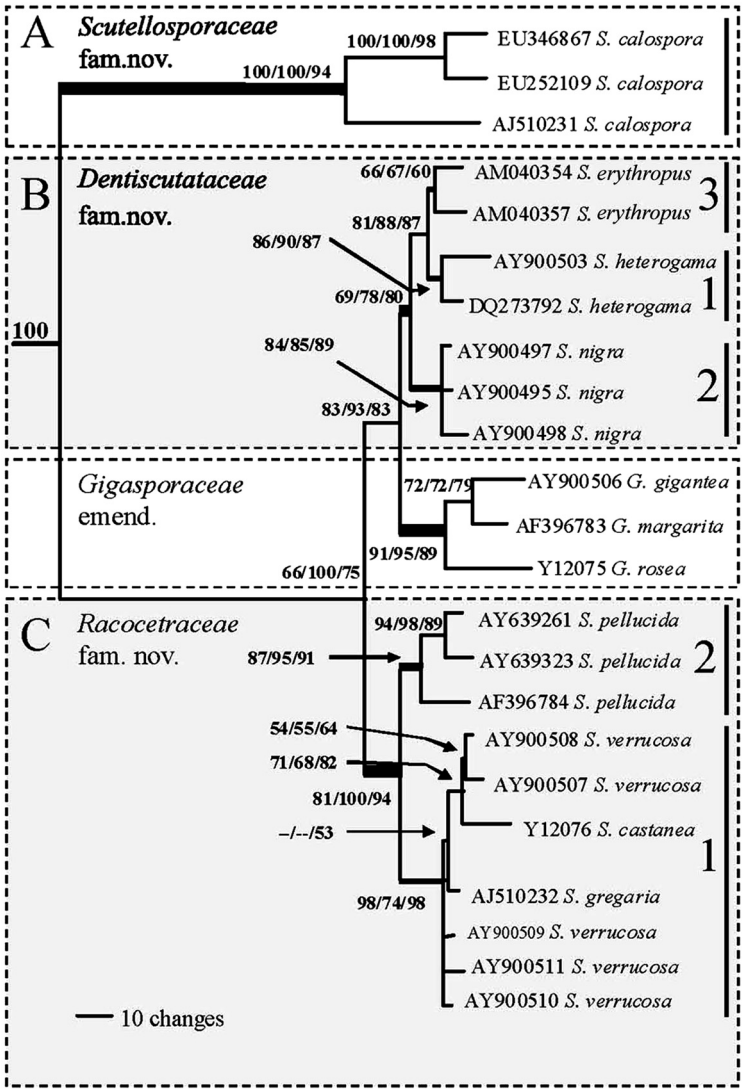


Plate 3–fig. 11. Phylogenetic reconstruction of the family *Gigasporaceae* based on partial 25S rDNA sequences. The tree was generated using Maximum Likelihood criteria with 1000 bootstrap replicates using the GTR substitution model (Lanave et al. 1984) + G substitution model with the following parameters: a = 0.4967; b: 7,3879; c: 2.4411; d: 0.8468; e: 15.7499; Nucleotide frequencies: pi(A) = 0.3057; pi(C) = 0.1423; pi(G) = 0.2535; pi(T) = 0.2985; Number of substitutions types 6, rates gamma, gamma distribution parameter alpha: 0.5688, proportion of invariable sites = 0. The tree was rooted with *Acaulospora* (AY900514, AJ510228) as outgroup. Bootstrap values for topologies obtained by Maximum Parsimony with gaps treated as missing character; Maximum

(see alignment ALIGN_001257 deposited at EMBL database) indicating a common evolutionary event. The 18S-based tree could not fully resolve this clade, as it appears as a polytomy. Group B encompasses species sequences of three morphological groups based on our analyses. The trees strongly support separation of Group B2 from B1 and B3 (PLATE 3). Separation between B1 and B3 is less evident as both seem to descend from the same last common ancestor. Nevertheless, the remarkable morphological differences between species in B1 and B3 provide full support for our proposed taxonomic changes. Subgroups C1 and C2 within Group C are clearly separated in both 18S and 25S rRNA trees.

Our analyses clearly show that *Gigasporaceae* with its two old genera *Gigaspora* and *Scutellospora* do not reflect the evolutionary history of this group. The polyphyletic/paraphyletic nature of *Scutellospora* and the position of the *Gigaspora* clade within the *Scutellospora* clades solicitates the revision of the *Gigasporaceae* and supports the erection of three new families based on the clades A, B and C within the *Diversisporales*. The molecular phylogenetic reconstruction also gives some support for the erection of five new genera within the families erected from clades B and C (PLATE 3). Although the subgroups within clade B were represented by sequences of only one or two species in both trees, the morphological differences are conspicuous (TABLE 2): B1, represented by *S. heterogama*; B2, represented by *S. nigra,* and B3 represented by *S. erythropus*. Clade C clearly shows two well supported sub-clades: C1 and C2 (PLATE 3), with species belonging to each sub-clade possessing significant morphological spore differences (TABLE 2). Finally, the family *Gigasporaceae* remains with a single genus *Gigaspora*.

Re-organization of *Scutellospora* based on morphological and molecular analyses

Based on the morphological differences of germination shields and spore wall numbers (TABLE 2) and supported by the above phylogenetic analyses, (PLATES 2–3) we can attribute current *Scutellospora* species to three higher level (A, B, C) groups and six subgroups (A, B1, B2, B3, C1, C2); *Gigaspora* species form another distinct higher level group. Based on the higher groups, we first emend the *Gigasporaceae* and describe three new families in the *Diversisporales*. Further, from within *Scutellospora* we assign 10 species to genetic group A, 9 to Group C1, 5 to Group C2, 4 to Group B1, 7 (including one new species see below) to Group B2, and 1 to Group B3. We hereafter emend the genus description of

Parsimony considering gaps as fifth state, and Maximum Likelihood criteria are shown respectively. The thickest branch lines designate monophyletic groups used to erect new families; the second thickest branch lines show the genera. The three major *Scutellospora* clades are indicated by capital letters A, B and C, as well as the sub-clades B1, B2, B3, C1 and C2.

Scutellospora, which corresponds to Group A, and describe five new genera corresponding to C1, C2, B1, B2 and B3. Following the International rules, we select a type species for each new genus from each genetically related group that was first described in literature. One exception was made for *S. heterogama* because of the particular taxonomic problems with this species (see below). Finally, we present identification keys to the families, genera and species with gigasporoid spore formation and list (TABLE 3–4) the morphological features obtained from all species in the former *Scutellospora* under the new generic headings.

Taxonomic problems encountered during study

While revising the *Gigasporaceae*, we encountered some species misidentifications and other problems noted below.

Isolates analysed and identified as *Scutellospora heterogama* by Franke & Morton (1994) and Jeffries et al. (2007) do not correspond to the *S. heterogama* type specimens deposited at FH (as *Endogone heterogama*). While *S. heterogama* sensu Franke & Morton (1994) and Jeffries et al. (2007) has a simple violin-shaped to oval germ shield, the germ shield of the FH type of *E. heterogama* is ellipsoid and has a dentate periphery and several gti. Also, the type spores are lighter in color (pale yellow-brown; Nicolson & Gerdemann 1968) than the red- to dark-brown spores of *S. heterogama* sensu Franke & Morton (1994) and Jeffries et al. (2007). For these reasons we cite below the type of *E. heterogama* in a new combination and describe *S. heterogama* sensu Franke & Morton and Jeffries et al. as a new species in a different genus.

Secondly, our examination of type materials deposited at OSC and FH revealed that *S. weresubiae* has only one inner wall, which does not stain in Melzer's, while the outer wall turns yellow to orange-yellow, a finding clearly different from the original description. We believe that Koske & Walker (1986) identified the thin innermost wall layer of the outer wall as a separating middle wall (Koske & Walker 1986).

Thirdly, *Scutellospora scutata* sensu Walker & Diederichs (1989) was described with three spore walls, while *S. scutata* sensu Silva et al. (2006b; based on INVAM reference accession BR243) has four spore walls. The germ shields in both have a dentated periphery in planar view but photographs of the germination shield in INVAM's *S. scutata* as published in Walker & Diederichs (1989) and here (FIG. 43, photographer F. Oehl) suggest two different species. We do not believe that the germ shield differences represent just an intra-species variation, but in the absence of complete information, we do not describe the INVAM specimen as a new species.

Next, we attributed *S. trirubiginopa* to Group B1 although it was not totally clear from the species description that the brown germination shield was indeed

bi-lobed. Specimens were not available, and photographs of spores and the shield were not published. However, the description says that the germination shield is simple and that two germ tubes might arise from the shield.

For *S. crenulata* only a few spores were available to us, and the shields, hidden under the thick and conspicuously ornamented outer spore wall, were difficult both to detect and to isolate from the IW. Moreover, several infolds prevented us from determining the number and organization of the lobe(s) (FIGS. 19–20) and whether more than one gti was present.

Finally, we did not consider a sequence of *S. dipapillosa* (Z14013) in our molecular analyses as we have doubts about the correct species identification – it is likely that there was a confusion of *S. dipapillosa* with *S. heterogama* sensu Franke & Morton (1994) and Jeffries et al. (2007).

Molecular problems encountered during study

In the *Glomeromycota* the 18S rDNA genes possess low phylogenetical signal, and trees generated from 18S rDNA sequences did not fully resolve the ancestry of the clades B, C and *Gigaspora,* with Clade C receiving the lowest bootstrap values (PLATE 2), which point to a conflict in the position of clade C on 18S-based trees. On the other hand, the 25S rDNA-generated trees were fully resolved; the main clades used to erect new families received bootstrap values >66% among all tree-building methods used, and >80% for Maximum Likelihood criteria. Despite the lower number of available species sequences, groups obtained on the 25S rDNA trees are congruent with the germination shield-based morphological groups.

CLADE A – At one hand, the 18S rDNA based trees could not resolve the terminal nodes, which appear as a polytomy. On the other hand, the 25S rDNA trees were built using only sequences of a single species, *S. calospora.* Nonetheless, both tree topologies (PLATES 2–3) agreed with the placement of clade A (see also de Souza et al. 2005).

CLADE B – The 25S rDNA trees show support for the three proposed genera on this clade, but they were represented by sequences of only one species for each genus, while in the 18S rDNA based tree, there is no sequence of group B3, and differences between B1 and B2 are less resolved, due to the position of *S. cerradensis* sequences. The germination shield in *S. cerradensis* (FIG. 42; see also *S. hawaiiensis*, FIG. 41) differs to some extent from the typical dentate shields in *D. nigra* and all other *Dentiscutata* species (FIGS. 38–44), although it clearly resembles more the latter than the bi-lobed shields in *Fuscutata* species (PLATE 8). Further work is needed to resolve the relationships of *S. cerradensis* and *S. hawaiiensis*.

CLADE C – Clade C is well resolved on the 25S rDNA trees, although the sub-clade C1 terminal nodes appear as a polytomy and the *S. verrucosa* sequences

appear non-monophyletic. We intentionally selected those *S. verrucosa* sequences to show this feature of AMF. The rDNA copies are polymorphic in the genome of all *Glomeromycota* species studied to date (see de Souza et al. 2004). Due to this intraspecific rDNA polymorphism, non-monophyly among rDNA copies obtained from a single species is common throughout the *Glomeromycota*. However, such polymorphism does not affect the resolution of the rDNA-based phylogeny.

Taxonomic revision of *Gigasporaceae*

Key to families and genera with spores formed on bulbous sporogenous cells (*Diversisporales*)

1a Spores with one spore wall; generally with germ warts on inner surface of the wall in mature spores ..... Family 1: *Gigasporaceae (Gigaspora)*
1b Spores with outer spore wall and one to three inner walls; germination shield on innermost wall 2
2a Spores with a hyaline to subhyaline seldom light yellow germination shield 3
2b Spores with a yellow brown to brown germination wall Family 4: *Dentiscutataceae* (3 genera) 4
3a Germ shield simple, generally (mono-) to bi-lobed, thus (1-)2 germ tube initiations (gti); spores with 3 walls ..... Family 2: *Scutellosporaceae (Scutellospora)*
3b Germ shield multiple-lobed, 4 or more compartments with each one gti Family 3: *Racocetraceae* (2 genera) 5
4a Germination shield simple, generally bi-lobed; spores with 3 walls ..... *Fuscutata*
4b Germination shield complex, >8 compartments, each with a gti; shield periphery generally dentate in planar view; spores with three or more walls 6
5a Spores with 2 walls ..... *Racocetra*
5b Spores with 3 walls ..... *Cetraspora*
6a Spores with 3 walls ..... *Dentiscutata*
6b Spores with >3 walls ..... *Quatunica*

Gigasporaceae J.B. Morton & Benny **emend. Sieverd., F.A. Souza & Oehl**

Emendation: Sporocarps are unknown. Spores formed singly in soil or rarely in roots. Spores are formed on bulbous sporogenous cells arising from subtending hyphae (sporophore) that differentiate from mycelia hyphae in soil. Spores with one spore wall consist of three layers: a unit, semi-persistent to persistent outer layer, a laminate middle layer and a thin inner germinal layer. The germinal layer has multiple, randomly or regularly organized germ warts. From one or sometimes several of the germ warts a germ tube arises during germination, penetrates directly the spore wall and generally branches in a short distance from the spore. Auxiliary cells formed in hyphal mycelium have spiny or nodulous elevations but not smooth round. Form arbuscular mycorrhiza; so far vesicles in roots unknown.

Type genus: *Gigaspora* Gerd. & Trappe emend. C. Walker & F.E. Sanders, Mycotaxon 27: 179. 1986.
Type species: *Gigaspora gigantea* (T.H. Nicolson & Gerd.) Gerd. & Trappe, Mycologia Memoir No. 5: 29. 1974.
Excluded genus: *Scutellospora*

Key to *Gigaspora* species

For the key we considered the original descriptions of the species. The synonomy of *G. ramisporophora* with *G. margarita* (Bentivenga & Morton 1995) is not accepted based on molecular (de Souza et al. 2004) and morphological (Spain et al. 1989) differences. *Gigaspora rosea* and *G. candida* are also treated as separate species. We exclude *G. tuberculata* from *Gigaspora* (see also Bentivenga & Morton 1995).

1a. Mean diameter of mature spores < 250 μm; spores rarely > 300 μm. 2
1b. Mean diameter of mature spores > 250 μm; spores generally > 300 μm. 3
2a Spores white when young 4
2b Spores white to cream, usually with a rose-pink tint; 230–300 μm in diam; sporogenous hyphae darker than the spore wall color . . . . . *G. rosea*
3a Spores pearly white when young, 200–490 μm in diam 5
3b Spores generally pigmented already when young; 290–810 μm in diam; mature spores brilliant yellow-green, color associated with spore contents; spore contents turning bright red in alkaline solutions; laminae of spore wall not turning dark purple in Melzer's reagent; composite wall thickness generally < 10 μm . . . . . *G. gigantea*
4a. Mature spores dull white with sporogenous hyphae similar in color with spore wall 6
4b. Spores (orange) white to white orange, 140–270 μm in diam . . . . *G. alboaurantiaca*
5a Spores with one sporogenous hyphae; 260–480 μm in diam mature spores pearly white to an opaque, creamy yellow color; composite wall thickness generally < 25 μm in diam. . . . . *G. margarita*
5b Spores with composite spore wall thickness generally > 25 μm, 200–500 μm in diam; mature spores yellow to brown 7
6a Spores with composite spore wall thickness generally < 8 μm, 200–300 μm in diam, average 250 μm, sporogenous cell dull white 30–50 μm diam . . . . . *G. candida*
6b. Spore with composite spore wall thichness < 12 μm, 143–350 μm in diam, average 265 μm, sporogenous cell hyaline to yellow 24–50 μm diam . . . . . *G. albida*
7a Spores with composite spore wall thickness generally > 25 μm, 320–490 μm in diam; mature spores golden yellow to brown, with one sporogenous cell . . . . . *G. decipiens*
7b. Spores as in 6a, subtending hypha forms one or more sporogenous cells one on top of other appearing as beads, spore formed on last cell; from any of the sporogenous cells another subtending hypha can emerge and may or may not form another sporogenous cell from which another spore forms . . . . . *G. ramisporophora*

Scutellosporaceae Sieverd., F.A. Souza & Oehl, **fam. nov.**
MB 511945

Sporae terminaliter efformatae anguste adiacetae ad cellulas sporogeneas; cum tunicis tribus; scutellum germinale coniunctum ad tunicam interiorem, viola-forme vel ovale vel cardioforme, hyalinum ad alboflavum, cum 1–2 lobis et depressionibus germationis; formans structuras mycorrhizarum arbuscularum.

Key characters: Sporocarps are unknown. Spores generally singly formed on bulbous sporogenous cells that are formed terminally on a subtending hypha that arises from mycelia hyphae in soil. Spores have 3 walls, an outer, a middle and an inner, germinal wall. Outer wall with 3 or several layers, middle wall with 1–2 layers, and inner wall with 2 or several layers. A germination shield is formed on the outer surface or between the outer and the subsequent layer of the inner wall. Germination shield is transparent, or hyaline to subhyaline, seldom light yellow, generally bi-lobed, rarely mono-lobed; often violin-shaped or oval to ovoid, rarely cardioid, circular or coiled; only a few folds cover the shield surface where in general two germ tube initiations (gti) are formed. Generally from one gti, seldom from both gti, a germ tube arises and penetrates the outer spore walls. Subtending hyphae form one to several septa in some distance to the sporogenous cells. Auxiliary cells in the hyphal mycelium are, as far as known, knobby without spines on the surface. Forming typical arbuscular mycorrhizae; vesicle formation in roots is so far unknown.

Type genus: *Scutellospora* C. Walker & F.E. Sanders emend. Oehl et al.

Scutellospora C. Walker & F.E. Sanders. **emend. Oehl, F.A. Souza & Sieverd.**

Emendation: Sporocarps unknown. Spores formed on sporogenous cells that form terminally on a hypha which arises from mycelia hyphae in soil. Outer spore wall generally is three-layered and continuous with the wall of the sporogenous cell. Outer layer of the outer spore wall generally rigid, second layer laminate and third layer thin, often membranous, tightly adherent to the laminate layer and thus, often difficult to observe. Pore between the spore and sporogenous cell is narrow and usually closed by a plug formed by spore wall material. Two hyaline walls ('mw' and 'iw') form de novo during spore formation and have 1–2 and 2–3 layers, respectively. The iw is two to three-layered forming a germination shield on its outer surface or between the outer and the subsequent layer of iw. Germination shield is transparent, or hyaline to subhyaline, seldom light yellow, bi- to mono-lobed; often violin-shaped to oval to ovoid to more rarely cardioids or coiled and then, either circular or apparently broad ellipsoid to irregular; only a few folds cover the shield surface where 1–2 rounded germ tube initiations ('gti', about 2–4 μm in diam)

are visible from where the germ tubes arise which penetrate the outer spore wall layers. Mycelia hyphae form one to several septa in some distance to the sporogenous cells. Auxiliary cells in the hyphal mycelium, as far as known, are knobby without spines on the surface. Forming typical arbuscular mycorrhizae, vesicle formation in roots so far unknown.

Type species: *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders

Key to *Scutellospora* species

1a Spores without ornamentation on outer wall 2
1b Spores with ornamentation on outer wall 3
2a Spores light colored: subhyaline, pink, creamy, straw to greenish or brownish yellow 4
2b Spores yellow brown to black 5
3a Spores with one homogenous ornamentation on spore surface 6
3b Spores with two-type ornamentation on spore surface 7
4a Spores subhyaline to creamy to pale straw to greenish yellow 8
4b Spores dark yellow to golden yellow to brown yellow 9
5a Spores yellow brown to orange-brown, 160–360 µm in diam, outer and innermost wall strongly staining in Melzer's reagent . . . . . *S. arenicola*
5b Spores dark brown to black, 300–460 µm in diam . . . . . *S. tricalypta*
6a Spores hyaline to pale yellow, 160–270 µm in diam; with knobs 3.5–6.5 µm high and 7.0–10.5 µm wide at base . . . . . *S. nodosa*
6b Spores golden yellow to ochraeous to sienna, 100–180 µm in diam; with 2.0–4.0 µm long protuberances formed by the structural, laminated layer of the outer wall . . . . . *S. projecturata*
7a Spores with two-type ornamentation on spore surface; spores pale orange brown to dark orange brown, 130–180 µm in diam; with small conical warts and additional blunt, bacilliform larger projections on the spore surface . . . . . *S. dipapillosa*
7b Spores with warted projections having central secondary projections in the tip; spores cream to yellow, 100–170 µm in diam . . . . . *S. crenulata*
8a Spores subhyaline to pale straw to pale greenish-yellow, (100–)150–300(–500) µm in diam, generally ellipsoid to oblang, with bi-layered middle wall . . . *S. calospora*
8b Spores yellow to greenish-yellow; 140–240 µm in diam, generally globose to subglobose, with one layered middle wall . . . . . *S. dipurpurescens*
9a Spores golden yellow to dull yellow, (130–)200–420(–520) µm . . . . . *S. aurigloba*
9b Spores dark yellow to brown yellow (to yellow brown), 105–150 µm, outer spore wall strongly expanding in lactic acid based mountants . . . . . *S. pernambucana*

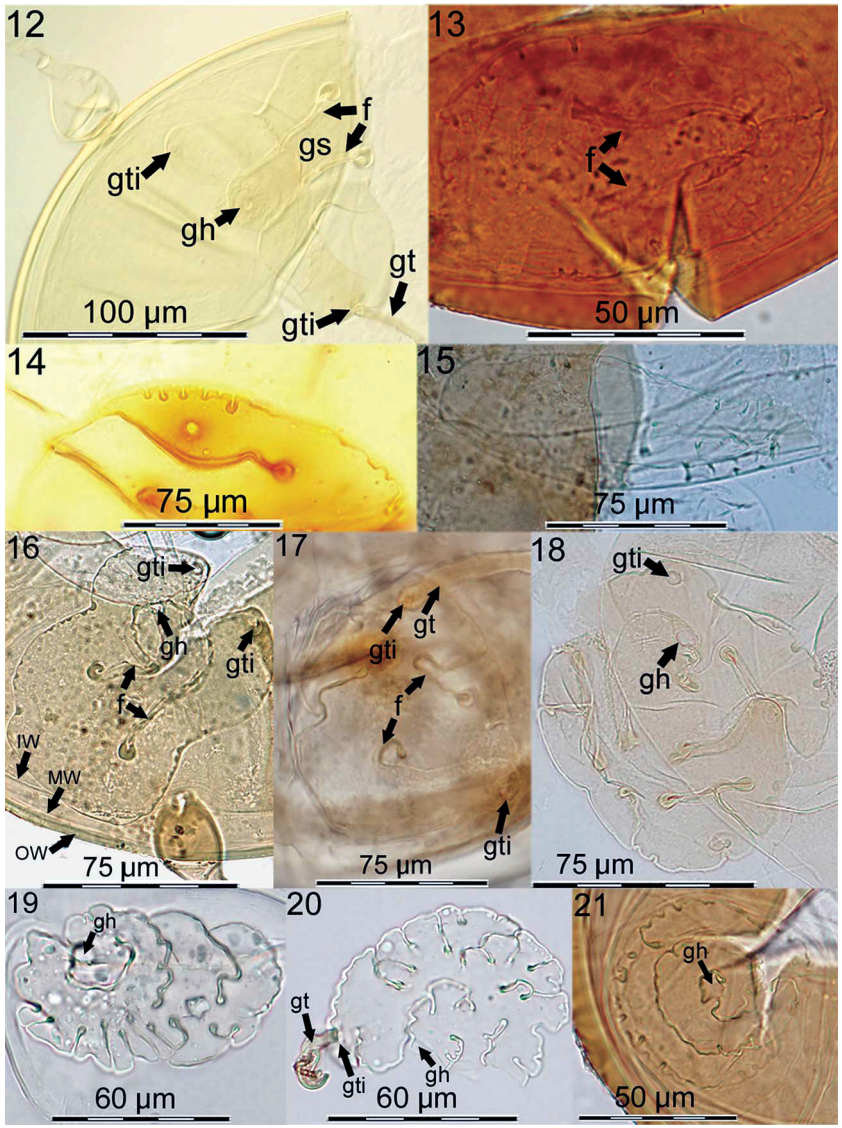


Plate 4—Typical simple, hyaline to subhyaline, violin-shaped to oval, to rarely cardioid, circular, oblong or irregular germination shields in *Scutellospora* spores. Spores have one outer (ow), one middle (mw) and one inner (iw) spore wall. Generally, shields have two compartments (lobes) with one germ tube initiation (gti) per lobe from where the germ tubes (gt) may arise (germination visible in fig. 12). Rarely shields are mono-lobed (figs. 19–21). Shield and inner wall in *S. tricalypta* darkened during 25 years mounted in Ferrant medium (fig. 14).

Scutellospora arenicola Koske & Halvorson,
Mycologia 81: 927. 1990 [‘1989’]. (FIG. 15)

Scutellospora aurigloba (I.R. Hall) C. Walker & F.E. Sanders.,
Mycotaxon 27: 180. 1986; emend. C. Walker & I.R. Hall,
Mycol. Res. 95: 400. 1991. (FIG. 17)
BASIONYM: *Gigaspora aurigloba* I.R. Hall, Trans. Brit. Mycol. Soc. 68: 351. 1977.

Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders,
Mycotaxon 27: 180. 1986. (FIG. 12)
BASIONYM: *Endogone calospora* T.H. Nicolson & Gerd., Mycologia 60: 322. 1968.
≡ *Gigaspora calospora* (T.H. Nicolson & Gerd.) Gerd. & Trappe,
Mycologia Memoir No. 5: 28. 1974.

Scutellospora crenulata R.A. Herrera, Cuenca & C. Walker,
Can. J. Bot. 79: 674. 2001. (FIGS. 19–20)

Scutellospora dipapillosa (C. Walker & Koske) C. Walker & F.E. Sanders,
Mycotaxon 27, 181. 1986. (FIG. 13)
BASIONYM: *Gigaspora dipapillosa* C. Walker & Koske, Mycologia 77: 709. 1985.

Scutellospora dipurpurescens J.B. Morton & Koske,
Mycologia 80: 520. 1988. (FIG. 16)

Scutellospora nodosa Błaszk., Mycologia 83, 537. 1991. (FIG. 18)

Scutellospora pernambucana Oehl, D.K. Silva, N. Freitas, L.C. Maia,
Mycotaxon 106: 363. 2008. (FIG. 21)

Scutellospora projecturata Kramad. & C. Walker, Annals Bot. 86: 22. 2000.

Scutellospora tricalypta (R.A. Herrera & Ferrer) C. Walker & F.E. Sanders,
Mycotaxon 27: 180. 1986. (FIG. 14)
BASIONYM: *Gigaspora tricalypta* R.A. Herrera & Ferrer, Rev. Jard. Bot. Nacional, Habana. 1:
49. 1981 [‘1980’].

Racocetraceae Oehl, Sieverd. & F.A. Souza, **fam. nov.**
MB 511946

Sporae terminaliter efformatae anguste adiacetae ad cellulas sporogeneas; scutellum germinale coniunctum ad tunicam interiorem, hyalinum subhyalinum ad alboflavum, undulo-lobatum cum 4–12 lobis et 4–12 depressionibus circularibus germinationis; formans structuras mycorrhizarum arbuscularum.

FIG. 12. Type species *S. calospora* (germination visible). FIG. 13. *S. dipapillosa* FIG. 14. *S. tricalypta* (25-year old spores in Ferrant medium). FIG. 15. *S. arenicola*. FIG. 16. *S. dipurpurescens*. FIG. 17. *S. aurigloba*. FIG. 18. *S. nodosa*. FIGS. 19–20. *S. crenulata* (monolobed). FIG. 21. *S. pernambucana* (monolobed).

Key characters: Spores formed singly in soils and rarely in roots formed on bulbous sporogenous cells which are formed terminally on a subtending hypha that arises from mycelia hyphae. Spores with two to three walls: an outer spore wall, either no middle wall or one middle wall, and an inner germinal wall. Each wall if present has one, two or several layers. A germination shield arises on the outer surface or beneath a thin outer layer of the inner wall; germination shield is hyaline to subhyaline seldom light yellow, generally oval to ellipsoid or subglobose, with several (4–12) conspicuously lobed, wave-like projections forming the outer surface of the shield; folds separate the lobes in compartments on the shield, and each compartment may have a germ tube initiation (gti). From one or rarely two gti a germ tube may arise and penetrate the middle and the outer spore wall. In the substending hypha of the sporogenous cell, often several septae are formed in some distance from pore. Auxiliary cells in the hyphal mycelium as far as they are known knobby without spines on the surface. Forming typical arbuscular mycorrhizae; vesicle formation in roots unknown.

Type genus: *Racocetra* Oehl et al.

Other genus: *Cetraspora*

Racocetra **Oehl,** F.A. Souza & Sieverd., **gen. nov.**

MB 511947

Sporae terminaliter efformatae anguste adiacetae ad cellulas sporogeneas; cum tunicis duabus; scutellum germinale coniunctum ad tunicam interiorem, hyalinum ad alboflavum, undulo-lobatum, 4–12 lobis et 4–12 depressionibus germinatione; formans structuras mycorrhizarum arbuscularum.

Etymology: from the Greek: (ρακος, racos = cloth, lobe), and from the Latin: cetra (light shield), referring to the wavy-lobed surface of the germination shield in planar view.

Key characters: Spores formed singly in soil and rarely in roots, on bulbous sporogenous cells that arise terminally on mycelia hyphae. Outer spore wall is generally three-layered and continuous with the wall of the sporogenous cell. Outer layer of the outer spore wall generally semi-persistent to persistent, rigid; second layer laminate; third layer thin, often membranous, tightly adherent to the laminate layer and thus, often difficult to observe. Pore between spore and sporogenous cell is narrow and usually closed by a plug formed by spore wall material. A single inner wall forms de novo during spore formation and has two to three layers. A germination shield arises on the outer surface or beneath a thin outer layer of the inner wall; germination shield is hyaline to subhyaline seldom light yellow, generally oval to ellipsoid or subglobose, with several (4–12) wave-like lobed projections forming the outer surface of the shield; folds separate the lobes on the shield, and each lobe may have a germ tube initiation

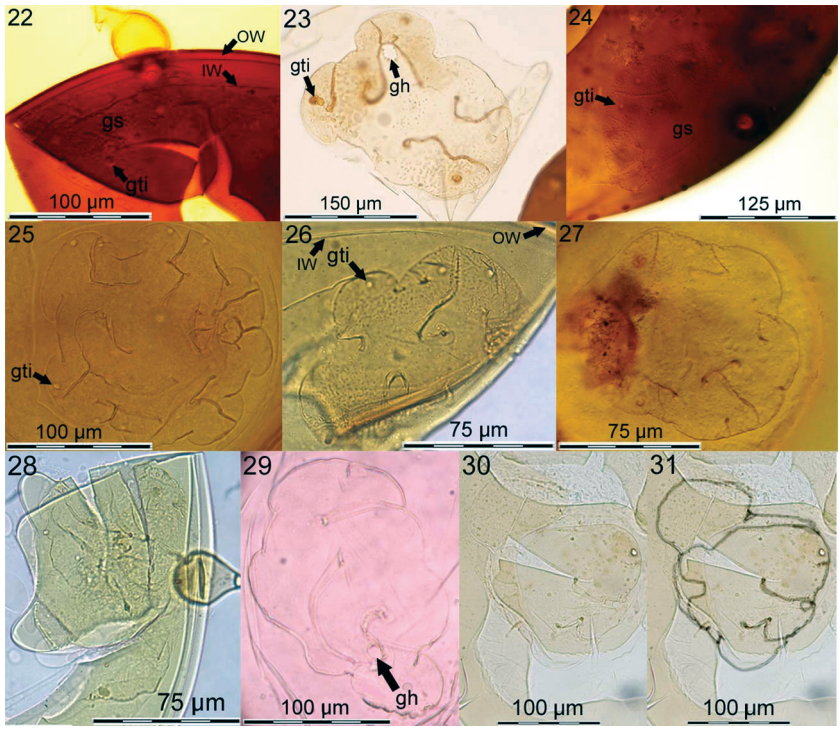


Plate 5—*Racocetra* germination shields (gs) have multiple compartments that give a multiple-lobed, wavy shield appearance. *Racocetra* spores have one outer (ow) and one inner (iw) spore wall. Generally one germ hole (gh) present with one germ tube initiation (gti) visible on each of the multiple lobes.

Fig. 22–23. Type species *R. coralloidea*. Fig. 23. Germ shield wall in the type specimen partly darkened after more than 30 years of storage in lactophenol. Fig. 24. *R. gregaria*. Fig. 25. *R. castanea*. Fig. 26. *R. persica*. Fig. 27. *R. verrucosa*. Figs. 28–29. *R. fulgida*. Fig. 29. Shield in a newly developed spore; gti's not yet formed. Figs. 30–31. *R. weresubiae* shield not completely visible in planar view and thus overlapping itself; slightly deformed through spore crushing; Fig. 31. Shield wall amplified using Adobe® Photoshop 9.0.

(about 2–5 µm in diam) from where the germ tubes arise and penetrate the outer wall. In the subtending hypha of the sporogenous cell one to several septa formed in some distance to the sporogenous cells. Auxiliary cells in the hyphal mycelium (as far as they are known) knobby without spines on the surface. Forming typical arbuscular mycorrhizae; vesicle formation in roots is so far unknown.

Type species: *Racocetra coralloidea* (Trappe, Gerd. & I. Ho) Oehl et al.

Key to *Racocetra* species

1a Spores without ornamentation on outer wall 2
1b Spores with ornamentation on outer wall 3
2a Spores light colored 4
2b Spores creamy-brown to brown-sienna, 170–370 µm in diam . . . . . *R. castanea*
3a Surface ornamentation with spines or warts generally < 2.5 µm in diam 5
3b Surface ornamentation with projections generally > 2.5 µm in diam 6
4a Spores hyaline to pale straw to pale yellowish-cream, 160–250 µm in diam . . . . . *R. fulgida*
4b Spores pale to dark pink, 120–170 × 130–420 µm in diam; outer wall staining yellow to yellow orange in Melzer's reagent . . . . . (*R. alborosea*)/*R. weresubiae*
5a Spore surface with fine spines; spores pinkish-orange to brownish orange, 270–390 µm in diam. . . . . *R. persica*
5b Spore surface with crowded rounded warts; spores pale straw to yellow to light orange brown, 220–480 µm in diam. . . . . *R. verrucosa*
6a Spores red brown to dark brown with rounded projections on surface 7
6b Spores dark brown, 300–460 µm in diam; with coralloidic warts. . . . . *R. coralloidea*
7a Spores red brown to dark brown, 250–450 µm in diam; with rounded dome-shaped warts. . . . . *R. gregaria*
7b Spores brown to dark brown; 90–180 µm in diam; warts on top with central depressions . . . . . *R. minuta*

Racocetra alborosea (Ferrer & R.A. Herrera) Oehl, F.A. Souza & Sieverd., **comb. nov.**
MB 511950
Basionym: *Gigaspora alborosea* Ferrer & R.A. Herrera, Rev. Jardin Bot. Nacional, Habana. 1: 55. 1981 ['1980'].
≡ *Scutellospora alborosea* (Ferrer & R.A. Herrera) C. Walker & F.E. Sanders, Mycotaxon 27: 180. 1986.

Racocetra castanea (C. Walker) Oehl, F.A. Souza & Sieverd., **comb. nov.**
MB 511952 (fig. 25)
Basionym: *Scutellospora castanea* C. Walker, Cryptogamie Mycologie 14: 280. 1993.

Racocetra coralloidea (Trappe, Gerd. & I. Ho) Oehl, F.A. Souza & Sieverd., **comb. nov.** (figs. 22–23)
MB 511948
Basionym: *Gigaspora coralloidea* Trappe, Gerd. & I. Ho, Mycologia Memoir No. 5: 30. 1974.
≡ *Scutellospora coralloidea* (Trappe, Gerd. & I. Ho) C. Walker & F.E. Sanders, Mycotaxon 27: 181. 1986.

Racocetra fulgida (Koske & C. Walker) Oehl, F.A. Souza & Sieverd., **comb. nov.** (figs. 28–29)
MB 511955
Basionym: *Scutellospora fulgida* Koske & C. Walker, Mycotaxon 27: 221. 1986.

Racocetra gregaria (N.C. Schenck & T.H. Nicolson) Oehl, F.A. Souza & Sieverd., **comb. nov**. (FIG. 24)
MB 511949
BASIONYM: *Gigaspora gregaria* N.C. Schenck & T.H. Nicolson, Mycologia 71: 185. 1979.
≡ *Scutellospora gregaria* (N.C. Schenck & T.H. Nicolson) C. Walker & F.E. Sanders, Mycotaxon 27: 181. 1986.

Racocetra minuta (Ferrer & R.A. Herrera) Oehl, F.A. Souza & Sieverd., **comb. nov.**
MB 511951
BASIONYM: *Gigaspora minuta* Ferrer & R.A. Herrera, Rev. Jardin Bot. Nacional, Habana. 1: 53. 1981 ['1980'].
≡ *Scutellospora minuta* (Ferrer & R.A. Herrera) C. Walker & F.E. Sanders, Mycotaxon 27: 180. 1986.

Racocetra persica (Koske & C. Walker) Oehl, F.A. Souza & Sieverd., **comb. nov.**
MB 511953 (FIG. 26)
BASIONYM: *Gigaspora persica* Koske & C. Walker, Mycologia 77: 708. 1985.
≡ *Scutellospora persica* (Koske & C. Walker) C. Walker & F.E. Sanders, Mycotaxon 27:181. 1986.

Racocetra verrucosa (Koske & C. Walker) Oehl, F.A. Souza & Sieverd., **comb. nov.**
MB 511954 (FIG. 27)
BASIONYM: *Gigaspora verrucosa* Koske & C. Walker, Mycologia 77: 705. 1985.
≡ *Scutellospora verrucosa* (Koske & C. Walker) C. Walker & F.E. Sanders, Mycotaxon 27: 181. 1986.

Racocetra weresubiae (Koske & C. Walker) Oehl, F.A. Souza & Sieverd., **comb. nov.**
MB 511956 (FIGS. 30–31)
BASIONYM: *Scutellospora weresubiae* Koske & C. Walker, Mycotaxon 27: 224. 1986.

Cetraspora Oehl, F.A. Souza & Sieverd., **gen. nov.**
MB 511957

Sporae terminaliter efformatae anguste adiacetae ad cellulas sporogeneas; cum tunicis tribus: scutellum germinale coniunctum ad tunicam interiorem, hyalinum ad alboflavum, undulo-lobatum, cum 4–12 lobis et 4–12 depressionibus germinatione; formans structuras mycorrhizarum arbuscularum.

ETYMOLOGY: from the Latin: *cetra* (= light shield) and spora (= spore), referring to the light colored germination shield which often is difficult to observe, as in *C. gilmorei* and especially in *C. pellucida*.

KEY CHARACTERS — Spores formed singly in soil and rarely in roots on bulbous sporogenous cells that arise terminally on a subtending hypha which is connected to mycelium. Outer spore wall generally is three-layered and continuous with the wall of the sporogenous cell. Outer layer of the outer spore wall generally semi-persistent to persistent, rigid; second layer laminate; third layer thin, often membranous, tightly adherent to the laminate layer and thus, often difficult to observe. Pore between spore and sporogenous cell is narrow

and usually closed by a plug formed by spore wall material. Two hyaline walls ('middle wall' and 'inner wall') form de novo during spore formation and have 1–2 and 2–3 layers, respectively. A germination shield arises on the outer surface of the inner wall or beneath a thin outer layer of the innermost wall; germination shield is hyaline to subhyaline seldom light yellow, generally oval to ellipsoid or subglobose, with several (4–12) wave-like lobed projections forming the outer surface of the shield; large folds separate the lobes on the shield, and each lobe may have an germ tube initiation (about 2–5 µm in diam) from where the germ tubes arise and penetrate the overlaying spore walls. Subtending hyphae form one to several septa in some distance to the sporogenous cells. Auxiliary cells in the hyphal mycelium as far as they are known knobby without spines on the surface. Forming typical arbuscular mycorrhizae; vesicle formation in roots is unknown.

Type species: *Cetraspora gilmorei* (Trappe & Gerd.) Oehl et al.

Key to *Cetraspora* species

1a Spores without ornamentation on outer wall 2
1b Spores with ornamentation on outer wall 3
2a Spores hyaline to white 4
2b Spores apricot yellow to yellow brown, 140–240 µm in diam . . . . . *S. armeniaca*
3a Spores with a fine dense spiny ornamentation on outer spore wall; spores ochraeous to fulvous or rust, 130–230 µm in diam . . . . . *C. spinosissima*
3b Spores ornamentated with finger-print-like processes in planar view; spores ochraeous yellow with a pinkish tint, 110–190 µm in diam . . . . . *C. striata*
4a Spores brilliant hyaline, white to rarely pale grey, generally globose to subglobose; (60–)120–250(–420) µm in diam; sporogenous cell a hyaline to subhyaline; germination shield generally not seen . . . . . *C. pellucida*
4b Spores hyaline, becoming creamy under storage in formalin; globose to subglobose to ellipsoid; 200–320 µm in diam; sporogenous cell brown; germination shield generally easy to observe . . . . . *C. gilmorei*

Cetraspora armeniaca (Błaszk.) Oehl, F.A. Souza & Sieverd., **comb. nov.** (fig. 35)
MB 511961
Basionym: *Scutellospora armeniaca* Błaszk., Mycologia 84: 939. 1993.

Cetraspora gilmorei (Trappe & Gerd.) Oehl, F.A. Souza & Sieverd., **comb. nov.**
MB 511958 (figs. 32–33)
Basionym: *Gigaspora gilmorei* Trappe & Gerd., Mycologia Memoir No. 5: 27. 1974.
≡ *Scutellospora gilmorei* (Trappe & Gerd.) C. Walker & F.E. Sanders, Mycotaxon 27: 181. 1986.

Cetraspora pellucida (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd., **comb. nov.** (fig. 34)
MB 511959

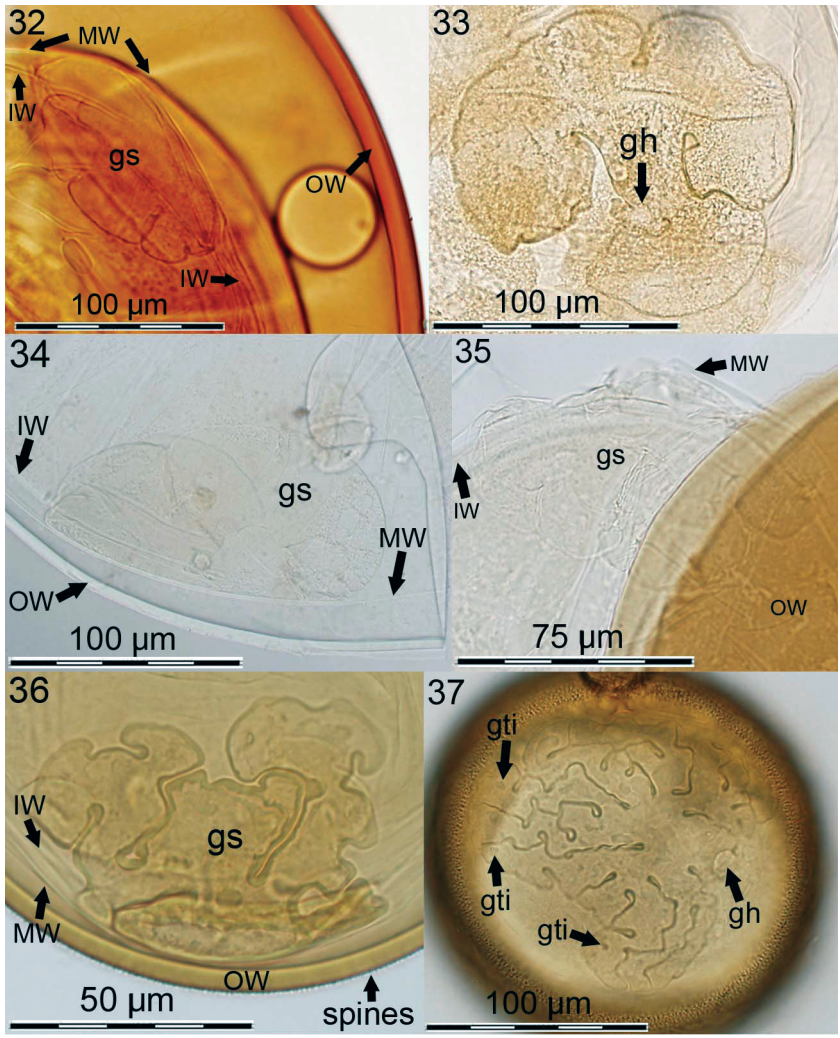


Plate 6—Multiple-lobed, wavy germination shield (gs) form in *Cetraspora* spores; spores with one outer (ow), one middle (mw) and one inner (iw) spore wall. On each of the lobes (compartments) a germ tube initiation (gti) might be formed from where the germ tubes arise. Shields and gti are generally light or transparent and less obvious than in *Racocetra*.

Fig. 32–33. Type specimen of *C. gilmorei*. Spore walls and shield have darkened after > 30 years stored in lactophenol. Fig. 34. *C. pellucida*. Fig. 35. *C. armeniaca*. Figs. 36–37. *C. spinosissima*.

Basionym: *Gigaspora pellucida* T.H. Nicolson & N.C. Schenck, Mycologia 71: 189. 1979.

≡ *Scutellospora pellucida* (T.H. Nicolson & N.C. Schenck) C. Walker & F.E. Sanders, Mycotaxon 27: 181. 1986.

Cetraspora spinosissima (C. Walker & Cuenca) Oehl, F.A. Souza & Sieverd., **comb. nov.** (figs. 36–37)
MB 511960
Basionym: *Scutellospora spinosissima* C. Walker & Cuenca, Annals Bot. 82: 723. 1998.

Cetraspora striata (Cuenca & R.A. Herrera) Oehl, F.A. Souza & Sieverd., **comb. nov.**
MB 512598
Basionym: *Scutellospora striata* Cuenca & R.A. Herrera, Mycotaxon 105: 81. 2008.

Dentiscutataceae F.A. Souza, Oehl & Sieverd., **fam. nov.**
MB 511962

Sporae terminaliter efformatae anguste adiacetae ad cellulas sporogeneas; cum tunicis tribus vel quartuoribus; scutellum germinale coniunctum ad tunicam interiorem, flavo-brunneum ad brunneum, viola-forme vel ovale vel cardioforme vel ellipsoideum. Formans structuras mycorrhizarum arbuscularum.

Key characters: Sporocarps are unknown. Spores formed singly on bulbous sporogenous cells which are formed terminally on a subtending hypha that arise from mycelia hyphae. Outer spore wall three- to four to rarely five-layered. Spores have 3–4 walls, an outer spore wall with several layers, one to several middle walls and an inner wall each of two or more layers. Germination shield generally formed on the outer surface of the innermost wall or beneath a thin outer layer of the inner wall, yellow-brown to brown, violin-shaped to oval to ovoid to heart-shaped to reniforme to ellipsoid, consisting of two lobe-like compartments or up to 30 small compartments. Lobes and compartments are separated by folds and generally have each one germ tube initiations; from one to a few gti a germ tube may arise penetrating the other walls. Subtending hypha forms one to several septa in some distance to the sporogenous cells. Auxiliary cells in the mycelium hyphae as far as they are known knobby without spines on the surface. Forming typical arbuscular mycorrhizae, vesicle formation in roots unknown.

Type genus: *Dentiscutata* Sieverd. et al.

Other genera: *Fuscutata*, *Quatunica*

Dentiscutata Sieverd., F.A. Souza & Oehl, **gen. nov.**
MB 511968

Sporae singulatim terminaliter efformatae anguste adiacetae ad cellulas sporogeneas; cum tunicis tribus; scutellum germinale coniunctum ad tunicam interiorem, flavo-brunneum ad brunneum; cum 8–30 compartimentibus et depressionibus germinationis; formans structuras mycorrhizarum arbuscularum.

Etymology: From the Latin denti(culata) (= dentate), and scutata (= 'shielded'), referring to the dentate form of the periphery of the brown germination shield.

Key characters: Sporocarps are unknown. Spores singly formed on bulbous sporogenous cells that are formed terminally on subtending hypha that arises

from mycelia hyphae. Outer spore wall is three- to five-layered and continuous with the wall of the sporogenous cell. Pore between the spore and sporogenous cell is narrow and usually closed by a plug formed by outer spore wall material. A hyaline middle wall and a hyaline inner wall form de novo during spore formation; middle wall one- to bilayered; inner wall two to three-layered. Germination shield generally arising on the outer surface of the innerwall or beneath a thin outer layer of the inner wall, yellow-brown to brown, generally ellipsoid (to oval), or rarely reniforme to cardioforme, usually with many (8–30) large folds separating the shield into 8–30 'small compartments'; each small compartment generally with one circular germ tube initiation; usually from one or a few gti germ tubes may arise and penetrate the middle wall and the outer wall; the periphery of the germination shield generally appears dentate in planar view. Septa often formed in subtending hypha in some distance to the sporogenous cells. Auxiliary cells formed in hyphal mycelium as far as they are known knobby without spines on the surface. Forming typical arbuscular mycorrhizae, vesicle formation in roots so far unknown.

Type species: *Dentiscutata nigra* (J.F. Redhead) Sieverd. et al.

Key to *Dentiscutata* species

1a Spores without ornamentation on outer wall 2
1b Spores with ornamentation on outer wall 3

2a Spores hyaline, white to light olive, 350–750 µm in diam
. . . . . .*D. scutata* (sensu Walker & Diederichs 1989)
2b Spores pale orange brown to red brown, 200–300 µm in diam; outer wall not staining in Melzer's reagent; second middle wall layer fracturing with a series of rectangular and V-shaped notches. . . . . *D. hawaiiensis*

3a Spores with a single ornamentation on outer spore wall 4
3b Spores with double ornamentation on outer spore wall. 5

4a Spores hyaline to white, globose to ovoid, 170–370 µm in diam; with spiny papillae ornamentation . . . . . *D. cerradensis*
4b Spore with papillae surface ornamentation on outer wall; Spores light brown, globose to ellipsoid, 150–260 µm in diam. . . . . *D. heterogama*

5a Spores with double ornamentation on the outer spore surface 6
5a Spores with each one papillae ornamentation both on outer and inner surface of the outer wall; yellow-brown to brown, (120–)260–450(–500) µm in diam . . . . . .*D. biornata*

6a Spores orange brown to dark red brown, 200–470 µm in diam; having spines in the depressions of a reticulum. . . . . *D. reticulata*
6b Spores dark brown to black, 290–500(–1050); with rounded pits and a sinuous secondary ornamentation below . . . . . .*D. nigra*

Dentiscutata biornata (Spain, Sieverd. & S. Toro) Sieverd., F.A. Souza & Oehl, **comb. nov.** (FIG. 40)
MB 511971
BASIONYM: *Scutellospora biornata* Spain, Sieverd & S. Toro, Mycotaxon 35: 220. 1989.

Dentiscutata cerradensis (Spain & J. Miranda) Sieverd., F.A. Souza & Oehl, **comb. nov.** (FIG. 42)
MB 511973
BASIONYM: *Scutellospora cerradensis* Spain & J. Miranda, Mycotaxon 60: 130. 1996.

Dentiscutata hawaiiensis (Koske & Gemma) Sieverd., F.A. Souza & Oehl, **comb. nov.** (FIG. 41)
MB 511972
BASIONYM: *Scutellospora hawaiiensis* Koske & Gemma, Mycologia 87: 678. 1995.

Dentiscutata heterogama (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl, **comb. nov.** (FIG. 44)
MB 511975
BASIONYM: *Endogone heterogama* T.H. Nicolson & Gerd., Mycologia 60: 319. 1968.
≡ *Gigaspora heterogama* (T.H. Nicolson & Gerd.) Gerd. & Trappe, Mycologia Memoir No. 5: 31. 1974.
≡ *Scutellospora heterogama* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders, Mycotaxon 27: 180. 1986.

Dentiscutata nigra (J.F. Redhead) Sieverd., F.A. Souza & Oehl, **comb. nov.**
MB 511969 (FIG. 38)
BASIONYM: *Gigaspora nigra* J.F. Redhead, Mycologia 71: 187. 1979.
≡ *Scutellospora nigra* (J.F. Redhead) C. Walker & F.E. Sanders, Mycotaxon 27: 181. 1986.

Dentiscutata reticulata (Koske, D.D. Mill. & C. Walker) Sieverd., F.A. Souza & Oehl, **comb. nov.** (FIG. 39)
MB 511970
BASIONYM: *Gigaspora reticulata* Koske, D.D. Mill. & C. Walker, Mycotaxon 16: 429. 1983.
≡ *Scutellospora reticulata* (Koske, D.D. Mill. & C. Walker) C. Walker & F.E. Sanders, Mycotaxon 27: 181. 1986.

Dentiscutata scutata (C. Walker & Dieder.) Sieverd., F.A. Souza & Oehl, **comb. nov.**
MB 511974 (FIG. 43)
BASIONYM: *Scutellospora scutata* C. Walker & Dieder., Mycotaxon 35: 357. 1989.

Fuscutata Oehl, F.A. Souza & Sieverd., **gen. nov.**
MB 511963

Sporae terminaliter efformatae anguste adiacetae ad cellulas sporogeneas; cum tunicis tribus; scutellum germinale coniunctum ad tunicam interiorem, flavo-brunneum ad brunneum; ovale vel ovoidum vel viola-forme, cum 2 lobis et depressionibus germinatione; formans structuras mycorrhizarum arbuscularum.

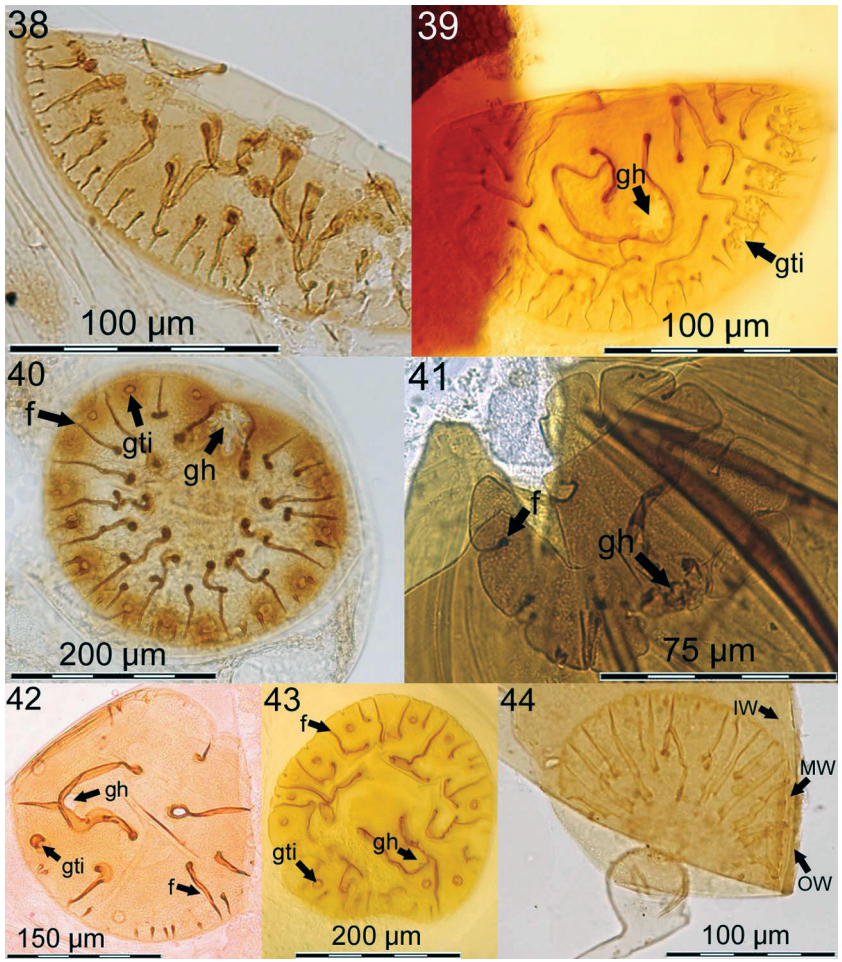


Plate 7—*Dentiscutata* with complex structured, yellow-brown to brown germination shields (gs) showing several to many (8–30) compartments, separated by large folds (f); often one initial germ hole (gh) and several to many (8–30) germ tubes initiations (gti) visible; germ tubes emerge from one or two gti during germination; shield periphery conspicuously dentate in planar view.

Fig. 38. Type species *D. nigra.* Fig. 39. *D. reticulata.* Fig. 40. *D. biornata.* Fig. 41. *D. hawaiiensis* Fig. 42. *D. cerradensis.* Fig. 43. *D. scutata* sensu Silva et al. (2006b). Fig. 44. *D. heterogama*.

Etymology: from the Latin fu(scus) (= brown), and scutata (= 'shielded'), referring to the color of the germination shield formed on the inner spore wall.

Key characters: Sporocarps are unknown. Single spores formed on bulbous sporogenous cells which are terminally formed on the subtending hypha that

arises from mycelia hyphae. Outer spore wall three- to four layered. Pore between the spore and sporogenous cell is narrow and usually closed by a plug formed by spore wall material. Two hyaline walls ('middle wall' and 'inner wall') form de novo during spore formation and have 1–2 and 2–3 layers, respectively. Germination shield generally formed on the outer surface of the innermost wall or beneath a thin outer layer of the inner wall, yellow-brown to brown, generally ovoid to violin-shaped to heart-shaped, consisting of 2 lobes and folds; both lobes with a germ tube initiation ('gti', about 3-6 µm in diam). Spore germination generally from one gti; germ tube penetrates from there the middle and outer spore wall. In the subtending hypha one to several septa are formed in some distance to the sporogenous cell. Auxiliary cells in the hyphal mycelium as far as they are known knobby without spines on the surface. Forming typical arbuscular mycorrhizae, vesicle formation in roots so far unknown.

Type species: *Fuscutata heterogama* Oehl et al.

Key to *Fuscutata* species

1a Spore without surface ornamentation on outer wall 2
1b Spore with surface ornamentation on outer wall 3
2a Spores hyaline to white; oblong-ellipsoid to long ellipsoid, 280–580 × 210–370 µm in diam. . . . . .*F. savannicola*
2b Spores dark orange brown to dark red brown, 140–220 µm in diam, outer and innermost wall strongly staining in Melzer's reagent. . . . . *F. rubra*
3a Spores hyaline to subyaline, globose to oval, µm in diam; spores with fine spiny ornamentation on the surface of the outer wall . . . . . *F. trirubiginopa*
3b Spore with papillae ornamentation on the surface of the outer wall; spores red brown to dark brown, globose to oval, 159–295 µm in diam . . . . . *F. heterogama*

Fuscutata heterogama Oehl, F.A. Souza, L.C. Maia & Sieverd., **sp. nov.**
MB 511964 (figs. 45–46)

Sporae singillatim in solo efformatae anguste adiacetae ad cellulas sporogeneas subterminales vel intercalares, brunneae vel rubro-brunneae vel oscuro-brunneae, globosae, 165–280 µm in diametro, vel subglobosae vel ovale (159–265 × 195–295 µm); sporae cum tunicis tribus: tunica exterior stratis tribus in totum 7.0–11.5 µm crassa, coniuncta tunicam cellulae sporogeneae et tunica hyphae; stratum exterius tunicae exterioris hyalinum, evanescens, 1.1–2.1 µm crassum; papillibus ornatum altis 1.1–2.1 µm et 0.9–1.5(–2.5) µm latis ornatum; stratum secundum brunneum vel rubro-brunneum vel oscuro-brunneum, laminatum, 5.5–8.5µm crassum; stratum interius tunicae exterioris brunneum vel rubro-brunneum, subtile; tunica media et tunica interior de novo formans; cum stratis hyalinibus; tunica media duobus stratis in totum 2.1–2.8 µm crassum; tunica interior tribus stratis, 2.1–3.4 µm crassa; stratum medium et stratum interiore tunicae exterioris purpureo-brunneum vel oscuro-brunneum colorans, stratum medium tunicae interioris purpureo colorans reagente Melzeri; scutellum germinale coniunctum ad tunicam interiorem, flavo-

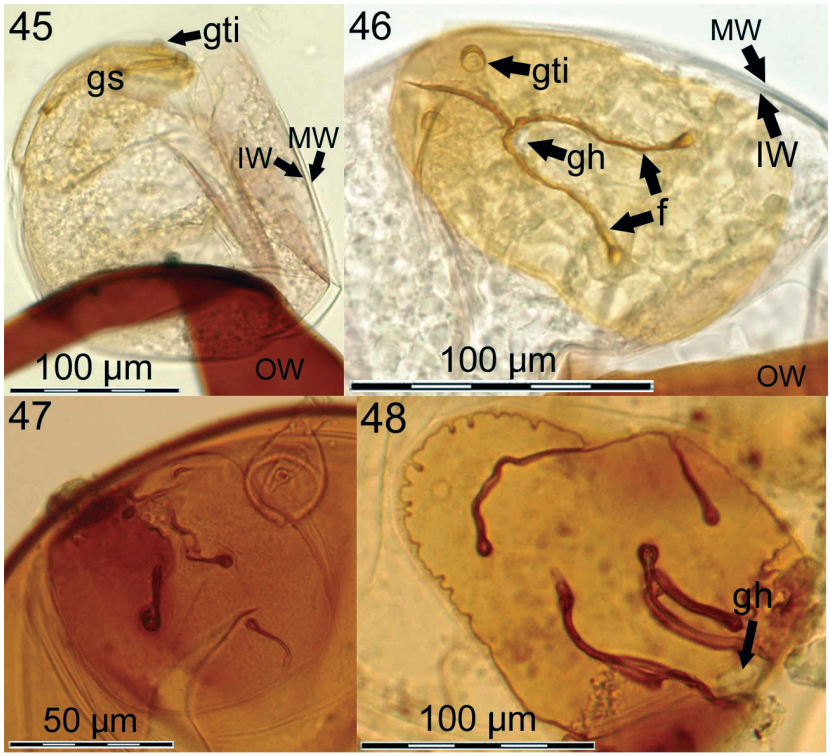


Plate 8—*Fuscutata* with yellow-brown to brown germination shields and three walls (ow, mw and iw). . Germination shields are generally bi-lobed with a germ hole (gh) and two germ tube initiations (gti). figs. 45–46. Type species *F. heterogama*. fig. 47. *F. rubra*. fig. 48. Old shield, in *F. savannicola*.

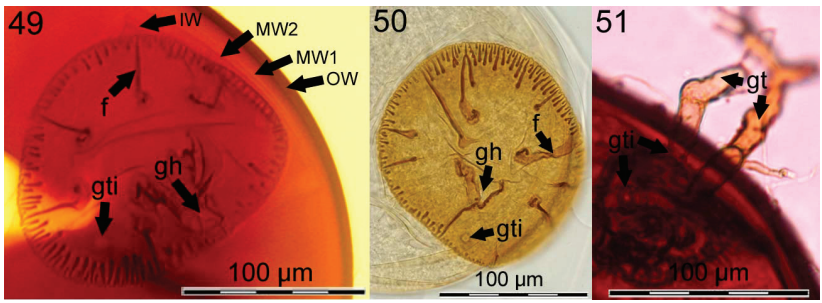


Plate 9—figs. 49–51: *Quatunica* with one species, *Q. erythropus*: Complex structured, yellow-brown to brown germination shields (gs) in spores of type species *Q. erythropus* having four spore walls (ow, mw1, mw2 and iw). Germination shield on the outer surface of iw. Shields with many (> 10) compartments, separated by large folds (f); generally one initial germ hole (gh) and several germ tubes initiations (gti) visible from where one or few germ tubes per spore emerge during germination (fig. 51); periphery of shield walls conspicuously dentate.

brunneum ad brunneum; ovale vel ovoid vel viola-forme, simplex bilobatumque cum 2 depressionibus germinationis; structurae mycorrhizarum arbuscularum formans.

Type specimens: BRAZIL. Pernambuco. Município de Camaragibe. Location: Aldeia. ***holotype*** (84-8401) and isotype (84-8402 & 84-8403) specimens deposited at URM (Recife, Brazil); isolated from field samples (10 June 1997) and established in a pure culture maintained at URM and INVAM; isotypes 84-8404& 84-8405 deposited at Z+ZT (accession number ZT Myc 642), and 84-8406 & 84-8407 deposited at OSC (OSC #134'504).

Etymology: heterogama referring to *Endogone heterogama* with which this new species has been very often confused.

Commentary: A detailed English species description of *F. heterogama* is not needed, since several isolates of the new species were intensively studied during the last 20 years (e.g. Franke & Morton 1994, Jeffries et al. 2007). Spores are globose (165–280 µm in diam) to subglobose to oval (159–265 × 195–295 µm) to rarely irregular, reddish-brown to dark red-brown to dark brown, with three spore walls (ow, mw and iw). The ow three-layered (owl1-3: owl1 hyaline, semi-persistent to persistent, 1.1–2.1 µm thick, with papillae projections, 0.9–1.5(–2.5) µm in diameter and at 0.8–2.4 µm distance from each other; owl2 red-brown to dark brown, laminate. 5.5–8.5 µm thick, owl3 subtile, concolorous with owl2; mw hyaline, bi-layered, in total 2.1–2.8 µm thick; iw bi-to-tree-layered, in total 2.1–3.4 µm thick; owl2 & owl3 and iwl2 staining dark red-brown and purple, respectively, in Melzer's reagent. Sporogenous cell globose to oblong, 32–51 × 31–45 µm; germ shield yellow-brown to brown, simple, bi-lobed, oval to ovoid to violin-shaped (75–105 × 55–75 µm) to rarely irregular, generally with 2 lobes rarely showing 4 folds; shield periphery generally smooth, rarely with scarse dentate structures. Forms typical arbuscular mycorrhiza without intraradical vesicles but extraradical auxiliary cells in the hyphal mycelium. *Fuscutata heterogama* can be easily distinguished from *D. heterogama* by spore color and especially by the morphology of the shield (see above; compare figs. 45–46 with fig. 44). It can be easily distinguished from other *Fuscutata* spp. by the ornamentation on the outer spore surface (see identification key below).

Distribution: *Fuscutata heterogama* has a major distribution in warmer climates (Mediterranean, Subropical and Tropical climates) but was confused with *Endogone heterogama* which might have a less broad distribution. The new species is frequent in Southern America as we have found it several times in Colombia and Brazil. It was recently found also in Benin (West Africa; Hountondji & Oehl, unpublished, Tchabi et al. 2008).

Other specimens examined: COLOMBIA. Eastern Plains. Sieverding collection. UNITED STATES. Florida. Illionois. Specimens deposited at INVAM, and specimens prepared by F. Oehl during his visit at INVAM in 2002 (slides deposited at Z+ZT). Specimens of the Embrapa Agrobiologia germplasm collection, Seropédica, Brazil,

CNPAB2. BENIN: Guinea and Sudan savannas under yam and peanuts (Tchabi et al. 2008).

Fuscutata rubra (Stürmer & J.B. Morton) Oehl, F.A. Souza & Sieverd., **comb. nov.** (FIG. 47)

MB 511965

BASIONYM: *Scutellospora rubra* Stürmer & J.B. Morton, Mycol. Res. 103: 951. 1999.

Fuscutata savannicola (R.A. Herrera &Ferrer) Oehl, F.A. Souza & Sieverd., **comb. nov.** (FIG. 48)

MB 511966

BASIONYM: *Gigaspora savannicola* R.A. Herrera & Ferrer, Rev. Jard. Bot. Nacional, Habana. 1: 57. 1981.

≡ *Scutellospora savannicola* (R.A. Herrera & Ferrer) C. Walker & F.E. Sanders, Mycotaxon 27: 180. 1986.

Fuscutata trirubiginopa (X.L. Pan & G.Yun Zhang) Oehl, F.A. Souza & Sieverd., **comb. nov.**

MB 511967

BASIONYM: *Scutellospora trirubiginopa* X.L. Pan & G.Yun Zhang, Mycosystema 16: 169.

Quatunica F.A. Souza, Sieverd. & Oehl, **gen. nov.**

MB 511976

Sporae terminaliter efformatae anguste adiacetae ad cellulas sporogeneas, quartuoribus tunicis; scutellum germinale coniunctum ad tunicam interiorem, flavo-brunneum ad brunneum, cum 8–30 compartimentibus et depressionibus germinationis; formans structuras mycorrhizarum arbuscularum.

ETYMOLOGY: From the Latin: 'qua' abreviation from quartuor (four) and 'tunica' (wall) referring to the four spore walls, a unique character in *Glomeromycota.*

KEY CHARACTERS: Sporocarps are unknown. Spores singly formed on bulbous sporogenous cells that are formed terminally on a subtending hypha that arises from mycelia hyphae. Spores have 4 walls. Outer spore wall three- to four-layered and continuous at least in part with the wall of the sporogenous cell; pore between the spore and sporogenous cell is narrow and usually closed by a plug formed by outer spore wall material. Two hyaline middle walls and a hyaline inner wall form de novo during spore formation. Germination shield generally arising on the outer surface of the inner wall or beneath a thin outer layer of the inner wall, yellow-brown to brown, generally ellipsoid to oval, or rarely reniforme to cardioforme, usually with a many (8–30) large folds separating the shield into 8–30 'small compartments'; each small compartment generally with one germ tube initiation ('gti). From one or few gti germ tubes may arise penetrating the middle walls and the outer wall; the periphery of shield wall usually conspicuously dentate in planar view. One to several septa often formed in the subtending hypha in some distance to the sporogenous cell.

Auxiliary cells formed in hyphal mycelium as far as they are known knobby without spines on the surface. Forming typical arbuscular mycorrhizae, vesicle formation in roots so far unknown.

Type species: *Quatunica erythropus* (Koske & C. Walker) F.A. Souza et al.

Key to *Quatunica* species

1a Spores white-yellow, to yellow-brown, 340–640 µm in diam
. . . . . *Q. scutata* (*S. scutata* sensu Silva et al. 2006b) [non editum]
1b Spores orange-brown to dark red brown, 200–650 µm in diam . . . . . *Q. erythropus*

Quatunica erythropus (Koske & C. Walker) F.A. Souza, Sieverd. & Oehl, **comb. nov.** (plate 9)
MB 511977
Basionym: *Gigaspora erythropus* Koske & C. Walker, Mycologia 76: 250. 1984.
≡ *Scutellospora erythropus* (Koske & C. Walker) C. Walker & F.E. Sanders, Mycotaxon 27: 181. 1986.

Discussion

Molecular analyses, based almost exclusively on 18S rRNA genes, have provided the means to describe the phylum *Glomeromycota* and to reorganize almost entirely the glomeromycotan phylogeny (Schüßler et al. 2001). This resulted in a new order, the *Diversisporales*, validly described by Walker & Schüßler (2004), which contains species and genera that can be distinguished morphologically by the different spore formation types: acaulosporoid, entrophosporoid, gigasporoid or glomoid. Such spore formation differences earlier served as the basis for higher order ranks (Morton & Benny 1990). Only recently, new families and genera have been proposed for the *Glomeromycetes* based on combined molecular genetic and spore morphological data. Oehl & Sieverding (2004) separated the *Pacispora* genus from *Glomus* in the *Glomeraceae*, order *Glomerales*, based on morphological and germination characteristics, and Walker et al. (2004) cited molecular data in describing the new *Pacisporaceae* family in the order *Diversisporales* (Walker & Schüßler 2004). Sieverding & Oehl (2006), Spain et al. (2006) and Walker (2007) also referred to molecular data to review *Entrophospora* and the *Archaeosporaceae*, which prompted a taxonomic reorganization of new fungal genera and families in the *Archaeosporales* based on 18S and ITS rRNA-generated phylogenies (Walker et al. 2007).

We used published DNA sequences available from 18S and 25S rDNA for a total of 17 former *Scutellospora* spp. and 7 *Gigaspora* spp. (figs. 10–11) and morphological data from all previously described *Scutellospora* species as a basis for separating the new families and genera in the *Diversisporales* from the *Gigasporaceae*. The molecular analyses showed that *Gigasporaceae*

Table 3. Spore morphology and spore wall characteristics in *Scutellospora*, *Racocetra*, *Cetraspora*, *Fuscutata*, *Dentiscutata* and *Quatunica*

	Spore size (µm)	Spore shape	Spore color	Suspensor size (µm)	# of wall layers on OW-MW-IW[A]	Ornamentation on outer wall	Melzer's rxn on wall layer [A,B]
Scutellospora							
S. arenicola	160–360 × 120–310	Subglobose to irregular	Yellow brown to orange brown	35–47	3–2–2	No	OWL2 IWL2
S. aurigloba	200–400 × 130–420 (–520)	Globose to rarely polymorph	Yellow to dull yellow	40–70	3–2–2	No	IWL2
S. calospora	90–290 × 125–510	Ellipsoid to ovoid to cylindrical, rarely globose	Hyaline to light yellow	22–35 × 28–48	3–2–2	No	IWL2
S. crenulata	120–170	Globose to subglobose to rarely ellipsoid	Ochraceus to ochre to yellow	22–46 × 18–27	3-1-2	Warts on domed papillae	OWL2 MWL2 IW
S. dipapillosa	150–165 × 135–180	Globose to subglobose to irregular	Pale orange brown to dark orange brown	27–40	3–2–2	Double, warts and blunts	Unknown
S. dipurpurescens	150–240	Globose to subglobose, to rarely ellipsoid	Yellow to greenish yellow	26–30	3–1–2	No	IWL2
S. nodosa	160–270	Globose to subglobose, rarely ovoid	Hyaline to pale yellow	27–50	3–2–2	Knobby	OWL2 MW IW
S. pernambucana	105–135 × 120–150	Globose to subglobose to rarely oval	Dark yellow to brown yellow	31–48 × 26–43	3–2–2	No	IWL2
S. projecturata	100–175 × 105–185	Globose to subglobose to rarely ellipsoid	Golden, dull yellow, ochre to sienna	32–60 × 29–47	3–1–2	Straight or hooked digitations	IW
S. tricalypta	300–390; 285 × 455	Globose to subglobose to ellipsoid	Brown to dark brown	14–47	3–2–2	No	Unknown

Table 3, continued

	Spore size	Spore shape	Spore color	Suspensor size	# wall layers	OW Ornamentation	Melzer's rxn
Racocetra							
R. alborosea	200–210 × 265–345	Globose to subglobose to irregular	Pale pink to dark pink [c]	21–50	3–0–2	No	Unknown
R. castanea	165–370 × 175–375	Globose to subglobose, or ovoid	Ochraceous to sienna	38–51	3–0–2	No	OWL1-2
R. coralloidea	300–395 × 320–455	Globose to subglobose to ellipsoid	Orange-red to dark orange brown	55–64	3–0–2	flattened warts with angular margins	OWL2, hardly to discern (spore color)
R. fulgida	160–240	Globose to subglobose	Hyaline to pale straw or pale yellowish-cream	26–46, sometimes darker than spore	3–0–2	No	OWL2
R. gregaria	250–450	Globose to subglobose	Red brown to dark brown	39–80	3–0–2	Rounded warts	OWL2
R. minuta	95–180	Globose to subglobose to irregular	Brown to dark brown to opaque	22–31	3–0–2	Concave pits in tips of rounded warts	Unknown
R. persica	270–360 × 280–384	Globose to subglobose to ellipsoid	Pale pinkish-orange to brownish orange	45–62 × 47–125	3–0–2	fine spines	OWL2
R. verrucosa	220–480	Globose to subglobose	Pale straw to yellow to yellow brown	55–100 × 35–70	3–0–2	verrucose warts	OWL2
R. weresubiae	125–265 × 135–415	Globose to subglobose to irregular	Pale pink to deep pink [c]	32–50	3–0–2	No	OWL2 (yellow); not IWL2! [type material!]
Cetraspora							
C. armeniaca	140–240	Globose to subglobose	Apricot yellow to yellow brown	32–42	3–2–2	No	OWL2 MWL2 IWL2
C. gilmorei	200–320	Globose to subglobose to rarely ellipsoid	Hyaline [D], suspensor light brown	27–40,	3–2–2	No	IWL2
C. pellucida	60–240 × 60–240 (–410)	Globose to subglobose to ellipsoid	Hyaline, white to pale grey	10–50	3–2–2	No	OWL2 IWL2
C. spinosissima	130–230 × 120–210	Globose to subglobose to broadly ellipsoid	Ochraceous to fulvous to rust	19–32	3–1–2	Fine spines	IWL2
C. striata	130–185 × 115–155	Globose to subglobose	Ochraceous yellow of a pinkish tint	22–29	3–1–3	Muri, finger-print-like processes	IWL2

Table 3, concluded

	Spore size	Spore shape	Spore color	Suspensor size	# wall layers	OW Ornamentation	Melzer's rxn
Fuscutata							
F. heterogama	159–265 × 195–295	Globose to ellipsoid to irregular	Brown to reddish brown	32–51 × 31–45	3–2–2	'minute projections'	OWL2 IWL2
F. rubra	140–220	Globose to subglobose	Dark orange brown to red-brown	18–35	3–2–2	No	OWL2 IWL2
F. savannicola	285–585 × 210–365	Oblong to ellipsoid	Hyaline to white	33–51	3–2–2	No	IWL2
F. trirubiginopa	210–310 × 210–300	Globose to subglobose	Hyaline to white, suspensor (reddish-) brown	ca. 42	3–2–2	Fine spines	OWL2 MW IWL2
Dentiscutata							
D. biornata	280–385 × 340–415	Globose to subglobose	Yellowish-brown to brown	(30–)50–65	3–1–2	Blunt projections on OWL1 and OWL3	OWL2 IWL2
D. cerradensis	(170–) 230–365	Globose to ovoid	(Sub)hyaline to straw with age, suspensor brown	35–55 × 50–75	4–2–2	Fine 'papillae'	OWL2 MW IWL2
D. hawaiiensis	200–360 × 180–290	Subglobose to irregular	Pale orange brown to dark orange brown	50–65	3–2–2	No	IWL2
D. heterogama	150–210; 175 × 255	Globose to ellipsoid to irregular	Pale brown to yellow brown	21-26	3–2–2	'Minute projections'	Unknown
D. nigra	295–500 (–1050)	Globose to subglobose	Dark brown to black	40–60 × 80–120	3–2–2	Largely pored pits over sinuous ornamentation	IWL2
D. reticulata	205–470 × 185–340	Globose to subglobose	Orange-brown to dark red brown	45–87 × 85–140	4–2–2	Spines between ridges of a reticulum	IWL2
D. scutata	340–640, 350–670 × 350–715	Globose to subglobose to ellipsoid to oblong	Hyaline to white, suspensor brown	47–100 × 90–125, yellow brown	3–2–2	No	OWL2 IWL2
Quatunica							
Q. erythropus	170–551 × 205–660	Globose to subglobose to ellipsoid to oblong	Orange brown to dark red brown	60–125 × 30–60	3– (2+2) –2	No	MW2-L2 IWL2

[A] outer, middle and inner wall (OW, MW, IW) with different layers (OWL1-4, MWL1-2, IWL1-3); *Q. erythropus* has two bi-layered middle walls (MW1 and MW2). Recent analyses suggest that all *Scutellosporaceae*, *Racocetraceae* and *Dentiscutataceae* species have a three layered IW including a thin innermost layer IWL3. [B] if OWL2 stains also thin OWL3 generally stains in Melzer's reagent; [C] strongly darkened in mounted slides of type material; [D] strongly darkened in type material after storage in lactophenol.

Table 4. Morphology of germination shields and auxiliary cells in *Scutellospora*, *Racocetra*, *Cetraspora*, *Fuscutata*, *Dentiscutata* and *Quatunica*

	Germ shield (µm)	Shape of germ shield	Color of germ shield	Size (µm) & shape of auxiliary cells
Scutellospora				
S. arenicola	120–140	Bi-lobed, violin-shaped to oval to cardioid, with two germ tube initiations (gti)	Pale yellow brown to brown	20–41 × 20–40; clusters of 2-7 cells
S. aurigloba	65–85 × 65×105	Bi-lobed; violin-shaped to oval to cardioid, with two gti	Hyaline to light yellow	Aggregations about 100 µm; in clusters of 3-9 knobby cells
S. calospora	35–70 × 50–90	Bi-lobed, violin-shaped, with two gti	Hyaline to light yellow	18–22 × 31–33; irregular, singly or in loose clusters (2-4 cells)
S. crenulata	50–100 × 75–120	Mono-(to bi-(?))lobed, oval to ellipsoid to rarely cardioid to irregular, with 1(–2?) gti	Hyaline	Unknown
S. dipapillosa	60–80 × 85–95	Bi-lobed, violin-shaped, with two gti	Hyaline to light yellow	Cluster of 12–30 knobby cells
S. dipurpurescens	60–85 × 90–140	Bi-lobed, violin-shaped to oval to cardioid, with two germ tube initiations	Hyaline to light yellow	16–25 × 22–32; globose to obovate to irregular
S. nodosa	65–80 × 130–140	Bi-lobed, oval to cardioid, with two germ tube initiations	Hyaline to pale yellow	18–33; loose clusters of 2–5 cells
S. pernambucana	50–85 × 63–98	Mono-lobed, orb-like or coiled, with one gti	Hyaline to subhyaline to rarely light yellow	Unknown
S. projecturata	50–90	Mono-lobed, coiled, with one (to two?) gti	Hyaline to light yellow	Unknown
S. tricalypta	95 × 150	Bi-lobed, violin-shaped to oval, with two gti	Hyaline to pale yellow[a]	Unknown
Racocetra				
R. alborosea	Unknown	Unknown	Unknown	Unknown
R. castanea	180–210	Multiple-lobed (5–12), with multiple gti	Hyaline to light yellow	21–28 × 23–32; single or in clusters of 2–6 knobby cells
R. coralloidea	80–140	Multiple-lobed (4–8), with multiple gti initiations	Hyaline to pale yellow	25–30 × 30–40; a single knobby, later coralloid cell
R. fulgida	40–90 × 80–130	Multiple-lobed (4–8), with multiple gti	Hyaline to light yellow	20–25 × 25–37; in clusters of 5–13 knobby cells
R. gregaria	80–150	Multiple-lobed (4–8), with multiple gti	Hyaline to pale yellow	14–32 × 22–48; cluster of 3–13 knobby cells
R. minuta	Rarely observed	Unknown	Hyaline to light yellow	Unknown
R. persica	130–240 × 60–180	Multiple-lobed (4–8), with multiple gti	Hyaline to light yellow	22–27; in clusters of 4–6 knobby cells
R. verrucosa	80–90 × 150–210	Multiple-lobed (4–8), with multiple gti	Hyaline to light yellow	12–30; in clusters of 5–15 knobby cells

R. weresubiae	70–80 × 100	(Multiple)-lobed (4–8), with multiple gti	Hyaline to subhyaline	17–33 × 25–40; in clusters of 5–14 knobby cells
Cetraspora				
C. armeniaca	45–70 × 80–95	Multiple-lobed (4–10), with multiple gti	Hyaline to pale yellow	15–32; in clusters of 2–12 knobby cells
C.gilmorei	80–140 × 110–175	Multiple-lobed (4–8), with multiple gti	Hyaline to pale yellow	15–25; in loose clusters of 3–14 knobby cells
C. pellucida	110–140 × 125–160	Multiple-lobed (4–8), with multiple gti	Hyaline to rarely pale yellow	12–15; in clusters of 1–12 knobby cells
C. spinosissima	70–130 × 90–140	Multiple-lobed (4–12), with multiple gti	Hyaline to pale yellow	Unknown
C. striata	72 × 64	Multiple-lobed (4–12); with multiple gti (?)	Hyaline to subhyaline (to pale yellow)	Unknown
Fuscutata				
F. heterogama	75–105 × 55–75	Bi-lobed, violin-shaped to oval to ovoid, with two gti	Yellow brown to brown	16–29 × 20–43; in clusters of 1–11 'flattened' cells
F. rubra	60–70 × 80–110	Bi-lobed, violin-shaped to oval to ovoid, with two gti	Pale yellow brown to brown	15–28; Clusters of 5–11 knobby cells
F. savannicola	100–160 × 90–140	Bi-lobed, oval to cardioid, with two gti	Pale yellow brown to brown	15–29; in clusters of 3–20 cells
F. trirubiginopa	132 × 106	Bi-lobed (?), circular to ovoid; with two gti (?)	Reddish-brown	Unknown
Dentiscutata				
D. biornata	(110–)185–240 × (110–)185–275	Ellipsoid, 12–30 small compartments and gti; dentate	Brown	32–48 × 37–48; in clusters of 10–20 knobby cells
D. cerradensis	90–155 × 100–175	Cardioid to ellipsoid, 6–10 small compartments and gti; slightly dentate	Pale yellow brown to brown	20–33; cluster of 1–12 rounded to knobby cells
D. hawaiiensis	40–60 × 60–80	Ellipsoid, 8–12 compartments and gti; slightly dentate	Yellow brown to orange brown	40–55; in clusters of 4–6 knobby cells
D. heterogama	90–120 × 130–160	Ellipsoid, 12–24 small compartments and gti; dentate	Yellow brown to brown	16–27 × 20–31; in clusters of 1–10 'flattened' cells
D. nigra	130–170 × 180–240	Ellipsoid, 12–24 small compartments and gti; dentate	Yellow-brown to brown	21–36; in clusters of 3–12 knobby cells
D. reticulata	94–156 × 156–208	Ellipsoid to circular, to rarely reniform[b]; 12–24 small compartments and gti; dentate	Yellow-brown to brown	25–30 × 30-40; in clusters of 10–40 knobby cells
D. scutata	240–325 × 205–305	Ellipsoid oval, cardioid or circular, 12–18 small compartments and gti; dentate	Yellow-brown to brown	17–55 × 15–45; in clusters of 4–25 smooth to knobby cells
Quatunica				
Q. erythropus	125–200 × 175–190	Ellipsoid to oval, 8–20 small compartments and gti; dentate	Yellow-brown to brown	18–27; in clusters of 6–15 knobby cells

[a] IW and germ shield has darkened in type material after being mounted in the medium Ferrant, [b] the reniform type might be an artifact due to a special position of the germ shield when spore was crushed.

are phylogenetically closer to *Racocetraceae* and *Dentiscutataceae* than to *Scutellosporaceae* and suggested that *Scutellosporaceae* is basal among the four families that produce spores from a bulbous sporogenous cell. Contrary to the evolutionary hypotheses expressed by Morton (1990) and Morton & Benny (1990), *Gigasporaceae* may well have co-evolved with *Racocetraceae* and *Dentiscutataceae*. Morphologically, however, *Gigasporaceae* differ as greatly from *Racocetraceae* and *Dentiscutataceae* as *Pacisporaceae* differs from *Glomeraceae*.

In this study, germination structures and spore wall composition were the morphological characters that were supported by the molecular data. On their basis the species could be organized within new or re-described genera and families. This was easily done for *Gigaspora,* the only genus in the *Gigasporaceae,* as the *Gigaspora* spore has only one wall and no germination shield. However, it was more difficult to classify species forming a germination shield. While striking genetical differences between the *Scutellospora* species have become recognized (e.g. Simon et al. 1992, Helgason et al. 1998, Lanfranco et al. 1999, de Souza et al. 2005), researchers apparently have encountered difficulties in grouping the species along genetic lineages. A recently published key (Silva et al. 2006b) still focuses exclusively on spore size, spore wall ornamentation, and wall characteristics. For the most part, the known morphological differences in germ shields (Walker & Sanders 1986, Spain et al. 1989) have been ignored in species taxonomies, so that this represents the first time that germ shields have been observed, photographed, and used for taxonomic classification. Little is known about the ontogeny of germination shields of the different genera, which has been actually only described for *S. calospora* by Walker & Sanders (1986). We do not know about the ontogeny of shield formation of *Racocetra*, *Cetraspora*, *Fuscutata* and *Dentiscutata*, nor is this knowledge necessary to identify the genera. Germ shield morphology alone is sufficient clear, and stable.We confirmed that germ shield morphology does not change with spore age for all examined species, as we could examine numerous spores from different populations and sometimes very different ecosystems (see TABLE 1: *S. calospora*, *S. aurigloba*, *R. fulgida*, *R. gregaria*, *R. verrucosa*, *C. pellucida*, *F. heterogama*, *D. reticulata*, *D. biornata*).

Researchers who attempt to determine colors of very old field spores and shapes when germination shield is hidden or incompletely visible may encounter difficulties, particularly as the germ shield can readily be observed only when seen in planar view. Spain et al. (1989) describe how to reveal germ shield structure better by slightly moving the cover slide on a not yet completely hardened fixative and by separating spore walls from crushed spores under the stereomicroscope. There are a few species where the color of the germ shield

may be ambiguous because parts of the originally hyaline shield brown slightly over time on specific storage media (as in *R. coralloidea* and *C. gilmorei*) or the spore wall turns so dark brown that the shield can no longer be seen (as in *S. savannicola* herbarium specimens). Nonetheless, the germ shield folds are in most cases easy to recognize, particularly when the shield is either very simple (e.g. *Scutellospora* spp.) or very complex (*Dentiscutata* spp.). Some experience is needed to identify the wavy folded, hyaline, multi-compartmented shields of *Racocetra* and *Cetraspora* (FIG. 5 + PLATES 5–6). In such shields it is often easier to count the number of germ tube initiations (gti) within the shield compartments. Determining the number of middle walls may also confuse, particularly during separation when the inner lamina of the outer wall can be mistaken for a middle wall, making differentiation between *Racocetra* and *Cetraspora* (or *Dentiscutata* and *Quatunica*) difficult. Here it is a matter of crushing several spores so as to determine whether the wall layers stick together or are easily separated. Finally, other spore features — size, color, wall ornamentation, swelling of wall layers in PVLG based mountants, staining in Melzer's reagent — may also be used to identify the species (and thus genus) morphologically, as done previously (Silva et al. 2006b). Our own experience with the new keys presented in this paper convinces us that identification of species with bulbous sporogenous cells will be much easier now that germ shields are used as diagnostic characters. Nonetheless, we suspect that the genera presented here may contain additional groups with diverging shield characters and possibly divergent phylogenies that may justify — in the future — erection of new genera based on the number of lobes and gti or due to shield composition (e.g. in *Scutellospora* and *Dentiscutata*, TABLE 4).

During our study we faced several difficulties related to the availability and quality of live and herbarium specimens. Pure cultures were either unavailable for most of the as-yet unsequenced species, (e.g. *R. minuta*, *R. alborosea*, *F. savannicola*) or pure cultures got lost with time (e.g. *D. biornata*). In a few cases the herbarium type material was so poor in both spore number and spore quality after 20–40 years of storage (e.g. for *R. minuta*, *R. alborosea,* and other species described before 1990) that germ shields could not be found in the type material. In a few other cases, the spores mounted or preserved in the type material had obviously been immature, since the germ shield and sometimes even the inner wall could not be detected on the majority of the spores. In such cases we had to rely for the identification of shield morphology either on the original species description or on our own specimens collected in different parts of the world. Some species, e.g. *R. alborosea* and *R. weresubiae,* may be conspecific but it was not our aim to correct this. We also noted that some species should be redescribed (e.g. *S. tricalypta*), also not the goal of this paper. There was, nonetheless, an urgent need to separate the species presented in the

type specimens for *S. heterogama* (Nicolson & Gerdemann 1968) from those erroneously identified as such. Germ shield morphology clearly showed that the two 'heterogama' species belong to two different genera, *Fuscutata* and *Dentiscutata* (see description above).

In conclusion, the previously most commonly used morphological spore characters like spore size, color and staining of different spore walls in Melzer's reagent or expanding of wall layers in lactic acid based mountants (Franke & Morton 1994, Morton 1995) could not explain the rDNA-based phylogenetic clades (PLATES 2–3; also Walker et al. 2004, de Souza et al. 2005, Ahulu et al. 2006, Redecker et al. 2007). We have not investigated for this study whether there are differences in the staining behaviour of the mycelium in the roots among our new segregate genera. Such differences are important in *Paraglomeraceae* and *Archaeosporaceae* (Morton & Redecker 2001), but we found no literature indicating that this is the case in *Gigasporaceae*. We also did not investigate whether there are specific differences between the genera in the morphology of their auxiliary cells in the external root mycelium. Walker & Sanders (1986) noted that such differences are only minor in *Scutellospora* and then only consistent in differentiating *Gigaspora* from *Scutellospora*. Also, color of the mycelium in soil may be different: some species (e.g., *F. heterogama*, *D. reticulata*) form light brown to brown walled mycelium while others, like *R. castanea*, form mycelia with hyaline walls (pers. obs.). Information on all these characters is available for only a few species and was considered unnecessary to reorganize the genera presented here. Nevertheless, we propose that living pure cultures be established and that detailed studies of the auxiliary cells on extraradical mycelia and the ontogeny of germination shields in different genera be carried out; studies of genetical and ecological characters are also recommended. Unfortunately, much basic biology remains unknown in the *Glomeromycetes,* including germination, the process so intimately linked with the fitness and survival of these obligatory biotrophic symbiotic fungi. Although axenic cultures may prove useful in the future, we believe that the important spore characters must be observed in soil-grown spores that represent spores found under field conditions that ecologists and taxonomists encounter in real life.

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