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Gerdemannia gen. nov., a genus separated from *Glomus*, and *Gerdemanniaceae* fam. nov., a new family in the *Glomeromycota*

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Molecular, morphological, and cytological evidence show that the arbuscular mycorrhizal fungus *Glomus scintillans* is more closely related to the genera *Gigaspora* and *Scutellospora* (*Gigasporaceae*) than it is to *Glomus s. str.*, and consequently it contributes to the non-monophyly of the genus *Glomus*. We transfer the species to *Gerdemannia* gen. nov., with consequent autonymy of an appropriate family, *Gerdemanniaceae* fam. nov. Small subunit (SSU) rDNA analysis places *Gerdemannia* in a clade sharing common ancestry with the *Gigasporaceae*, and therefore within the *Diversisporales* (*Glomeromycota*). *Glomus dominikii* is considered to be conspecific with *G. scintillans*, and these species are synonymised. *Glomus chimonobambusae* is also transferred to the new genus as *Gerdemannia chimonobambusae* comb. nov.

INTRODUCTION

The phylum *Glomeromycota* (Schüßler, Schwarzott & Walker 2001) encompasses an ecologically significant group of symbiotic fungi, the arbuscular mycorrhizal (AM) and related fungi. These organisms have limited morphological characters for species definition and recognition, but through molecular analyses the phylogenetic relationships within AM fungi are being elucidated. Combined morphological, cytological and molecular evidence reveals a new clade within the *Glomeromycota*, which is described here as a new, as yet monogeneric family.

Within the *Glomeromycota* as presently defined, a large grade is referred to the genus *Glomus*. This group was shown to be non-monophyletic through analysis of the small subunit (SSU) rRNA gene (Schwarzott, Walker & Schüßler 2001). As a contribution towards uncovering the monophyletic groups in this unnatural assemblage, organisms that shared characters appearing to represent a distinctive clade were recognised. Three described species, *G. scintillans* (Rose & Trappe 1980), *G. dominikii* (Błaszkowski 1988a), and *G. chimonobambusae* (Wu *et al.* 1995) comprise one such group.

Representative samples, including type or authentic material, of the three described species were examined to determine any synonymy. Besides morphological examination, when suitable material was available, tests with Melzer's reagent, germination attempts, and SSU rRNA gene sequences were determined and compared. The symbiotic nature of *G. scintillans* was investigated by closed pot-culture techniques.

MATERIALS AND METHODS

Molecular analyses

To avoid focussing on just one sample, DNA was extracted from spores from soil or cultures from four geographic localities, with sampling dates distributed over almost two years. The samples originally identified as *G. scintillans* or *G. dominikii* therefore represent one spore each from four different origins, one in Poland, one in England, and two (about 1 km apart) in Germany. SSU rDNA was sequenced from four clones for each spore. Isolation of DNA from single spores, primers and conditions for PCR, and methods used for cloning and sequencing are described in Schwarzott & Schüßler (2001). The SSU rRNA gene was analysed phylogenetically as described earlier (Schwarzott *et al.* 2001, Schüßler *et al.* 2001). The aligned data set used for the analyses, comprising all diversisporalean near full-length sequences available in the databases (45 sequences), consisted of 1708 sites. Nine sequences,

representing the three main subclades within the *Glomerales* (the sister clade of the *Diversisporales*), were used as outgroups. Sequences published here are deposited in the EMBL sequence database under accession nos. AJ619940–AJ619955.

Alignment was carried out manually, with the freeware program ALIGN 4.0 (http://domix0.tripod.com), taking secondary structure into account. Consensus trees were constructed from 1000-fold bootstrapped neighbour-joining (NJ, based on F84 parameters), 100-fold bootstrapped maximum likelihood (ML), and 1000-fold bootstrapped maximum parsimony (MP) analyses. PHYLIP 3.6a3 (Felsenstein 1989) was used for NJ, ML and MP computations. Maximum likelihood (ML) quartet puzzling (QP) analyses (ML-QP) were performed with TREE-PUZZLE 5.0 (based on HKY as well as TN models) using 10 000 puzzling steps, and transition/transversion ratios and nucleotide frequencies are estimated from the dataset. To investigate influence of rate heterogeneity, additional ML-QP analyses (using KN and HKY models) were performed with gamma distributed heterogeneous rates, which also were estimated from the dataset. Although having a different base, QP analysis support values can be interpreted in much the same way as bootstrap values. Branches showing a QP reliability from 90 to 100 % can be considered very strongly supported.

The phylogenetic distances shown in the trees (Figs 1–2) were derived from non-bootstrapped ML analyses. For the topology of the *Gerdemannia scintillans* clade within the *Diversisporales* there was no difference among MP, NJ, ML, and QP-ML trees. Differences in bootstrap support or QP-ML analysis support values (both in %) are shown in Fig. 1. The alignment is available in different formats at www.amf-phylogeny.com, with accompanying further details on the cultures and software used.

Sequences

Accession numbers are given in parenthesis.

Outgroup taxa

Glomus coronatum (AJ276086); *G. geosporum* (consensus sequence from AJ132664, Y17643, AJ245637); *G. mosseae* (consensus sequence from U96139, U31995); *G. intraradices* (consensus sequence from AJ301859, X58725); *G. proliferum* (AF213462); *G. sinuosum* (AJ133706); *G. claroideum* (consensus sequence from AJ301851, AJ276075, AJ301852, Y17636); *G. etunicatum* (consensus sequence from Y17639, Z14008); *G. luteum* (consensus sequence from AJ276089, U36591, Y17645).

Diversisporales *taxa*

Acaulospora laevis (?) (Y17633); *A. laevis* (AJ250847); *A. longula* (AJ306439); *A. rugosa* (Z14005); *A. foveata* (?) (AJ306442); *A. spinosa* (Z14004); *A. undulata* (?) (AJ306441); *A.* sp. (AJ306440); *Entrophospora colombiana* (Z14006); *E.* sp. (Z14011); *Gigaspora albida* (Z14009); *Gi. candida* (AJ276091); *Gi. gigantea* (Z14010); *Gi. rosea* (X58726); *Gi.* aff. *margarita* (AJ276090); *G. etunicatum* (?) (consensus sequence from AJ276076, AJ301860, AJ301863, Y17644); *G. versiforme* (consensus sequence from AJ132666, AJ276088, X86687, Y17651); *G. spurcum* (consensus sequence from AJ276077-78, Y17649-50); *Scutellospora aurigloba* (consensus sequence from AJ276092, AJ276093); *S. calospora* (consensus sequence from AJ306445, AJ306446); *S. castanea* (consensus sequence from AF038590, U31997); *S. cerradensis* (consensus sequence from AB041344, AB041345); *S. fulgida* (AJ306435); *S. gilmorei* (AJ276094); *S. heterogama* (AJ306434); *S. heterogama* (U36593); *S. heterogama* (Z14013); *S. nodosa* (AJ306437); *S. pellucida* (Z14012); *S. projecturata* (AJ242729); *S. spinosissima* (AJ306436); *S. weresubiae* (AJ306444).

Specimens examined

Specimens examined are listed by country, state, locality, ecological details, collection date, collector, field sample number, herbarium accession number, and herbarium (in brackets).

Glomus scintillans

The numbering of samples and vouchers follows Walker & Vestberg (1998).

Australia: *Western Australia*: Perth, Bakers Hill, Allandale Farm, pasture with *Trifolium subterraneum*, *Arctotheca calendula* and *Lolium rigidum*, 2 Apr. 1996, *R. Rafique* [Walker sample 848, voucher W2936] (E); *loc. cit*., barley grass (*Hordeum leporinum*), 2 Apr. 1996, *R. Rafique* [Walker sample 843, voucher W2950] (E). – **Finland**: Rovaneimi, experimental field station, in a field under *Fragaria vesca*, from a soil-trap pot culture with *Trifolium pratense* 18 Apr. 1995, *M. Vestberg* [Walker sample 696, voucher W2216] (E). – **Germany**: Darmstadt, forest clearing under power lines, from sandy heathland, a single spore from a single-spore pot culture with *Plantago lanceolata* [Attempt 947-0]. No further spores were found, and no mycorrhizas established. Presumed to be the original spore used as inoculum, 1 Aug. 2002, *C. Walker* [Walker sample 998, voucher W4047] (E); *loc. cit*., from sandy heathland, beneath *Centaurea stoebe* and associated plants, 11 July 2000, *C. Walker* [Walker sample 935, voucher W3472] (E); *loc. cit*., from sandy heathland, beneath *Cynodon dactylon* and associated plants, 10 June 2001, *C. Walker* [Walker sample 962, voucher W3683] (E); *loc. cit*., from sandy heathland, beneath *Dactylis glomerata* and associated plants, 29 Oct. 2001, *C. Walker* [Walker sample 998, voucher W3793] (E); *loc. cit*., from sandy heathland, beneath *Anthericum liliago* and associated plants, 11 July 2000, *C. Walker* [Walker sample 1155, voucher W4545] (E). – **Poland**: Lipki, from a pot culture with *Plantago lanceolata* made with soil from beneath *Triticum aestivum* in an agricultural field, 2 Mar. 2002, *J. Błaszkowski* [Walker sample 1005, voucher W3849] (E); locality unknown, date unknown,

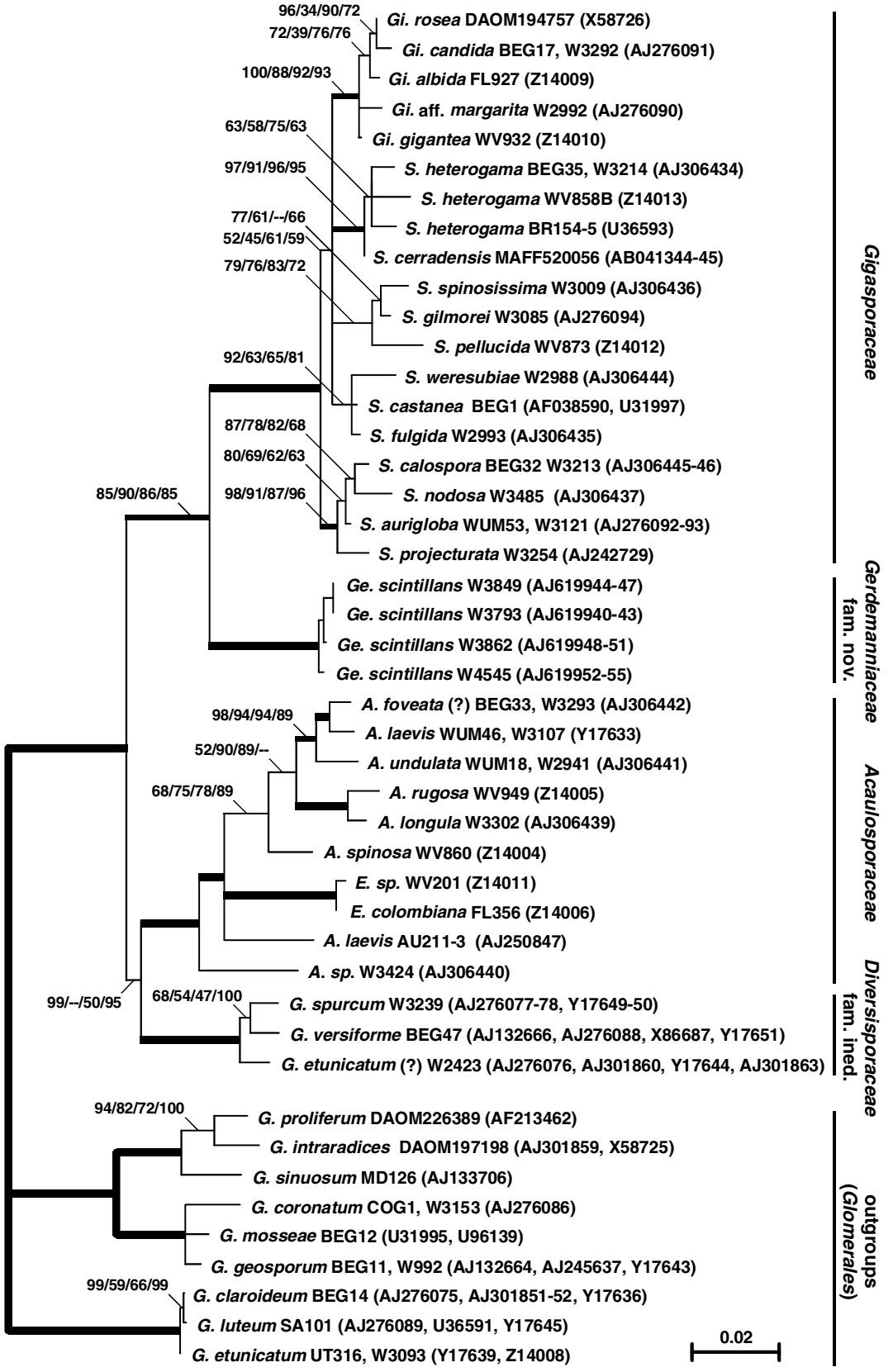


Fig. 1. Phylogenetic tree of the *Diversisporales* with members of the *Glomerales* (*Glomus* groups Aa, Ab, B) as outgroups. Distances are derived from a ML analysis. The values given above the branches correspond to supports in NJ/MP/ML/ML-QP analyses. The thick lines represent branches which are supported by at least 95% in all analyses, the medium ones to branches supported by at least 85% in all analyses. Where no value is given, the topology is not reflected in the respective analysis. Topologies that are not supported by at least 50% in at least three of the analyses are collapsed to polytomies. A., *Acaulospora*; E., *Entrophospora*; Ge., *Gerdemannia*; Gi., *Gigaspora*; G., *Glomus*; and S., *Scutellospora*.

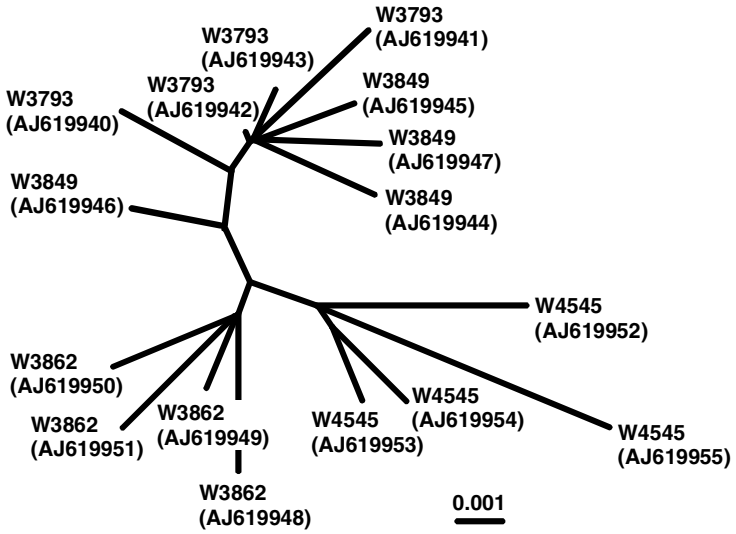


Fig. 2. ML analysis of the 16 *Gerdemannia scintillans* sequences, computed from 1722 aligned sites. Note that sequences from the spore from Poland (W3849) and those from Germany (W3793) form a mixed cluster.

J. Błaszkowski [Walker sample 1143, voucher W1523] (E) [note: specimens from these two Polish samples were determined to be *G. scintillans* by C.W., but *G. dominikii*, by J.B.]. – **UK**: *Dorset*: East Lulworth, Lindens Farm, a relatively undisturbed ancient meadow, beneath *Lolium perenne* and associated weeds, 27 Mar. 2002, *C. Walker* [Walker sample 1009, voucher W3862] (E). *Northumberland*: Kielder, Bakethin Reservoir, from an open pot culture [Attempt 209-0] made with unsterilised field soil and a sod containing plants from the field, 2 Apr. 1984, *C. Walker* [Walker sample 3, voucher W964] (E); *loc. cit*., a second sample from the same pot culture, 22 May 1984, *C. Walker* [Walker sample 3, voucher W969] (E); *loc. cit*., from a closed pot culture with *Plantago lanceolata* [Attempt 209-59] made from a single spore taken from Attempt 209-0, 8 May 1985, *C. Walker* [Walker sample 3, voucher W1050] (E). *East Lothian*: Edinburgh, Colinton, West Colinton House, from soil beneath a garden lily in an old garden, 8 Aug. 1985, *C. Walker* [Walker sample 279, voucher W1503] (E). *Shetland Isles*: Keen of Hammar, from pasture on serpentine soil, 15 Dec. 1984, *T. H. Nicolson* [Walker sample 1141, voucher W1003] (E). – **USA**: *North Carolina*: Burke Co., from a walnut plantation on sandy soil; *Juglans nigra* with understory of clover, trumpet creeper, *Smilax* sp. and *Mimosa* sp., 22 Apr. 1980, *D. Egel* (*neé Fardelmann*) [Walker sample 292, vouchers W292, W847] (E). *Oregon:* Lake Co.: near Picture Rock Pass, 1500 m elevation, beneath *Cercocarpus ledifolia*, Sept. 1978, *S. Rose* [Walker sample 479; Rose S-220] (OSC 41352 holotype; W185, E – isotype).

Glomus dominikii

Numbers of specimens refer to microscope slide numbers and sample numbers recorded in Poland by J.B., and follow a different system from those of C.W.

Poland: *Western Pomerania Province*: Kolbacz, from a cultivated field with *Trifolium pratense*, 10 July 1985, *J. Błaszkowski* [Sample 12] 256 (DPP) – holotype, microscope slide; 257–260 (DPP) – isotypes, microscope slides; Chynow, from cultivated *Avena sativa*, 20 July 1985, *J. Błaszkowski* [Sample 64] slides 261–265 (DPP); Prusinowo, from *Fragaria vesca*, 5 Aug. 1985, *J. Błaszkowski* [Sample 59] slides 266–268 (DPP); Czciradz, from cultivated *T. pratense*, 27 Aug. 1985, *J. Błaszkowski* [Sample 57] vouchers on microscope slides 271–280 (DPP); Nowogard, from cultivated *Lupinus luteus*, 5 Aug. 1985, *J. Błaszkowski* [Sample 42] slides 281–285 (DPP); Kamien Pomorski, from cultivated *Pisum sativum*, 25 July 1985, *J. Błaszkowski* [Sample 61] slide 286 (DPP); Brzozowo, from cultivated *T. aestivum*, 25 July 1985, *J. Błaszkowski* [Sample 62] slide 287 (DPP); Trzebiatow, from cultivated *Allium porrum*, 5 Aug. 1985, *J. Błaszkowski* [Sample 67] slide 289 (DPP); *Pomerania Province*: Przybierow, from cultivated *Secale cereale*, 25 July 1985, *J. Błaszkowski* [Sample 65] slide 288 (DPP); *Lubuskie Province*: Lasocin, from an agricultural field with *Zea mays*, 27 Aug. 1985, *J. Błaszkowski* [Sample 42] slides 269–270 (DPP); *Lublin Province*: Zwierzyniec, from *Festuca rubra* growing in a forest, 18 Nov. 1986, *J. Błaszkowski* [Sample 73] slides 290–291 (DPP). – **Israel**: near Tel-Aviv, from *Cenothera drummondi* colonizing maritime dunes of the Mediterranean Sea, 16 Dec. 1997, *J. Błaszkowski* [Sample 1182] slides 2427–2433 (DPP). – **Turkey**: Karabucak-Tuzla, from *Ammophila arenaria* colonizing maritime dunes of the Mediterranean Sea, 9 June 2001, *J. Błaszkowski* [2199], slides 2434–2443 (DPP).

Glomus chimonobambusae

Taiwan: Nan-Tou, National Taiwan University, Chi-tou Experimental Station, field collected from beneath *Chimonobambusae quadrangularis* and associated plants, date unknown, *C-G Wu*, *Wu 82041201* (TARI – holotype E [W2308] – isotype).

Pot cultures

C.W. attempt 209-0: a plant trap from native sward plants with soil lifted directly from the field and transferred to a pot. C.W. attempt 209-59: a single spore placed on roots of *Plantago lanceolata* before planting into sterile substrate. A multispore culture attempt by J.B., also allocated a C.W. attempt number, 961-1.

In addition, repeated attempts (more than 100) at producing living pot cultures from spores failed to sporulate or to establish mycorrhizas.

Morphological analyses

Isotype material of the three formally described species (i.e. *Glomus dominikii*, *G. chimonobambusae*, and *G. scintillans*) was available in the C.W. and J.B. herbarium collections; the former is lodged at the Royal Botanic Garden Edinburgh (E), and the latter in the Department of Plant Pathology, University of Agriculture, Szczecin (DPP), as specimens on semi-permanent microscope slides. The holotype of *Glomus scintillans* was borrowed from Oregon State University herbarium (OSC) for comparison.

Spores (Figs 3–10) were extracted from soil by centrifugation and sugar floatation (Walker, Mize & McNabb 1982) or by agitating or swirling in a container of water, allowing the contents to settle for a few seconds, and decanting the supernatant through a sieve with 35 or 40 μm openings. Extracted spores were transferred to small dishes of water and observed for surface characters and pigmentation under a dissecting microscope at magnifications up to 50× (Walker, Gianinazzi-Pearson & Marion-Espinasse 1993). Selected spores were then mounted in aqueous polyvinyl alcohol lacto-glycerol without (PVLG), or with the addition of Melzer's (PVLG/M) reagent (4:1 v/v), and observed through a Zeiss Photoscope III or a Zeiss Axioskop compound microscope, with or without Nomarski differential interference contrast optics, at magnifications up to 1600× to provide details of subcellular structure and organisation.

For germination tests, spores were selected with fine forceps under a dissecting microscope and placed on individual squares of membrane filters which, in turn, were placed on a complete membrane filter placed on moist, disinfested sand in a 6-cm Petri dish. These were inspected daily under a Leica Wild MZ8 dissecting microscope, at magnifications up to ×50, and microphotographs of germinated spores were taken with a digital camera (Nikon D1). Germinated spores were gently lifted, along with their supporting membrane filter square, and inverted onto a drop of PVLG at the centre of a microscope slide. The membrane filter square was then gently removed with tweezers, leaving the spore with its germ tube attached in the medium. A coverslip was then added and the preparation observed under the compound microscope.

Attempts at producing living pot cultures (Walker 1999) were made as soil traps, plant traps, and multi- and single-spore culture attempts in open (Gilmore 1968) and closed (Walker & Vestberg 1994) pot cultures. Seedlings from wild seed of *Plantago lanceolata* were transplanted to disinfested sand or soil and inoculated with one or more spores, selected from their appearance under a dissecting microscope. Spores, either pre-germinated or directly selected from soil samples were placed directly on the root *in situ* before re-filling the planting hole. Some plants were dug up, and after thorough washing of the roots under a stream of tap water, transplanted to disinfested sand or soil from their original localities. In other culture attempts, unsterilised field soil was introduced to a pot containing disinfested substrate into which mycorrhiza-free seedlings of *P. lanceolata* were transplanted.

To check for inoculation success, cores of the substrate were removed, swirled in a container of water and decanted gently through a 50 μm sieve. Root samples were also taken, stained with a procedure modified from Vierheilig *et al.* (1998) in which the roots were put in 1 M KOH in a 60 ml plastic jar, heated in a microwave to boiling point, and then left overnight at room temperature. They were then rinsed quickly in tap water, and immersed in about 20 ml of 1% HCl containing a few drops of permanent blue Parker Quink™ ink, methyl blue, or cotton blue. After a minimum period of 5 min in this stain, the roots were transferred directly to PVLG on a microscope slide, covered by a 22 × 22 mm coverslip, and examined under a compound microscope for arbuscular mycorrhizal structures.

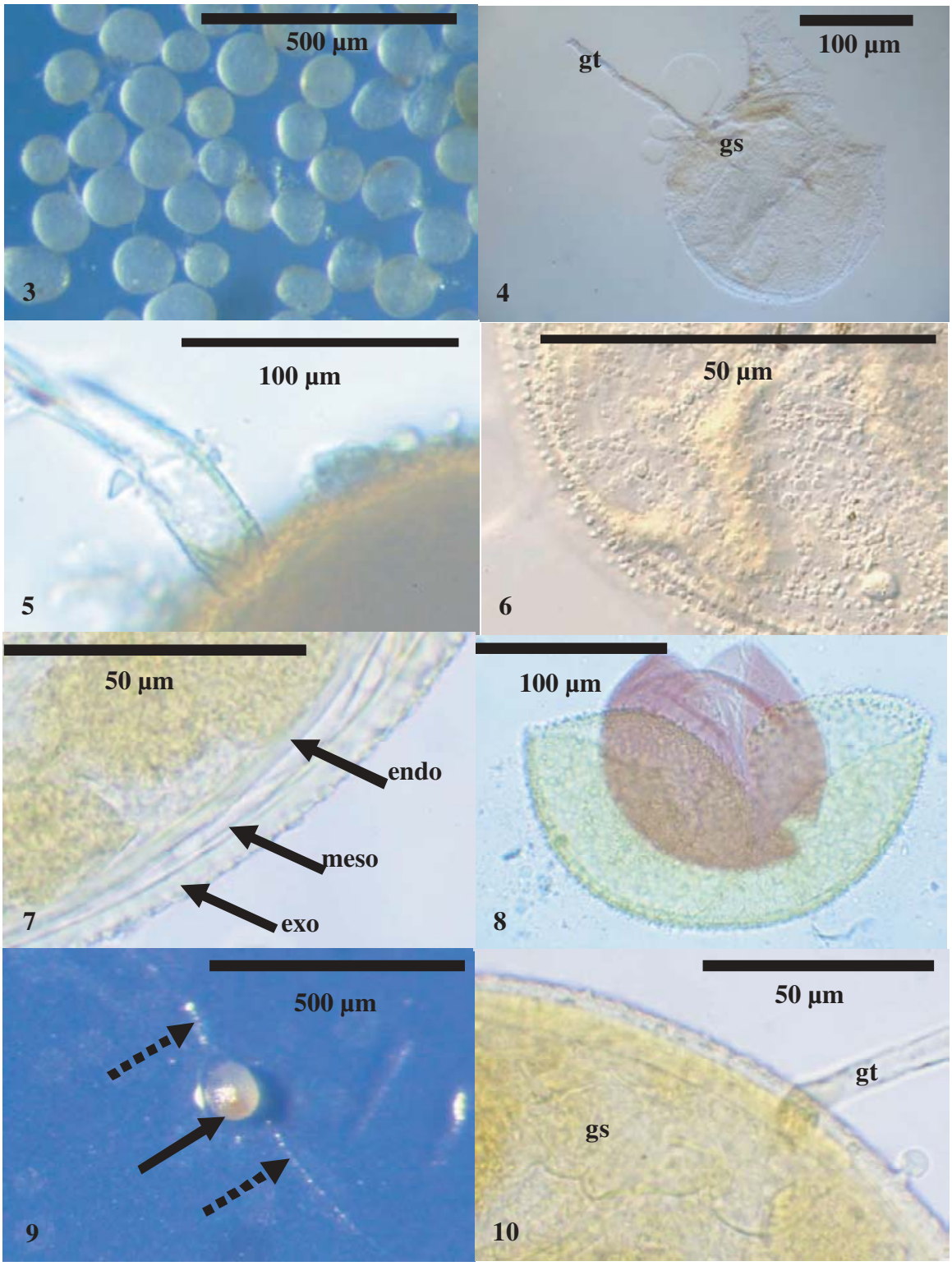
RESULTS

SSU rDNA analysis

Sixteen new SSU rDNA sequences were derived from this study and deposited in the EMBL database (Table 1).

The spores revealed intraspecific sequence variability, but all 16 sequences are very closely related (Figs 1–2). The four sequences from W3862 (England) and W4545 (Germany) form two independent clusters. The sequences of W3849 (Poland) and W3793 (Germany, sampling site close to that of W4545) form a mixed clade (Fig. 2). None of the sequences was identical with any other. Differences ranged from 6 to 16 sites over the 1768 bp sequenced, and the two highest values (13 and 16) were found within one spore (W4545).

NJ and MP analysis of all glomeromycotan near full length sequences available in the databases revealed that the *G. scintillans* sequences cluster within the *Diversisporales* with ⩾99% bootstrap support (not shown). Further phylogenetic analyses were performed with nine sequences of the *Glomerales* (the sister clade of the *Diversisporales*) as outgroups. Phylogenetic trees with all individual *G. scintillans* sequences, a consensus sequence for each spore (Fig. 1), and a consensus sequence from all 16 sequences were computed. These different sequence samplings and all the different phylogenetic methods applied always gave the same result, showing *G. scintillans* to belong to the *Diversisporales* with ⩾99% support in NJ, MP and ML analyses. ML-QP analyses, using KN and HKY models (also with gamma distributed heterogeneous rates)



Figs 3–10. *Gerdemannia scintillans*. **Fig. 3.** Spores in water, freshly extracted from soil. **Fig. 4.** Spore (from the isotype) showing the lightly-pigmented germination shield (gs) and the germ tube (gt). **Fig. 5.** Spore (from the isotype) showing the attachment of the subtending hypha at the spore base and discolouration as the result of storage in 0.025% sodium azide solution. **Fig. 6.** Spore surface showing ornamentation viewed with Nomarski differential interference contrast. **Fig. 7.** Wall structure of exospore ('exo'), mesospore ('meso') and endospore ('endo'). The mesospore can be seen adherent to the exospore (lower left), crossing over to the endospore (upper, right). **Fig. 8.** Melzer's reaction of the endospore; note the apparent difference in colour where the yellow-stained exospore is acting as a coloured filter; the true colour reaction can be seen only where the endospore is extruded from its encompassing wall components. **Fig. 9.** Spore germinating on a membrane filter; the lightly pigmented germination shield is indicated by a solid arrow, and the germination hyphae by dotted arrows. **Fig. 10.** Details of germination shield with crenulated edges (beneath gs) and germ tube (gt) (from the isotype).

Table 1. New SSU rDNA sequences derived from *Gerdemannia scintillans* spores. W3849 came from a pot culture; the remainder were field-collected.

Voucher no.	Clone	Collection date	Origin	Sequence accession no.
W4545	pWD200-2-3	11 July 2000	Germany	AJ619952
W4545	pWD200-2-4	11 July 2000	Germany	AJ619953
W4545	pWD200-2-5	11 July 2000	Germany	AJ619954
W4545	pWD200-2-6	29 Oct. 2001	Germany	AJ619955
W3793	pWD245-1-1	29 Oct. 2001	Germany	AJ619940
W3793	pWD245-1-2	29 Oct. 2001	Germany	AJ619941
W3793	pWD245-1-5	29 Oct. 2001	Germany	AJ619942
W3793	pWD245-1-6	29 Oct. 2001	Germany	AJ619943
W3849	pWD273-3-1	2 Mar. 2002	Poland	AJ619947
W3849	pWD273-3-2	2 Mar. 2002	Poland	AJ619944
W3849	pWD273-3-3	2 Mar. 2002	Poland	AJ619945
W3849	pWD273-3-5	2 Mar. 2002	Poland	AJ619946
W3862	pWD274-3-1	27 Mar. 2002	England	AJ619949
W3862	pWD274-3-2	27 Mar. 2002	England	AJ619948
W3862	pWD274-3-3	27 Mar. 2002	England	AJ619950
W3862	pWD274-3-4	27 Mar. 2002	England	AJ619951

gave support values from 96 to 99%. It moreover was resolved in all analyses that this species belongs to a sister clade of the *Gigasporaceae*, with supports ranging from 85 (NJ and ML) to 90% (MP). Conservative usage of 1642 sites in alignment for MP analysis resulted in the same topology and similar bootstrap support as using the longer (1708 sites) alignment.

Pot cultures

Most attempts at establishing mycorrhizas with spores failed. C.W. attempt 209-59 was successful, producing spores and vesicular–arbuscular mycorrhizas, but was eventually lost from the living collection in temporary housing of the collection during change of laboratory address. C.W. attempt 961-1 (Poland) produced abundant spores, but two morphs appeared, one with smooth or lightly ornamented spores corresponding to the 'white reticulate' spore of Mosse & Bowen (1968) or the 'white smooth-walled azygospore' of El Giahmi, Nicolson & Daft (1976) or Dodd & Krikun (1984). The other was identified as *Glomus dominikii* by J.B., and (independently) as *G. scintillans* by C.W. Work continued with single spore isolation attempts to clarify the relationship between these two morphs. Of 50 single-spore isolation attempts with spores from this culture, none was successful. C.W. attempt 209-0 was initially successful in mixed pot culture, followed by a slow loss of sporulation (assumed loss of species from culture) after which it was discarded.

Morphology

Subtending hypha

Regardless of origin or determination of species, the spores were produced from a single subtending, sporogenous hypha (Fig. 5) that readily became detached if handled robustly. Some of these subtending hyphae possessed a small peg-like protuberance, some with a thin, hyaline attached hypha.

Wall structure

Mature spores developed from a single somewhat fragile subtending sporogenous hypha. At maturity, the spore wall structure consisted of three groups (*sensu* Walker 1983) which are here interpreted as exospore, mesospore and endospore after Ferrer & Herrera (1981) (Fig. 7). The exospore outer wall layer is ornamented with knobs, spines, warts or ridges. The mesospore wall is thin and flexible and appears single-layered. The endospore wall reacts with Melzer's reagent in PVLG.

Germination shields

Germination was by means of a germination shield, followed by emergence of the germination tube directly through the spore wall (Fig. 4) in a similar manner to species of *Scutellospora*. The shields are delicate and sometimes difficult to discern. They are slightly pigmented (ochraceous), and possess crenulated edges. The shields deteriorate over time and become difficult to see.

Melzer's reaction

Spores identified as *Glomus scintillans* reacted positively, though somewhat variably in Melzer's reagent. Most specimens exhibited a purple or pinkish purple reaction of the endospore, though in spores with a moribund appearance or those that had been dried and re-hydrated, this reaction was relatively weak or failed to occur at all. In all instances, the purple colouration faded away within two weeks of mounting and crushing spores in PVLG/M. The wall components of the exospore stained yellow with this reagent, whereas the flexible mesospore's sole wall component remained unaffected. According to the protologue, spores of this species do not react distinctively to Melzer's reagent.

Those spores originally identified as *G. dominikii* all had a reaction to Melzer's reagent. The exospore wall components stained yellow, whilst there was a positive purple reaction of the endospore. Because of the yellow staining of the exospore wall, the colour of the reaction in the endospore wall could be misinterpreted as various shades of pink or red when viewed through it (Fig. 8). However, if the spore was crushed sufficiently the reaction could be seen to be purple or purplish-pink.

In the protologue, *G. chimonobambusae* is described as having a 'positive' Melzer's reaction. Wall components 1 and 2 (corresponding with the exospore wall) stain yellow and wall 4 (comprising the endospore wall) reacts to become pinkish red. No mention was made of the third wall component's reaction (interpreted here as the mesospore wall).

Root staining

Mycorrhizal structures could be seen immediately after staining, though their clarity increased with time until after 24 h, when the roots had de-stained. The mycorrhizas are similar to those of some members of the genus *Acaulospora*. They possess arbuscules and vesicles that are revealed when cleared and stained with ink, cotton blue or aniline blue (data not shown).

DISCUSSION

Although no identical sequences were found, the variation is within the amount to be expected from intraspecific variation, and the spores originally identified as *Glomus scintillans* and *G. dominikii* cannot be separated at the species level by SSU rDNA analysis. This is reinforced by the sequences from Poland of W3849 and those from the German sample (W3793) (the latter from a location very close to the origin of W4545) which formed a mixed clade (Fig. 2). Sequence variation are mainly due to differences caused by transitions (C–T or A–G). This type of mutation is frequently found in rDNA of closely related organisms and is well characterized as intraspecific polymorphism in AM fungi of the genus *Gigaspora* (Lanfranco, Delpero & Bonfante 1999, de Souza *et al*. 2004). In some instances differences could also be caused by polymerase errors.

The observed clustering of sequences stemming from W3862 (England) and W4545 (Germany) could possibly be a PCR artefact caused by selectivity. If a certain type of the variable sequences is amplified only (or mainly) during early PCR cycles, which could happen by chance, the resulting clones would represent mainly this sequence. Nevertheless, neither J.B. nor C.W. could find any morphological differences among the spores determined as *G. dominikii* and *G. scintillans*, including type and authentic material, strengthening our determination of conspecificity of the two species.

The strong statistical support for the relationship of *Gigasporaceae* (Fig. 1) from the NJ, ML and MP analyses shows that a common ancestry of *Gerdemanniaceae* and *Gigasporaceae* is highly probable.

Comparison of the published species descriptions was difficult because of the differences in approach to species description and the variability in terminology. Examination of type and authentic material allowed a new, integrated evaluation. The pot culture attempts were generally unsuccessful. Germination of individual spores showed that at least some were viable, but only one single-spore attempt was successful. The production of two morphs from the Polish pot culture was instrumental in stimulating the comparisons that resulted in the present work. Because this was a multispore culture attempt, it is impossible to be certain if only one variable species was present, or if two superficially similar species had been inadvertently used as inoculum. Determination of species from observation of spores under a dissecting microscope cannot be guaranteed, especially when superficially similar spores of different species are present (usually the case with spores from natural soil samples). SSU rDNA was successfully sequenced from the ornamented (*dominikii-scintillans*) morph, but not from smooth (white reticulate) morph. The white reticulate organism has been reported previously and has been found without accompanying *G. scintillans* spores in the past. Both morphs produced a rapid purple reaction of the endospore wall when crushed in PVLG with Melzer's reagent. We are attempting to isolate the white reticulate fungus, but have as yet insufficient information to assign it unequivocally to a species. It seems, however, to possess the characteristics of the new genus, *Gerdemannia*.

The plant and soil trap comprising attempt 209-0 produced abundant spores of *G. scintillans* along with *A. laevis*, *A. undulata*, *S. calospora*, *A. foveata* and *G. constrictum*. The *G. scintillans* spores corresponded with the description in the protologue, though the Melzer's test was not carried out. Walker attempt 209-59 was the only successful single-spore isolate, maintained in a sealed system, and therefore the only one that could be accepted as incontrovertible evidence for the vesicular–arbuscular mycorrhizal status of the organism. Neither of these pot cultures produced the white reticulate morph.

Spore morphology

In all three species, the spores were originally described as globose to subglobose. *Glomus chimonobambusae* was also described as having spores that are obovoid, ellipsoid or irregular. Similar variation was noted in new collections from Europe determined as *G. scintillans* and *G. dominikii*, extending the range of spore shape to all three species. The size ranges given for the three species overlapped somewhat, with *G. dominikii* being the smallest and *G. scintillans* the largest. The ornamentation of *G. scintillans* (Fig. 6) was described by Rose & Trappe (1980) as '… hyaline knobs 1–3 × 0.4–1.2 (–3) μm …'; for *G. dominikii* (Błaszkowski 1988a) as '… fine warts 1.7–5.7 μm …; and for *G. chimonobambusae* (Wu *et al*. 1995) as '… tiny warts and coarse clavate projections up to 12.5 μm long …' Such precision is not, however, possible from light microscopy; the measurements of the first two overlap sufficiently to be indistinguishable, whereas those of *G. chimonobambusae*, as given, have a much greater maximum height.

Colour is very difficult to interpret from species descriptions because of differences in interpretation among observers and because of the use of different optical instruments, illumination systems, and colour charts. The term 'hyaline' often is used rather loosely and may mean colourless (glass-like), but may also be used when the spores are opaque and white. Spores also may appear discoloured due to ageing or staining. In the protologues, *G. scintillans* was described as having

hyaline spores, but isotype material was lightly pigmented after storage, and other collections were sometimes hyaline, sometimes hyaline to white, and sometimes lightly pigmented due to age or staining from the soil. Similarly *G. dominikii* was described as being white or slightly pink in water, or becoming lightly pigmented (yellow to orange-yellow), but observation of fresh specimens under controlled lighting conditions (Walker *et al.* 1993) showed the colouration to be the result of deterioration with the passage of time.

Description of wall components and wall groups in the protologues is confusing because the methods of the different authors are not standardised. Both *G. scintillans* and *G. dominikii* are described as having three wall components in one and two groups, respectively, whereas *G. chimonobambusae* is said to have four in two groups. Such variation in interpretation of wall structures is common and the wall structure is reassessed as part of this work. No differences were noted amongst type specimens of the three species, and wall structure was consistent in all newly examined material, consisting of a complex structure that could be interpreted as being made up of an exospore-, mesospore- and endospore-wall.

The reaction to Melzer's reagent for *G. scintillans* is given as 'not distinctive' in the protologue, but by analogy with other collections corresponding to the species description in all other characteristics, and the failure of preserved material to react, we consider this to be an error, possibly because of the condition of the spores tested. *Glomus dominikii* and *G. chimonobambusae* were described as reacting in similar ways, though the endospore-wall reaction was described as becoming various shades of red. The reaction is amyloid (purple), but because the yellow-stained exospore-wall acts as a filter, the impression is given that the endospore reaction is red or orange-red. This effect led to a similar interpretation of the reaction to Melzer's reagent exhibited by *Acaulospora scrobiculata* (Trappe 1977).

The mycorrhizal status was unknown for all three species when originally described. It has been common practice in the past to describe supposedly arbuscular mycorrhizal fungi without having established whether or not they are symbiotic, often because repeated attempts at pot culturing have failed. Whenever possible, the establishment of pot cultures is to be encouraged before new species in the *Glomeromycota* are described. Although the *Code* would allow descriptions directly from remnants taken directly from soil, the probability of error is greatly increased if isolates are not available. However, the development of molecular methods now allows much more secure species descriptions, adding reliable and informative characters for species descriptions and systematic interpretation.

The germination of spores was described as directly through the spore wall for both *G. scintillans* and *G. chimonobambusae* (Rose & Trappe 1980, Wu *et al.* 1995), but was not mentioned in the description of *G. dominikii* (Błaszkowski 1988a). Recent observations of living specimens show germination to be by means of a germination shield for spores determined as *G. dominikii* and a new examination of isotype material of *G. scintillans* shows it to germinate in the same way (Figs 9–10). This is not a characteristic of organisms in the genus *Glomus*.

At the species level, we could find no consistent characteristics to separate *G. scintillans* from *G. dominikii*, supporting our hypothesis of conspecificity. The ornamentation on the spores of *G. chimonobambusae* is generally more pronounced (more than twice the maximum height of the other two). We conclude that *G. dominikii* is synonymous with *G. scintillans*. *G. chimonobambusae* has not been sequenced, but it shares most of the characteristics of *G. scintillans* and cannot reasonably be placed in a separate genus. Because it has considerably more pronounced spore wall ornamentation, it is retained as a separate species, pending future more detailed examination of living material. The species description of *G. scintillans* was published in 1980, eight years before that of *G. dominikii*, and so the former epithet has priority and the latter must be treated as a heterotypic synonym of the former.

TAXONOMY

There are some matters of terminology that must be addressed in explanation of the use of terms in spore description that may not accord precisely with other published work. We use the term 'glomoid' in the manner introduced by Morton & Redecker (2001), i.e. '… spores [which] develop blastically from the tip of a sporogenous hypha … and [that] are indistinguishable from spores of species in *Glomus* … ' The subtending hyphae usually are prominent and are thickened proximally, tapering distally to the point of origin (Mosse & Bowen 1968: figs 2–3). The attachment of the main structural spore body of species in *Gigaspora* or *Scutellospora*, in contrast, appear as a bulbous base that readily becomes detached during spore extraction (Spain, Sieverding & Schenck 1989).

The genus *Scutellospora* was partly defined by the presence of a pre-germination structure termed a 'germination shield' by Koske & Walker (1985) and described in more detail by Walker & Sanders (1986). Spores of species in the genus *Acaulospora* also germinate by way of a specialised pre-germination structure which was recognised as a germination shield by Spain (1992) but which is also known as a germination orb by Stürmer & Morton (1999) based on an assertion that the two structures are analogous rather than homologous. We do not offer an opinion on the matter of homology, but have retained the use of the single term for simplicity. The pre-germination structure of *G. scintillans* has the morphology and apparent developmental characteristics similar to one of the

more simple germination shields in *Scutellospora* (e.g. *S. castanea*; Walker *et al*. 1993) or one of the more complex germination shields of *Acaulospora* (e.g. *A. scrobiculata*; Spain 1992).

The use of the terms 'exospore', 'mesospore' and 'endospore' was introduced by Ferrer & Herrera (1981), but this has not been widely accepted to date. Nevertheless, it has considerable merit as a terminology to distinguish the simple chlamydospores produced by many members of the *Glomeromycota* (particularly those of *Glomus s. lat*.) from the more complex structures produced by species in *Acaulospora* and *Scutellospora*. The 'spores' of these organisms follow a serial development sequence in which a rigid-walled, structural entity is first produced (exospore), followed by the development of a thin, flexible component (mesospore), and then an internal, completely separate structure (endospore), from which the germination shield forms. Although we retain the term 'spore' for the entire complex structure, it could be considered to be a highly modified sporangiole, with the exospore corresponding to a sporangiophore, the mesospore to a sporangium, and the endospore to a sporangiospore.

The morphological investigations showed that the three species examined differed from all other fungi in the *Glomerales sensu* Walker & Schuessler (Schüßler *et al*. 2001). Although the spores were formed on a superficially glomoid subtending hypha, more detailed examination showed the attachment to be atypical of *Glomus* spp. *s. str*. and to have similarities with the bulbous spore base of members of the genera *Scutellospora* and *Gigaspora*. The hypha was not persistent, but was fragile and easily detached, and in some instances possessed a small peg-like structure similar to those found on the bulbous base of members of the *Gigasporaceae*. The multi-grouped wall construction of the spores is reminiscent of members of *Scutellospora*, and also of fungi belonging to the genus *Acaulospora*. No other species in the genus *Glomus* has a flexible innermost wall component that reacts to become purple in Melzer's reagent, and none forms a germination shield from which multiple germ tubes can arise. These characteristics occur commonly in fungi from the *Acaulosporaceae* and *Gigasporaceae*.

The phylogenetic analysis of the SSU rRNA gene firmly placed *G. scintillans* and *G. dominikii* within the *Diversisporales*, with *Gigaspora* as a sister group. Although it was not possible to carry out a similar analysis on *G. chimonobambusae*, the morphological characteristics indicate that it belongs to the same genus as the other two species. The three species therefore cannot naturally be accommodated in the genus *Glomus*, and we therefore herein transfer them to a new genus, *Gerdemannia*.

Because *Gerdemannia scintillans* and *G. chimonobambusae* belong to a sister clade of the *Gigasporaceae* within the *Diversisporales*, they cannot remain in the *Glomerales*. Comparing the genetic distances (Fig. 1) between this newly uncovered clade with the *Acaulosporaceae* and *Gigasporaceae* clades, the latter being the best characterised family at the molecular level (de Souza *et al*. 2004), and the *Diversisporaceae* fam. ined. (Schüßler *et al*. 2001), the clade containing *G. scintillans* is best represented by the rank of a family. This is also supported by the isolated position in the tree topology and we therefore erect a new family, the *Gerdemanniaceae*, to contain the new genus.

Gerdemanniaceae C. Walker, Błaszk., Schuessler & Schwarzott, **fam. nov.**

A familiis ceteris in *Glomeromycota* distincta combinatione sporae glomoideae elementis tunicae internae amylodeis, sporophoro glomoideo, et sequentio DNA differenti. Mycorrhizas vesiculares-arbusculares formans.

Typus: *Gerdemannia*.

Differs from other families in the *Glomeromycota* by possession of glomoid spores with a combination of amyloid inner wall components and germination by means of a germination shield, coupled with the formation of vesicular–arbuscular mycorrhizas and by the possession of the SSU rRNA gene sequence signature TTATCGGTTRAATC, corresponding to homologous position 650 of the *Sacharomyces cerevisiae* SSU rRNA sequence J01353, and ACTGAGTTMATYT, corresponding to homologous position 1481 of the *S. cerevisiae* SSU rRNA sequence J01353, with the underlined nucleotides being specific for the taxon.

Gerdemannia C. Walker, Błaszk., Schuessler & Schwarzott, **gen. nov.**

Etym.: in honour of James W. Gerdemann, a pioneer of the modern approach to morphological species description of AM fungi.

A generibus ceteris in *Glomeromycota* distincta combinatione sporae hyalinae tunicis internis flexibilibus, elemento tunicae internae in solutione Melzeri purpurascenti, scutello germinationis, et sporophoro glomoideo.

Typus: *Glomus scintillans* S. L. Rose & Trappe 1980.

Differs from other glomoid genera in the *Glomeromycota* by possessing the combination of non-pigmented spores with flexible inner wall, a germination shield, an innermost wall component that reacts to Melzer's reagent to become purple, and a glomoid subtending hypha.

Gerdemannia scintillans (S. L. Rose & Trappe) C. Walker, Błaszk., Schuessler & Schwarzott, **comb. nov.** (Figs 3–10).

Glomus scintillans S. L. Rose & Trappe, *Mycotaxon* **10**: 417 (1980).

Glomus dominikii Błaszk., *Karstenia* **27**: 37 (1988).

Spores formed singly in the soil; hyaline, subhyaline or white with a sparkling appearance or the appearance of frosted glass beads due to ornamentation. Becoming light yellow to orange yellow when moribund or due to attached fine soil particles. Globose, (70–) 110–210 μm

diam, to subglobose, ovoid, obovoid, ellipsoid to irregular; 100–180 × 110–200 μm; with one subtending hypha. Spore consisting of an exospore, mesospore and endospore. *Exospore* wall double: wall layer 1 permanent, rigid, hyaline to orange white; 0.5–1.5 μm thick; ornamented mainly with warts or blunt spines, 1.5–6 × 0.5–2 μm, rarely with ridges, 0.5–1 μm high, tightly adherent to a laminate, smooth, hyaline layer, (2–) 3.5 (–4.5) μm thick. Exospore wall rapidly staining yellow in Melzer's reagent. *Mesospore* wall flexible, hyaline, 0.5–1.5 μm thick; when crushed, usually separating from the endospore but sometimes adherent and difficult to discern. Not reacting to Melzer's reagent. *Endospore* wall flexible, coriaceous, hyaline, 1–3 μm thick, reacting to become purple in Melzer's reagent. Not reacting to Melzer's reagent when moribund or dead. Melzer's reaction in PVLG-Melzer's microscope slides sometimes fading within a fortnight. *Germination shield* hyaline to orange white, circular (45–53 μm diam), to elliptical (30–80 × 75–100 μm), with a sinuous or invaginated margin, appearing in section to consist of compartments, *ca* 0.5–1 μm thick from which may emerge a hyaline to orange white germ tube (3.8–12.5 μm diam). A germ tube develops from a germination shield and penetrates the spore wall. *Subtending hypha* hyaline; straight or slightly curved; cylindrical, sometimes slightly constricted at the spore base; (6.4–) 9 (–12.5) μm wide at the spore base: rather easily detached. Wall of subtending hypha hyaline; (1.2–) 1.5 (–2) μm thick at the spore base. Pore open or closed by a plug.

Distribution: *Gerdemannia scintillans* has a wide distribution, having been extracted from soil in North America, Central, Northern and Western Europe, Australasia, and the Middle East.

Notes: The distinctly ornamented spores of the three fungal species are easy to separate from those of the other species with glomoid spores. However, when observed under a dissecting microscope, spores of *G. scintillans* with small warts or low ridges resemble the smooth spores of *Glomus eburneum Gl. diaphanum*, *Gl. laccatum*, *Gl. viscosum*, and *Paraglomus occultum* (Walker 1982, Morton & Walker 1984, Błaszkowski 1988b, Walker *et al.* 1995, Kennedy, Stutz & Morton 1999, Morton & Redecker 2001), but none of these has a flexible endospore with a positive Melzer's reaction.

Gerdemannia chimonobambusae (C. G. Wu & Y. S. Liu) C. Walker, Błaszk., Schuessler & Schwarzott, **comb. nov.**

Glomus chimonobambusae C. G. Wu & Y. S. Liu, *Mycotaxon* **53**: 284 (1995).

Despite the lack of any published data of the formation of a germination shield, the diagnostic descriptions and illustrations of spores of *Glomus chimonobambusae* (Wu *et al.* 1995) suggest that it is congeneric with *Gerdemannia scintillans*, and it is likely, by analogy, to germinate in the same way.

Pending further investigation, we do not emend the original species description of this fungus, but only move it into the new genus.

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