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Cetraspora helvetica, a new ornamented species in the *Glomeromycetes* from Swiss agricultural fields

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Abstract — A new arbuscular mycorrhizal fungus, *Cetraspora helvetica*, was found in three Swiss agricultural soils: a no-till crop production system and two temporary grasslands. It forms white spores, 210–270 µm diam, on dark yellow sporogenous cells. The spores have three walls: a triple-layered outer, a bi-layered middle and a triple-layered inner wall. The spore surface is crowded with convex warts, 5–12 µm diam at the base and 1.5–5.0 µm high. The germination shield is hyaline with multiple (6–10) lobes. Glomerospores of two other *Gigasporineae* spp. have also three walls, multiple-lobed hyaline germination shields, and projections on the outer spore surface: *C. spinosissima* and *C. striata*. However, spores of these fungi are substantially pigmented (ochraceous yellow to rust) and crowded with short, thin spines or fingerprint-like processes, respectively. Partial sequences of the 28S ribosomal gene place the new species adjacent to *C. spinosissima*, *C. pellucida,* and *C. gilmorei*. Phylogenetic analyses demonstrate the monophyly of the two genera *Racocetra* and *Cetraspora* within the *Racocetraceae*.

Key words — *Gigasporaceae*, *Glomeromycota*, *Scutellospora*, conservation tillage

Introduction

Several species of the *Gigasporineae* sensu Morton & Benny (1990) have been recently described (e.g. Silva et al. 2008, Goto et al. 2009, 2010; Tchabi et al. 2009). However, most of the so far nearly 50 species described in this old sub-order have been known only for the warmer climates, and indeed species

richness of the *Gigasporineae* appears to be much lower in colder climates, especially in Europe north of the Alps (e.g. Jansa et al. 2002, Oehl et al. 2009b, 2010). In Northern and Central Europe, so far only ten species of this group has been found: *Gigaspora margarita* W.N. Becker & I.R. Hall 1976, *G. gigantea* (T.H. Nicolson & Gerd.) Gerd. & Trappe 1974, *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders 1986, *S. dipurpurescens* J.B. Morton & Koske 1988, *S. arenicola* Koske & Halvorson 1990, *S. nodosa* Błaszk. 1991, *Racocetra castanea* (C. Walker) Oehl et al. 2009, *R. persica* (Koske & C. Walker) Oehl et al. 2009, *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl et al. 2009, and *C. armeniaca* (Błaszk.) Oehl et al. 2009 (e.g. Błaszkowski 1991, Błaszkowski & Tadych 1997, Tadych & Błaszkowski 2000, Vestberg et al. 1995, Merryweather & Fitter 1998, Jansa et al. 2002, 2003, Oehl et al. 2005, 2010). Our morphological and molecular analyses revealed that one isolate, registered at the International Bank for *Glomeromycota* (BEG) and identified as *Scutellospora pellucida* (= *C. pellucida*) is not *C. pellucida* but a closely related, undescribed species whose spores have conspicuous warty ornamentation on the outer surface. The fungus, collected from three agricultural soils in Switzerland, is here described under the epithet *Cetraspora helvetica*.

Material and methods

Study area and sites

Between 1998 and 2009, AMF species richness was determined in > 100 Swiss agricultural soils distributed all over the country and including permanent grassland, conservation and no-tillage, biological and conventional agroecosystems (e.g. Jansa et al. 2002, Oehl et al. 2003, 2004, 2010, Oehl unpublished). The AMF communities of these sites were propagated in bait cultures for 8–32 months, in most of the cases for 18–24 months. At only three sites, the AM fungus, which is hereafter described, was detected. The sites are a long-term tillage experiment at Agroscope ART in Tänikon (Kanton Thurgau, 47°29'10.0"N, 8°55'10.1"E, at 540 m a.s.l.), a temporary grassland in the community Langnau im Emmental (Kanton Bern, 46°56'35.0"N, 7°45'46.8"E, at 656 m a.s.l.), and a temporary grassland in the community Grasswil (Kanton Bern, 47°08'34.0"N, 7°39'58.2"E, at 525 m a.s.l.). The locations have a temperate climate (typical for Central Europe), with mean annual temperatures of 8.5, 8.0, and 9.2 °C and mean annual rainfall of about 1200, 1450, and 1100 mm, respectively.

Soil sampling and soil parameters

In Tänikon, soils were sampled in January 1999 as described in Jansa et al. (2002). The soil samples in Langnau were sampled accordingly in April 2009. The soil type in Tänikon and Grasswil is a Haplic Luvisol developed on Moräne, while the soil type in Langnau was a Fluvic Cambisol developed on alluvial sediments. The pH (H_2O) of the topsoil was 6.0 at all three sites. The organic carbon content was 19.1, 21.1 and 12.2 mg C g^{-1} soil at Tänikon, Langnau and Grasswil, respectively. Total N and available P (according to Dirks & Scheffer 1930) were 2.3, 2.5 and 3.0 mg N g^{-1} soil and 2.3, 2.2, and 7.4 mg P kg^{-1} soil, respectively.

AMF bait and pure cultures

The AMF bait cultures for the soil from Tänikon were established at ETH Zurich in Eschikon-Lindau (Kanton Zurich) as described in Jansa et al. (2002) using *Zea mays* L., *Allium porrum* L., *Plantago lanceolata* L., *Helianthus annuus* L. and *Glycine max* (L.) Merr. as bait plants. The bait cultures for the soils from Langnau and Grasswil were established at Agroscope ART in Zürich-Reckenholz on *P. lanceolata*, *Lolium perenne* L., *Trifolium pratense* L., and *Hieracium pilosella* L. as host plants, as described for *Acaulospora alpina* (Oehl et al. 2006) but with the pots substantially larger than in the former work (volume 3.5 L instead of 1.0 L).

Pure cultures of the new fungus were established by inoculating leek plants with 15 spores obtained from the Tänikon soil bait cultures. The cultures have been maintained for several cycles of 15-24 months at ETH (alternating *A. porrum* and *Tagetes erecta* L. as host plants, and on *Medicago truncatula* L.). The isolate was also deposited in the European Bank of *Glomeromycota* under the accession number BEG153 and is maintained in the Swiss Collection of Arbuscular Mycorrhizal Fungi (SAF) at Agroscope ART in Zürich-Reckenholz under the accession number SAF15.

Morphological analyses

Glomerospores were extracted from field soils by wet sieving (Gerdemann & Nicolson 1963) and sucrose centrifugation (Jenkins 1964). The spores were thereafter mounted in polyvinyl-alcohol-lacto-glycerin (PVLG), PVLG + Melzer's reagent, and water (Brundrett et al. 1994, Spain 1990). About 100 spores of the fungus were examined. For the species description, terminology follows that used for the *Diversisporales* by Oehl et al. (2006), Sieverding & Oehl (2006), and Palenzuela et al. (2008, 2010), for germ shield structures by Walker & Sanders (1986), Oehl et al. (2009a), and Goto et al. (2010), and for spore denomination by Goto & Maia (2006).

Spore wall composition was compared with that observed in spores in type specimens of *Cetraspora armeniaca* [ex type: Błaszkowski collection], *C. gilmorei* (Trappe & Gerd.) Oehl et al. 2009 [Holotype OSC 30'990; paratype OSC 31'018; paratype OSC 30'921, *C. pellucida* [Holotype OSC 37'515], *C. spinosissima* (C. Walker & Cuenca) Oehl et al. 2009 [Ex type: (Gisela Cuenca collection, Oehl collection], and *C. striata* (Cuenca & R.A. Herrera) Oehl et al. 2009 [Ex type: Gisela Cuenca collection, slides 1642-7 & 1641-3].

Molecular analyses

The DNA from single spores was extracted according to Sanders et al. (1995). Single spores were crushed in 10 µl of PCR-grade water by freshly flamed Pasteur pipette. After 5 µl of Chelex-100 (20%, Bio-Rad Laboratories, Hercules, California, USA) were added, samples were placed onto a 95°C hot plate for 3 minutes and then incubated on ice at 0°C for 5 minutes. Five µl of the liquid phase were taken as template for PCR amplification of the large ribosomal subunit gene, 28S.

Spore DNA samples underwent a nested PCR procedure, first using eukaryotic-specific primers ITS3 and NDL22 (White et al. 1990), followed by fungal-specific primers LR1 and FLR2 (van Tuinen et al. 1998; Turnau et al. 2001). There were 30 cycles with each primer pair. The product of the first PCR was cloned or further diluted 1000 times, and 5 µL of the diluted mixture was used as a template for the second PCR reaction. PCR conditions followed van Tuinen et al. (1998), the annealing temperature

being 60°C in both PCR steps. The PCR products were then purified using QIAquick PCR Purification Kit (Qiagen Sciences), cloned into a blue script vector (pGEM-T Easy, Promega-Catalys AG, Wallisellen, Switzerland), and transformed into bacterial strain *E. coli* JM109 by the heat-shock method. The size of the insert in growing bacterial colonies was checked after PCR amplification using M13f and M13r primers that were targeted to the cloning site of the vector. Plasmid DNA was isolated from transformed bacteria following standard miniprep procedure (Sambrook et al. 1989), and used as a template for cycle sequencing using BigDye Terminator (Applied Biosystems, Faster City, California, USA). Sequencing analysis was performed on ABI-310 Capillary Sequencer (PerkinElmer, Wellesley Massachusetts, USA). Four sequences were obtained and deposited at GenBank (National Center for Biotechnology Information, Bethesda, Maryland, USA) under the accession numbers AF396784 and HM565944–HM565946.

The sequences of the new species were aligned with other glomeromycotean sequences from the GenBank using ClustalX (Larkin et al. 2007) and edited by BioEdit (Hall 1999) to obtain a final alignment.

For phylogenetic analyses and tree construction, maximum parsimony (MP) and neighbor joining (NJ) analyses with 1000 bootstrap replications for each, were performed using the Phylogenetic Analysis Using Parsimony program version 4 (Swofford 2003). The NJ analysis was performed using parameters obtained from ModelTest 3.7 (Posada & Crandall 1998). Sequences from *Pacispora scintillans* were used as outgroup.

Taxonomy

Cetraspora helvetica Oehl, Jansa, F.A. Souza, G.A. Silva, **sp. nov.** Figs. 1–19

MycoBank MB 518578

Sporocarpia ignota. Sporae singulatim in solo efformatae anguste adiacetae ad cellulas sporogeneas subterminales vel intercalares flavasque, albae, globosae (210–270 µm in diametro) vel subglobosae vel ovales (205–265 × 210–280 µm); sporae tunicis tribus: tunica exterior stratis tribus, in totum 8.4–15.0 µm crassa; stratum exterius tunicae exterioris hyalinum, semi-persistens, 0.9–1.6 µm crassum, cum verrucis exiguis 1.5–5.0 altis et 5–12 µm latis; stratum medium laminatum, album, 8.4–15.0 µm crassum; stratum interius tunicae exterioris album, 1.0–1.6 µm crassum; tunica media stratis duobus hyalinibus, 1.5–2.5 µm crassa in totum; tunica interior stratis tribus hyalinibus, 2.5–5.9 µm crassa in totum; stratum secundum et stratum interius tunicae exterioris et stratum secundum tunicae interioris purpureum vel oscuro-purpureum colorantes reagente Melzeri; scutellum germinale in superficie exteriore tunicae interioris, hyalinum ad subhyalinum ad albo-flavum; subglobosum vel ovale vel ellipsoidum, 120–150 × 120–200 in diameter, lobatum, paucioribus 6–10 lobis; structurae mycorrhizarum arbuscularum colorantes caeruleae Trypan blue; cellulae auxiliares formans.

Type: Switzerland: Kanton Thurgau, Tänikon, Agroscope Reckenholz-Tänikon Research Station (ART), from agricultural soil under no-till wheat–maize–canola production, 1999 by J. Jansa". (**Holotype**: 88-8801 (Z+ZT Myc 3037); pure cultures—ETZ Zürich (Eschikon) and Swiss Collection of Arbuscular Mycorrhizal Fungi (SAF; Zürich Reckenholz). **Isotypes**: 88-8802, 88-8803, 88-8804, 88-8805, 88-8806 (Z+ZT Myc 3038); 88-8807, 88-8808 (OSC 136'595); 88-8809, 88-8810 (URM).

Etymology: *helvetica* (Latin) = Swiss, referring to the country where the fungus was detected first.

Glomerospores formed singly in soil, terminally on subterminal or sometimes intercalary bulbous suspensor cell (= 'sporogenous' cell; Figs. 1–6). Glomerospores are brilliant white when young, and may slightly darken to creamy-white when ageing in soils, trap culture substrates or after several months in lactic acid based mountants (Figs. 1–3). The spores are globose (210–270 µm in diameter) to subglobose (205–265 × 210–280 µm), become dark purple to black purple when exposed to Melzer's reagent (Fig. 5), and have three walls: an outer, a middle, and an inner wall (Fig. 7).

Outer wall is 8.4–15.0 µm thick in total and consists of three layers (Figs. 7–8): outermost wall layer (owl1) is hyaline, semi-persistent and 1.1–1.6 µm thick crowded with convex warts that are 5-12 µm in diameter at their base and 1.5–5.0 µm high (Figs. 8–9). owl2 is brilliant white, and may become creamy-white with age. It is laminate, persistent and 5.0–12.0 µm thick. Third layer (owl3) is also white, semi-flexible (1.0–1.6 µm thick). owl2 and owl3 stain dark purple to black purple in Melzer's reagent, while owl1 generally does not stain (Fig. 8). The straight pore channel at the spore base (about 2.5–3.9 µm broad) is often closed by a plug formed by spore wall material of owl2, and by owl3, but also can appear open.

Middle wall (mw) is 1.8–2.7 µm thick in total and consists of two hyaline layers: a flexible outer layer mwl1 and a semi-flexible layer mwl2 (Figs. 7, 10). mwl1 is 0.7–1.2 µm thick and generally does not separate from underlying mwl2 but often shows several folds in crushed spores (Fig. 10). mwl2 is 1.1–2.0 µm thick, and generally more rigid than mwl1.

Inner wall (iw) is triple-layered (Figs. 7), 2.5–4.5(–5.9) µm thick, bearing a germ shield on the outer surface (Fig. 4, 11). The outer iw layer (iwl1) is hyaline, semi-flexible and 0.6–0.8 µm thick. The second layer (iwl2) is semi-flexible, unite to finely laminate, amorphous when slightly expanding in PVLG based mounting, and is 2.0–2.7(–3.9) µm thick. The innermost layer (iwl3) is relatively thin (0.6–1.2 µm thick), flexible, mostly tightly adherent to iwl2, and therefore generally difficult to observe. iwl2 stains purple to dark purple to black purple in Melzer's reagent (Fig. 11).

Sporogenous cell (sc) is globose to elongate, 34–70 µm long and 30–48 µm broad (Figs. 1–4, 6) and generally dark yellow. Two wall layers are visible on the young sporogenous cell, which are continuous with owl1 and with laminated owl2. owl1 is 0.4–1.0 µm thick and semi-persistent, and owl2 is 1.5–2.8 µm thick and persistent as long as sc remains attached on the spore. One to (rarely) two 'hyphal pegs' are often formed on the sporogenous cells, and are 4–10 µm thick at the sporogenous cell base tapering to 3.0–4.5 µm within its 12–30 µm length. Sometimes one peg continues as mycelial hypha or as a sporogenous hypha that may bear another sc in 400–800 µm distances from the first sc.

Then, the formation of the sc can be called intercalary instead of sub-terminal. The sporogenous hypha attached to the cell is also bi-layered, 12–25 µm thick and tapering to 5–7 µm within 100–450 µm distances from the sporogenous cell. Within these distances, the sporogenous hyphal wall tapers from 1.5–2.5 µm to 1.1–1.6 µm, and 2–9 septa originating from OWL2 may be visible in the sporogenous hypha (Fig. 2).

Germination shield is hyaline to subhyaline (Figs. 6, 10–15), infrequently light yellow in aged spores, subglobose to oval to rarely ellipsoid, 120–125 × 135–180 µm in diameter, and generally has 6–10 lobes (Figs. 10, 12–15). Large folds (~7–30 µm long) arise from the shield wall separating the lobes (Figs. 10, 12–15). The one-layered shield wall and the folds are hyaline to subhyaline and generally only 0.9–1.8 µm thick. The shield periphery regularly appears slightly dentate until the germination has started. Each lobe may bear one rounded germ tube initiation (gti, Figs. 10, 12–15), 2.5–4.5 µm in diameter. The majority of the gti's may remain undetected in young spores or in crushed mature spores due to the pressure applied on the cover slide, especially when the shields are completely separated from the spores by applying harsh pressure (Fig. 10). Single germination tubes may simultaneously emerge from 1 to 3 gti's during early germination (Figs. 3–4, 15). They penetrate the OW (Figs. 3–4) and branch in the spore periphery within a short distance.

Spore development — The key stages of spore development observed in the pure and bait cultures are the same as known for other species in the *Racocetraceae*: First the outer spore wall differentiated into one semi-persistent outer layer (OWL1), the laminate, structural layer (OWL2) which differentiates the characteristic convex projections, and the adherent inner layer (OWL3). The MW and IW developed de novo with no visible connection with the outer wall. Finally, the germination shield differentiated its multiple-lobed structure, beginning from the initial germ hole (= germ pore) and forming a gti at the end

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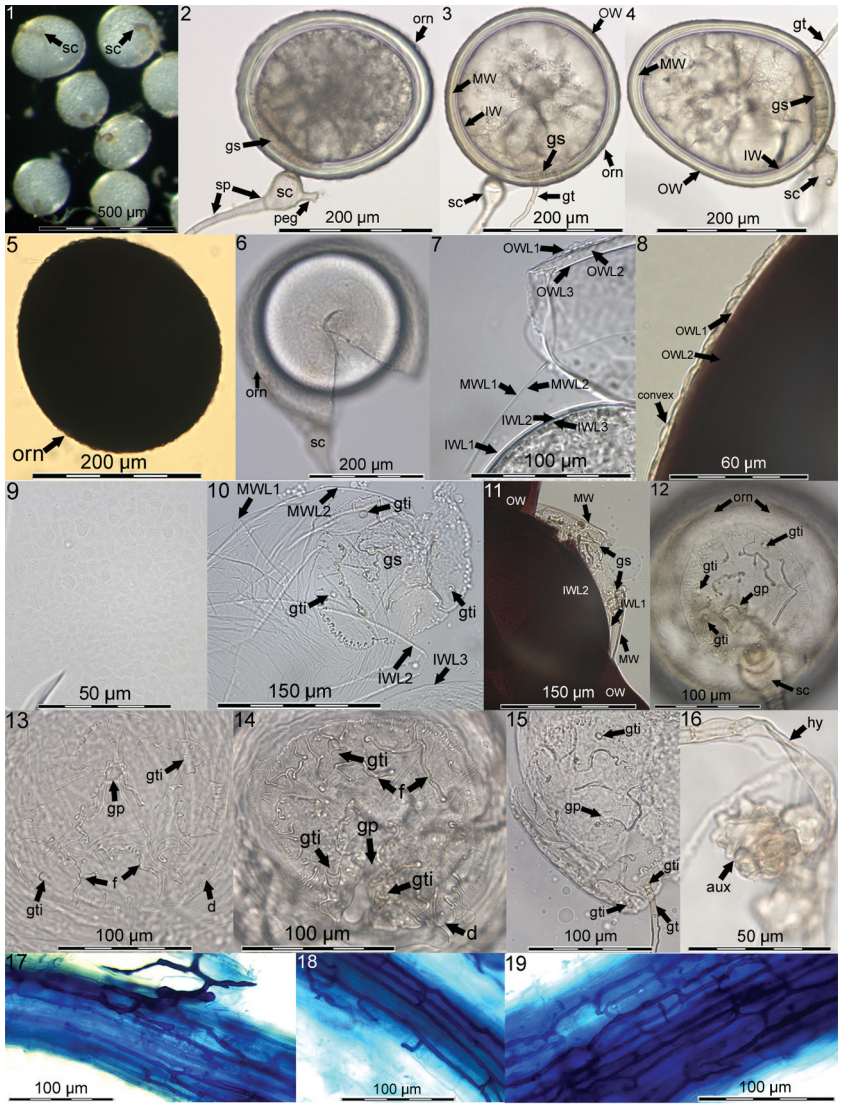
Figs. 1–19. *Cetraspora helvetica.* Fig. 1. White spores with pigmented sporogenous cells (sc) in a Petri dish. Figs. 2–4. Spores have three walls: an outer, middle and inner wall (OW, MW, IW). On sc, short hyphal pegs (peg) may be differentiated, and one to several septa (sp) may be visible in the sporogenous hypha. Germination shields (gs) are formed on the outer IW surface, and sometimes germ tubes (gt) are visible in germinating spores. The convex warty projections (orn) are not obvious under low magnification in PVLG mountants. Fig. 5. Spores stain dark purple to purple black in Melzer's reagent. Here, the convex projections are conspicuous. Fig. 6. Crushed spore with focus on the ornamentation in planar view. Fig. 7. Triple-layered OW (OWL1-3), bi-layered MW (MWL1-2) and triple-layered IW (IWL1-3). Fig. 8. Laminate OWL2 stains dark purple to black purple in Melzer's reagent, while OWL1 with the convex projections (here in cross view) does not stain. Fig. 9. Ornamentation in planar view. Fig. 10. MW with thin MWL1 that slightly wrinkle, and thus, shows several folds; three germ tube initiations (gti) are in focus on the slightly crushed germ shield. Fig. 11. Crushed spore in Melzer's with gs between MW and IW; MW does not stain, while OW



and IWL2 stain dark purple to black purple. Figs. 12–15. Germ shields (gs) in (semi-)planar view; shields have a initial germ pore (gp; = germ hole) and several lobes that are generally separated by large folds (f); the lobes may regularly bear one germ tube initiation (gti) each but the gti's often become invisible following pressure needed to present the gs in planar view or to separate the gs from overlaying OW and MW; shield periphery is slightly dentate (d) in mature spores. Fig. 16. Light yellow to yellow, knobby auxiliary cells (aux) formed on light yellow to yellow mycelial hyphae. Figs. 17–19. Mycorrhizal structures (here roots of *Medicago truncatula*, 12 weeks after inoculation) lack intraradical vesicles.

of the shield development usually in each of the lobes; from 1–3 of these gti, the germination tubes emerge during initial germination.

Germination — One to two germ tubes may arise. They are light yellow to bright yellow, 5–7 µm in diameter and emerge from one or two gti's (Figs. 3–4, 15). Germ tubes directly penetrate the ow and branch then almost immediately in the soil environment. The mono- to bi-layered germ tube walls are ~1.2–2.0 µm thick in close spore vicinity.

Auxiliary cells are formed singly or in small aggregates (2–4 cells) on light yellow to yellow mycelial hyphae (Fig. 16). They are yellow, knobby and 20–25 µm in diameter.

Arbuscular mycorrhiza formation is without formation of vesicles (Figs. 17–19).

Additional collections: **Switzerland**: **Kanton Bern**, Langnau im Emmental, temporary grassland in April, 2009, specimens from 8 trap cultures (in July 2009; Z+ZT Myc 3040a–h); Grasswil, temporary grassland in April, 2009, specimens from 2 trap cultures (in July 2010; Z+ZT Myc 3202a–b).

Distribution —*Cetraspora helvetica* has thus far been detected only at the cited locations in the Kantons Thurgau and Bern, Switzerland.

Molecular analyses — Four partial sequences of the large (LSU, 28S) subunit (~700 bp) of the ribosomal gene were obtained. Phylogenetic analyses firmly placed the newly described fungus into the genus *Cetraspora* adjacent to *C. spinosissima*, *C. pellucida* and *C. gilmorei* (Fig. 20). The analyses also demonstrate the monophyly of the two genera *Racocetra* and *Cetraspora* of the family *Racocetraceae* recently described (Fig. 20).

Discussion

The three-walled glomerospores and the multiply lobed, hyaline germination shield place the newly described species in the genus *Cetraspora* in the *Racocetraceae* (Oehl et al. 2009a) of the *Diversisporales* (Schüßler et al. 2001). The molecular analyses using the 28S ribosomal gene confirmed the morphological findings: *Cetraspora helvetica* clustered in the phylogenetic tree next to *C. spinosissima*, *C. pellucida,* and *C. gilmorei*. *Cetraspora helvetica* is readily distinguished from all other known species in the *Racocetraceae* by spore color, staining features in Melzer's reagent, and the spore wall characteristics, including the characteristic convex warts on the outer spore surface.

There are only five species known within *Cetraspora* sensu Oehl et al. (2009a), i.e. species of *Scutellospora* group C sensu de Souza et al. (2005) with three spore walls and multiple-lobed germination shields. These species are: *C. armeniaca*, *C. gilmorei*, *C. pellucida*, *C. spinosissima,* and *C. striata* (Oehl et al. 2009a). However, these species have either smooth spore surfaces

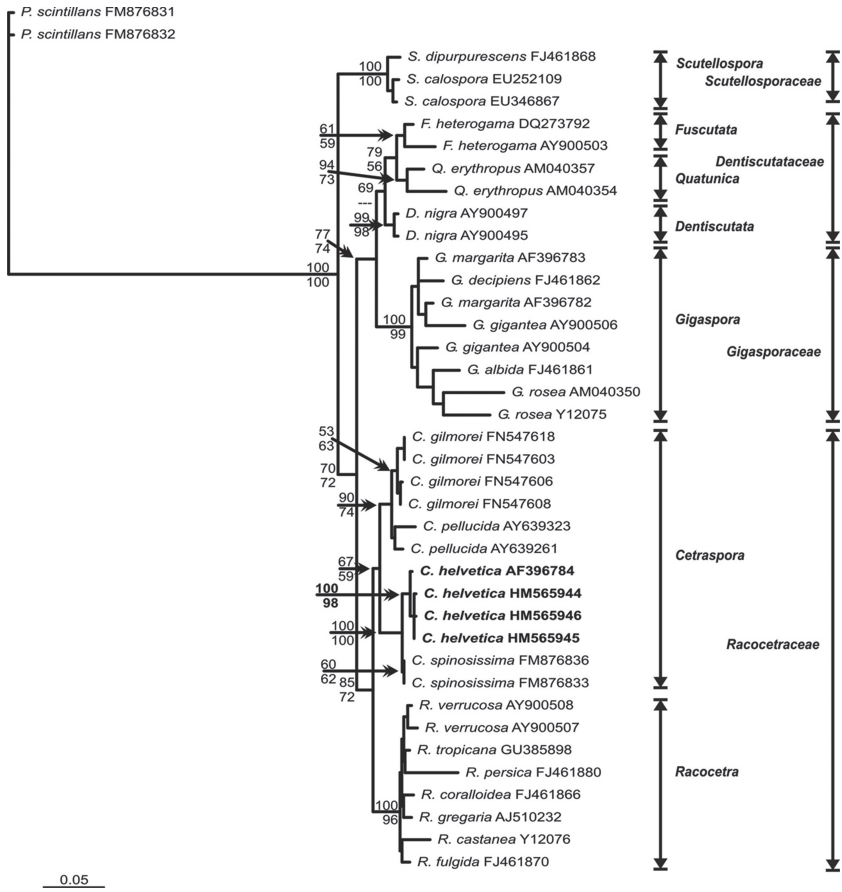


Fig. 20. Phylogenetic reconstruction of the *Gigasporineae* sensu Morton and Benny (1990) obtained from partial LSU rDNA sequences (~700 bp). The neighbor-joining (NJ) analysis was performed with GTR substitution model using the following ModelTest parameters: rate matrix (a = 0.9199, b = 10.3397, c = 2.6872, d = 0.6133, e = 21.1774); number of substitutions types = 6; nucleotide frequencies (A = 0.32210, C = 0.13470, G = 0.23930, T = 0.30390); rates = gamma; shape=0.6497 and proportion of invariable sites = 0. The four new sequences obtained are indicated in bold. Sequences are labeled with their database accession numbers. Bootstrap values (in %) are from neighbor-joining (NJ) and maximum parsimony (MP) analyses (1000 bootstraps), respectively. Only topologies with bootstrap values of at least 50% are shown. The lines to the right show the current genera and families of the *Gigasporineae*. (Consistency Index = 0.6077; Retention Index = 0.7799).

(*C. armeniaca*, *C. gilmorei*, *C. pellucida*; Błaszkowski 1993, Gerdemann & Trappe 1974, Nicolson & Schenck 1979, Koske & Walker 1986) and/or do not form white spores (*C. armeniaca*, *C. spinosissima* and *C. striata*; Błaszkowski 1993, Walker et al. 1998, Cuenca et al. 2008). Moreover, the ornamentations of *C. spinosissima* and *C. striata* consist of spines and fingerprint-like procedures, respectively, and not of convex warts (Walker et al. 1998, Cuenca et al. 2008).

Besides *C. helvetica*, there is only one other fungus in the *Racocetraceae* forming hyaline or white spores with a warty surface ornamentation. This is *Racocetra beninensis* Oehl et al. 2009 (Tchabi et al. 2009). However, *R. beninensis* has only two spore walls and its projections are smaller and more irregular than those of *C. helvetica*. Moreover, its inner spore wall does not stain in Melzer's reagent, while the outer wall stains bright yellow to dark yellow but not purple to dark purple as in *C. helvetica*.

Only *Scutellospora nodosa* (Błaszkowski 1991), which phylogenetically belongs to *Scutellospora* group A sensu de Souza et al. (2005) and to the monogeneric family *Scutellosporaceae* sensu Oehl et al. (2009a), has a similar warty ornamentation as *C. helvetica*. However, differences in sporogenous cell color, germination shield size and structure, inner wall structure, and the staining behavior in Melzer's clearly differentiate *C. helvetica* and *S. nodosa*. *Scutellospora nodosa* has sc's that are concolorous with the spore, a simpler and substantially smaller germ shield, and an outer wall that stains brownish-red instead of dark to black purple. Additionally, of the inner wall only IWL3 stains purple in *S. nodosa*, while in *C. helvetica* it is IWL2. The IWL2 stains purple in all known *Cetraspora* spp.

It is remarkable that species of *Racocetraceae* and *Dentiscutataceae* generally have pigmented sporogenous cells (sc) even when the spore color is hyaline or white to light creamy. This is known for *R. beninensis* and *R. fulgida*, *C. pellucida*, *C. gilmorei,* and *C. helvetica*, and for *Dentiscutata cerradensis*, *D. scutata,* and *Fuscutata savannicola*, which all form light colored, hyaline to subhyaline or white spores. In *C. helvetica*, the germ tube, the mycelial hyphae, and the auxiliary cells are also concolorous with the sc, i.e. bright yellow to dark yellow. It will be interesting to determine later whether this feature is common for all (or a majority) of the *Racocetraceae* and *Dentiscutataceae* spp. Our observation is even more remarkable when considering that *Racocetraceae* spp. form hyaline to subhyaline germ shields while *Dentiscutataceae* spp. have yellow-brown to brown shields. However, the database for the mycelial hyphae and auxiliary cell morphologies is, to our knowledge, still incomplete and in need of improvement.

Notably, our study is the first to report that sporogenous cells can form not only sub-terminally, but also intercalarly. It will be interestingly to follow up in the future if this feature is unique within the *Glomeromycota*.

Our phylogenetic analyses demonstrate the monophyly of the genera *Racocetra* and *Cetraspora* in the *Racocetraceae* and fully support the analyses and classification of Oehl et al. (2009a), which have been recently criticized by Morton & Msiska (2010), who did not find major congruency between spore morphology and molecular phylogeny in this species group. In our opinion, those authors included some characters in their morphological-phylogenetic analyses that weakened their analyses. The authors also found a much higher intraspecific variability of the shields than Oehl et al. (2009a) and Oehl and co-workers who investigated the intraspecific variability of mature shields for a series of *Scutellosporaceae*, *Racocetraceae* and *Dentiscutataceae* spp (e.g. Silva et al. 2008, Tchabi et al. 2009, Goto et al. 2010, 2011, Oehl unpublished results). This discrepancy is due partly to the fact that in their attempt to include ontogeny in their analyses, Morton & Msiska considered also young, immature shields, which was not particularly helpful. Moreover, we believe that their isolates did not always derive from completely pure cultures but from oligospecies cultures — especially evident for *C. pellucida* where *Fuscutata savannicola*, *Dentiscutata scutata,* or similar species most probably co-existed in the cultures, which would invalidate the analyses and the conclusions drawn from those isolates. After investigating many specimens from several locations worldwide, we have never found brown shields in *C. pellucida*, nor have we found brown shields in the other five known *Cetraspora* spp. (e.g. Oehl et al. 2009a).

Cetraspora helvetica has been found thus far only in Switzerland. However, it was found in two different soil preservational agro-ecosystems —a no-till crop rotation system and two temporary grasslands that are rarely ploughed and characterized by long-interval (5–7 year) crop rotations dominated by 3–4 years of continued grass-clover production. It will be interesting to elucidate the biogeographical distribution of our new species in Switzerland and in the surrounding countries in more detail. This would be especially interesting in that the sporulation of *C. helvetica* appears to differ from that of *C. pellucida* and other sporogenous cell-forming arbuscular mycorrhizal fungi such as *S. calospora* and *G. margarita* that most commonly sporulate in late fall (e.g. Oehl et al. 2004, 2009b); in contrast, under more or less ambient light and temperature conditions, *C. helvetica* has formed spores only in early summer during our experiments (Oehl, unpublished).

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