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Septoglomus nigrum, a new arbuscular mycorrhizal fungus from France, Germany and Switzerland

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With 6 figures and 1 table

Abstract: A new arbuscular mycorrhizal fungus, *Septoglomus nigrum*, was found in several agricultural field sites in France, Germany and Switzerland, especially in extensively to intensively managed natural meadows and pastures and in extensively managed cropping systems. The fungus was propagated in trap pots and single species cultures on *Lolium perenne*, *Trifolium pratense*, *Plantago lanceolata* and *Hieracium pilosella*. It differentiates black spores with triple-layered walls, 95–175× 90–170 µm in diameter, formed singly in soils or rarely in roots. Phylogenetically, it forms a distinct clade close to *S. altomontanum* and *S. africanum*, which can morphologically be distinguished from spores of *S. nigrum* by the characteristics of the spore wall and by the color, size and shape of the subtending hyphae. An identification key is provided that differentiates all species so far described in *Septoglomus*.

Key words: farming systems; Europe, Glomeraceae; Glomerales; Glomeromycota; grasslands; soil tillage

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Introduction

In 2011, a new genus in the Glomeraceae, *Septoglomus* Sieverd. et al., was described based on concomitant spore morphological and molecular phylogenetic analyses (Oehl et al. 2011). Only a few species were transferred to the new genus, namely the type species *S. constrictum*, and *S. africanum*, *S. deserticola*, and *S. xanthium* (Trappe 1977, Trappe et al. 1984, Błaszkowski et al. 2004, 2010). During the following years, several new species emerged in the genus *Septoglomus* that were morphologically similar to the type species but phylogenetically different (Błaszkowski et al. 2013a, 2013b, 2014, Goto et al. 2013, Palenzuela et al. 2013, Symanczik et al. 2014).

Already in 2003, a black spored *Glomus* sp. strain BR3 resembling *Glomus constrictum* was mentioned in the literature (Oehl et al. 2003), which forms exclusively black spores singly in soils or in loose clusters, in between an enormously dense network of hyaline mycelia hyphae. We detected this fungus repeatedly also in other grass- and cropland sites in France, Germany and Switzerland. The objective of the present study was to describe this fungus as sp. nov. based on both, morphological and phylogenetic analyses and to evaluate its biogeographical distribution in Central Europe. An identification key is included that differentiates all *Septoglomus* species hitherto described.

Material and Methods

Study sites, soil sampling: During the last 18 years, soil samples were repeatedly taken in agricultural field sites in France, Germany and Switzerland. The sites comprise extensive meadows, and organic and conventional cropping sites of a wide range of soil pH, organic carbon and available P contents subjected to no-tillage, reduced tillage or conventional soil tillage, but with almost permanent plant cover throughout the years. The soil types ranged from Leptosols and Regosols to Cambisols and Luvisols. Samples were generally taken at 0–10 cm soil depth, as described in Oehl et al. (2003). Selected chemical soil characteristics were determined as also described in Oehl et al. (2003). A summary of selected isolation sites is given in Table 1.

AM fungal trap and monosporic cultures: AM fungal trap cultures and monosporic cultures were established as described in Oehl et al. (2003). The field soil for trap cultures from that the type was isolated, had been collected in Niederösch (Canton Bern, Switzerland). The cultures were established in April 2009 and maintained for two vegetation periods until December 2010 using *Hieracium pilosella* L., *Lolium perenne* L., *Plantago lanceolata* L. and *Trifolium pratense* L. as plant hosts in pot cultures. Thereafter the trap culture substrates were harvested and air-dried. In May 2011, spores of the new species were isolated from the substrates and inoculated for the establishment of monosporic cultures. Single spores were placed in several pipette tips (one mL) filled with Loess soil (Oehl et al. 2003, Tchabi et al. 2010). *Hieracium pilosella* was seeded on the surface of the pipette tip substrate, and exactly above each spore that was placed at about 5 mm depth with a Pasteur pipette (Oehl et al. 2003, Tchabi et al. 2010). The pipette tips were

placed in a rack, and the bottom of the tip reached into water so that the soil was watered by capillary forces of the soil. After 5 weeks, the pipette tips with the inoculated plant were directly transplanted into bigger pots (350 mL) filled with sterile soil substrate, after cutting the tips horizontally in two halves to ease the root growth from the pipette tips into the pots. The cultures were maintained for 14 months before checking for mycorrhizal root colonization and spore formation. In one of the initiated monosporic cultures, single spores, small spore clusters and sporocarps of different sizes, and mycorrhizal roots were found and analyzed applying morphological and molecular analyses. The successful cultures were deposited in the Swiss collection of arbuscular mycorrhizal fungi (SAF) at Agroscope in Zürich (Switzerland) under the accession number SAF86, among other cultures from the same and other farming sites (e.g. SAF47 from Vogtsburg, SAF84-85 from Niederösch, SAF136-138 from Frick, SAF171-175 from Rubigen).

Morphological analyses: Spores of the new fungus were separated from the soil samples, trap cultures and monosporic culture by a wet sieving process as described by Sieverding (1991). The described morphological spore characteristics and their subcellular structures are based on observations of specimens mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG; Koske & Tessier 1983), Melzer's reagent, in a mixture of PVLG and Melzer's reagent (Brundrett et al. 1994), a mixture of lactic acid to water at 1:1, and in water (Spain 1990). The terminology of the spore structure basically is that presented in Błaszkowski (2012) and Oehl et al. (2015) for species with glomoid spore formation. Photographs were taken with a digital camera (Leika DFC 295) on a compound microscope (Leitz Laborlux S) using Leica Application Suite Version V 4.1 software. Specimens mounted in PVLG and a (1:1) mixture of PVLG and Melzer's reagent were deposited at Z+ZT (ETH Zurich, Switzerland).

Molecular analyses: All the isolated spores, derived from the monosporic pure culture (see above) established on *H. pilosella*, were surface-sterilized (Mosse 1962) using chloramine T (2%), streptomycin (0.02%) and Tween 20 (2–5 drops in 25 mL final volume). Crude extracts were obtained by crushing 5–6 spores from a loose spore clusters with a sterile disposable micropestle in 23 µL milli-Q water, as described by Palenzuela et al. (2013). Direct PCR of these crude extracts were performed in an automated thermal cycler (Gene Amp PCR System 2400, Perkin-Elmer, Foster City, CA, USA) with a pureTaq Ready-To-Go PCR Bead (Amersham Biosciences Europe GmbH, Germany) following manufacturer's instructions with 0.4 µM concentration of each primer. A two-step PCR was conducted to amplify the ribosomal fragment consisting of partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA using the primers SSUmAf/LSUmAr and SSUmCf/LSUmBr consecutively according to Krüger et al. (2009). PCR products from the second round of amplifications (~1500 bp) were separated electrophoretically on 1.2% agarose gels, stained with Gel Red™ (Biotium Inc., Hayward, CA, USA) and viewed by UV illumination. The band of the expected size was excised with a scalpel and isolated from the gel with the QIAEX II Gel Extraction kit (QIAGEN, USA) following the manufacturer's protocol, cloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA, USA), and transformed into One Shot® TOP10 chemically competent *Escherichia coli* (Invitrogen, Carlsbad, CA, USA). Recombinant colonies were selected by blue/white screening and

the presence of inserts detected by PCR amplification with GoTaq® *Green* Master Mix (Promega) using universal forward and reverse M13 vector primers. After isolation from transformed cells, plasmids were sequenced on both strands with M13R/T7 primers using the BigDye Terminator kit 3.1v (Applied Biosystems). The products were analyzed on an automated DNA sequencer (Perkin-Elmer ABI Prism 373). Just the partial LSU rDNA fragment was sequenced satisfactorily. Sequence data were compared to gene libraries (EMBL and GenBank) using BLAST (Altschul et al. 1990). The new sequences were deposited in the EMBL database under the accession numbers MK234700 and MK234701.

Phylogenetic analyses: The AM fungal sequences (partial LSU of the rDNA) obtained were aligned with other related glomeromycotan sequences from GenBank in ClustalX (Larkin et al. 2007). *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler was included as outgroup. Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (Milne et al. 2004). Bayesian (two runs over 2×10^6 generations, with a sample frequency of 200 and a burnin value of 25%) and maximum likelihood (1,000 bootstrap) analyses were performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), respectively, launched from Topali 2.5, using the GTR + G model.

Results

Septoglomus nigrum Oehl, Sánchez-Castro, Palenz. & G.A. Silva (Figs 1–5)

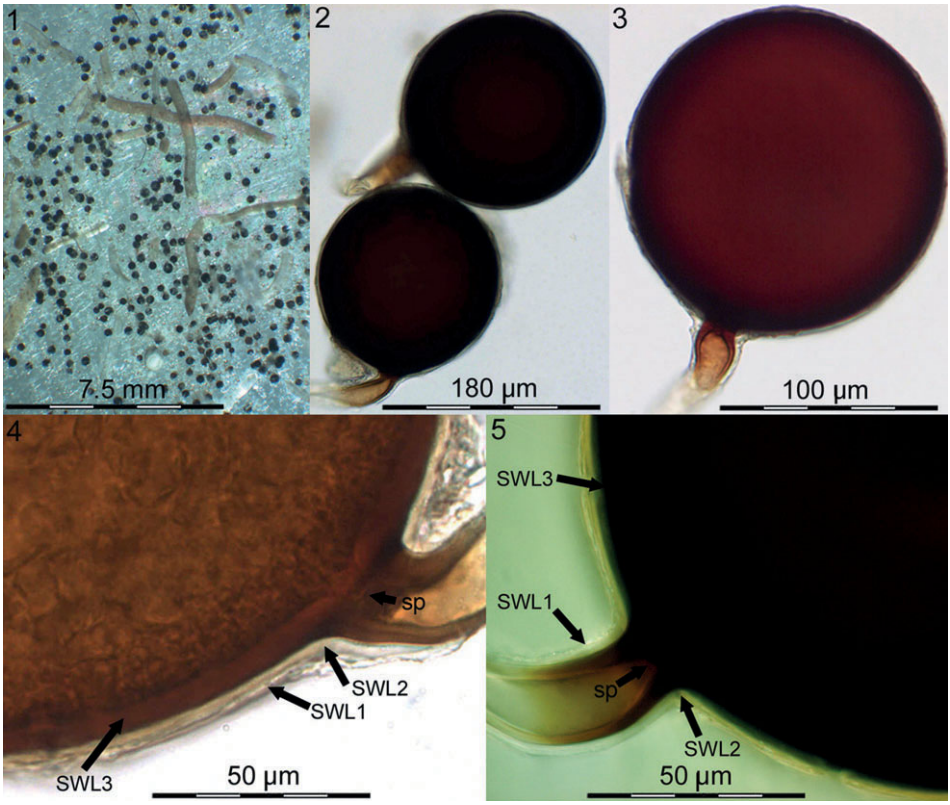
MycoBank MB 828735

Etymology: *nigrum* referring to the black spores of the new species, found both in field soils and in trap and single species cultures, when observed under stereo-microscopes.

Diagnosis: Differing from *Septoglomus constrictum* in forming black, triple layered and smaller spores (90–175 µm) instead of dark brown to brown black spores with bi-layered walls, and generally larger than 175 µm in diam.

Type: Holotype, deposited at Z+ZT (accession ZT Myc 59679), derived from a monosporic culture established on the host plant *Hieracium pilosella* in the greenhouse of the Swiss collection for Arbuscular mycorrhizal fungi (SAF) at the Institute for Sustainability Sciences, Agroscope, in Zürich, Switzerland. Spore for the culture originated from an extensively managed grassland site in Niederösch (Kanton Bern, Switzerland; 47°06'56''N; 7°36'32''E). Collector was F. Oehl and collection date 15.6.2012. Isotypes (ZT Myc 59680) and paratypes from other sites in France, Germany and Switzerland were also deposited at Z+ZT. Living cultures of the fungus are currently maintained at SAF under the accession numbers SAF86 and SAF175.

Description: Spores formed terminally on subtending hyphae (SH) either singly, or in small, very loose spore clusters within an extensive net of extraradical mycelial hyphae. They are white to coffee brown when young and developing, but are dark brownish black



Figs 1–5. *Septoglomus nigrum* 1. The dark spores of *S. nigrum* are formed in trap cultures frequently after > 1 year of cultivation. 2–3. Mature spores are regularly dark brown black to black. 4. Segment of a young, developing spore becoming coffee-brown, while formation of a septum at spore base has started. Spore wall with three layers (SWL1–3). 5. Mature, black spore of *S. nigrum*. Rarely all three spore wall layers are present on mature spores, as SWL1 is evanescent and short-lived, while SWL2 is semi-persistent, but might also lack completely in older spores.

to generally completely black when mature, globose to subglobose, (95–)105–165(–175) × (90–)100–155(–170) µm.

Spore wall (SW) triple-layered with a hyaline to subhyaline, evanescent outer layer (SWL1), 0.8–1.8 µm thick and a subhyaline to light brown, semi-persistent second layer (SWL2), 1.1–2.1 µm thick. Innermost, structural layer (SWL3) finely laminate, dark brownish black to generally black, 3.8–7.1 µm thick. Sometimes, SWL1 stains pinkish to purple in Melzer's reagent.

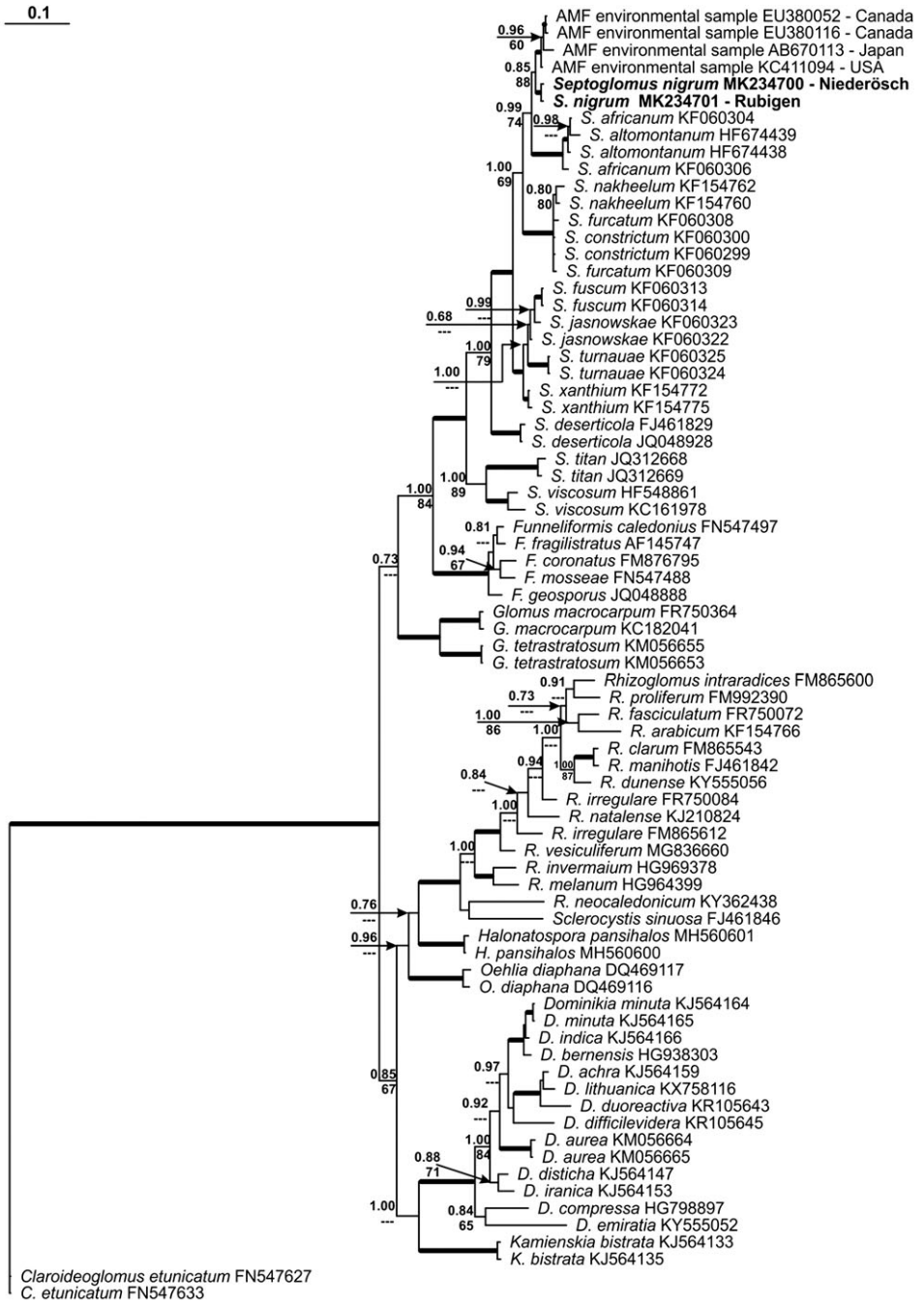
Subtending hyphae (SH) of spores often recurved to rarely straight, constricted to rarely cylindrical, 11.0–17.3 µm broad and 15–70 µm long, with a wall thickening toward the spore base, generally forming a plug-like, straight septum at the spore base, 2.0–5.1 µm

Table 1 Isolation sites of *Septoglomus nigrum* in France, Germany and Switzerland, with geographical data, soil pH, organic Carbon and available P.

Community, province/ country	Geographical position	Land use	pH (H_2O)	Corg (g kg^{-1})	Available P (P-DL; mg kg^{-1})
Leymen, Alsace/France	47°30'28" N; 7°28'27" E	Extensively managed meadow	8.0	45.8	8.4
Wintzenheim, Alsace/ France	48°03'27" N; 7°14'19" E	Extensively managed meadow	5.7	38.2	41.9
Vogtsburg, Baden/ Germany	48°05'17" N; 7°41'29" E	Extensively managed meadow	7.7	38.9	5.7
Therwil, Basel Land/ Switzerland	47°29'33" N; 7°31'40" E	Extensively managed meadow	7.5	36.5	8.4
Frick, Aargau/Switzerland	47°30'38" N; 8°01'25" E	Extensively managed meadow	7.8	31.8	32.5
Niederösch, Bern/ Switzerland	47°06'56" N; 7°36'32" E	Extensively managed meadow	5.8	18.4	23.1
Rubigen, Bern/ Switzerland	46°54'08" N; 7°32'44" E	Extensively managed meadow	6.0	20.2	49.9
Läufelfingen, Basel Land/Switzerland	47°24'05" N; 7°51'23" E	Extensively managed meadow	7.6	47.6	95.2
Chur, Graubünden/ Switzerland	46°52'44" N; 9°30'50" E	Extensively managed pasture	5.5	40.0	6.0
Therwil, Basel Land/ Switzerland	47°30'09" N; 7°32'22" E	Organic farming, tillage	6.4	15.8	7.9
Frick, Aargau/Switzerland	47°30'42" N; 8°01'25" E	Organic farming, reduced-tillage	7.6	24.54	149.3
Niederösch, Bern/ Switzerland	47°06'54" N; 7°36'33" E	Conv. farming, tillage	6.0	13.4	60.9

thick. Pore (and the closing septum) at base 6.2–9.1 µm broad. SH wall triple-layered, as continuous with the triple-layered spore wall. Outer wall layer usually evanescent and often missing in fully developed spores, second layer 1.2–1.8 µm, and third layer tapering from 4.0–7.5 to 1.8–3.5 µm within 50 µm distance to the spore base.

Fig. 6. Phylogenetic tree of the Glomeraceae obtained by analysis from partial LSU sequences of different Glomeraceae spp. Sequences are labeled with their database accession numbers. Support values (from top) are from Bayesian inference (BI) and maximum likelihood (ML), respectively. Sequences obtained in this study are in boldface. Only support values of at least 65% are shown. Thick branches represent clades with more than 95% of support in all analyses. The tree was rooted by *Claroideoglomus etunicatum.*


0.1
AMF environmental sample EU380052 - Canada
AMF environmental sample EU380116 - Canada
AMF environmental sample AB670113 - Japan
AMF environmental sample KC411094 - USA
Septoglomus nigrum MK234700 - Niederösch
S. nigrum MK234701 - Rubigen
S. africanum KF060304
S. altomontanum HF674439
S. altomontanum HF674438
S. africanum KF060306
S. nakheelum KF154762
S. nakheelum KF154760
S. furcatum KF060308
S. constrictum KF060300
S. constrictum KF060299
S. furcatum KF060309
S. fuscum KF060313
S. fuscum KF060314
S. jasnowskae KF060323
S. jasnowskae KF060322
S. turnauae KF060325
S. turnauae KF060324
S. xanthium KF154772
S. xanthium KF154775
S. deserticola FJ461829
S. deserticola JQ048928
S. titan JQ312668
S. titan JQ312669
S. viscosum HF548861
S. viscosum KC161978
Funneliformis caledonius FN547497
F. fragilistratus AF145747
F. coronatus FM876795
F. mosseae FN547488
F. geosporus JQ048888
Glomus macrocarpum FR750364
G. macrocarpum KC182041
G. tetrastratosum KM056655
G. tetrastratosum KM056653
Rhizoglomus intraradices FM865600
R. proliferum FM992390
R. fasciculatum FR750072
R. arabicum KF154766
R. clarum FM865543
R. manihotis FJ461842
R. dunense KY555056
R. irregulare FR750084
R. natalense KJ210824
R. irregulare FM865612
R. vesiculiferum MG836660
R. invermaium HG969378
R. melanum HG964399
R. neocaledonicum KY362438
Sclerocystis sinuosa FJ461846
Halonatospora pansihalos MH560601
H. pansihalos MH560600
Oehlia diaphana DQ469117
O. diaphana DQ469116
Dominikia minuta KJ564164
D. minuta KJ564165
D. indica KJ564166
D. bernensis HG938303
D. achra KJ564159
D. lithuanica KX758116
D. duoreactiva KR105643
D. difficilevidera KR105645
D. aurea KM056664
D. aurea KM056665
D. disticha KJ564147
D. iranica KJ564153
D. compressa HG798897
D. emiratia KY555052
Kamienskia bistrata KJ564133
K. bistrata KJ564135
Claroideoglomus etunicatum FN547627
C. etunicatum FN547633

Mycorrhiza formation: forming AM associations with *H. pilosella*, *L. perenne*, *P. lanceolata* and *T. pratense* as plant hosts in pot cultures. The mycorrhizal structures consist of arbuscules, vesicles, and intra- and extraradical hyphae and stain dark blue in 0.05% trypan blue.

Distribution: A summary of locations where the new species were collected is given in Table 1. The fungus seems to be frequently occurring in Central Europe, with grasslands, and no-tillage to reduced tillage or other low-input cropping systems as preferred habitats when compared to intensively managed, periodically ploughed habitats (Oehl et al. 2003; Table 1).

Molecular analyses: Phylogenetic analyses of the partial LSU rDNA reveal that the sequences of the new species group with environmental sequences obtained from soil (KC411094) and roots from green needle grass (EU380052), switch grass (EU380116), and *Ixeris repens* (AB670113). The nearest species phylogenetically to *S. nigrum* are *S. altomontanum* and *S. africanum* (Fig. 6).

Key to *Septoglomus* species:

Septoglomus species generally form spores in loose clusters or singly in soils, rarely in roots (Oehl et al. 2011). Most of the species, if not all, regularly form a septum at the spore base, or at a short distance to the base in the subtending hyphe (SH).

1 Spores generally < 150 µm 2

1 Spores generally > 150 µm 3

2 Spores with 2 wall layers 4

2 Spores > 2 wall layers 8

3 Spores with 2 wall layers 12

3 Spores with 3 wall layers . . . . . ***Septoglomus titan*** B.T. Goto & G.A. Silva

Spores yellow brown to orange brown, to dark red brown at maturity, (225–)245–285 × 308–325 (–400) µm. SW in total 15.3–25.6 µm thick. SWL1 sub-hyaline to light yellow, 0.5–1.4 µm; SWL2 yellow brown to orange brown, 2.5–5.1 µm; darker than SWL3 in young spores; SWL3 orange brown to dark red brown, 12.8–19.2 µm thick. No staining in Melzer's reagent. SH cylindrical to constricted, light yellow to orange brown, 17.9–33.2 µm wide at spore base. SH wall 7.6–8.9 (–10.2) µm thick at spore base, tapering to approximately 2.5–5.2 µm at 100 µm distance; bridging septum arising from SWL3

4 Spores light yellow to brownish yellow 5

4 Spores light brown to dark brown 6

5 Spores pale yellow to brownish yellow; (60–)80–110(–125) × (60–)90–140 µm . . . . . ***Septoglomus africanum*** (Błaszk. & Kovács) Sieverd. et al.

SWL1 semipermanent to evanescent, hyaline, (1.5–)3.1(–8.6) µm thick; in young and freshly matured spores, the upper surface of this layer usually covered with irregular blister-like outgrowths, rarely is smooth. SWL2 pale yellow to brownish yellow, (1.0–)1.7(–2.7) µm thick. Layers do not stain in Melzer's. Subtending hyphae pale yellow to brownish yellow; flared to slightly funnel-shaped, sometimes slightly constricted at the spore base; (3.7–)5.7(–9.3) µm wide at the spore base. SH wall pale yellow to brownish yellow, (2.9–)4.4(–5.4) µm thick at spore base. Pore (1.0–)2.1 (–2.9) µm in diam, often occluded by a curved septum formed by SWL2.

5 Spores pale yellow to brownish yellow; 28–42(–98) ×31–60(–98) µm . . . . . ***Septoglomus jasnowskae*** Błaszk. et al.

SWL1 mucilaginous, hyaline, (0.8–)1.2(–2.0) µm thick, usually frequently completely sloughed in older specimens; in young and freshly matured specimens often swelling and then separating up to 15.5 µm from SWL2, when mounted in PVLG. SWL2 laminate, smooth, pale yellow to brownish yellow, (1.3–)2.4(–5.0) µm thick. SWL1 stains purplish pink to deep Magenta in Melzer's reagent. SH pale yellow to brownish yellow, cylindrical to funnel-shaped, sometimes slightly constricted at the spore base; (3.3–)6.8(–11.0) µm wide at the spore base. SH wall pale yellow to brownish yellow; (1.0–)1.8(–3.5) µm thick at the spore base. Pore (1.0–)3.3(–6.8) µm diam, open or closed by a curved septum arising from SWL2; septum up to 9.0–14.0 µm below the spore base.

6 Spores deep yellow or range to light brown 7

6 Spores brownish orange to dark brown, 21–50(–90) × 23–60(–90) µm . . . . . ***Septoglomus fuscum*** Błaszk. et al.

SWL1 semi-persistent, orange white to golden yellow, rarely hyaline, (1.0–)1.6(–2.5) µm thick, rarely partly deteriorated in mature and older spores, usually easily separating from SWL2 in crushed spores. SWL2 brownish orange to dark brown, (2.0–)3.8(–7.0) µm thick. Layers do not stain in Melzer's. SH brownish orange to dark brown, cylindrical to funnel-shaped, sometimes slightly constricted; (5.5–)7.4(–13.6) µm wide at the spore base. SH wall brownish orange to dark brown, (1.0–)2.9(–6.2) µm thick at the spore base, continuous with spore wall layers 1 and 2. Pore (1.2–)1.7(–2.4) µm diam, with age gradually narrowing due to thickening of wall SWL2 of the subtending hypha toward the center of its lumen.

7 Spores (deep yellow to light) brown, (50–)59–71 × (50–)72–155 µm . . . . . ***Septoglomus deserticola*** (Trappe et al.) G.A. Silva et al.

SWL1 evanescent, hyaline, (0.5–)1.3(–2.3) µm thick. SWL2 deep yellow to light brown, (1.5–)2.0–2.5(–4.0) µm thick, frequently thickened at the spore base to form a collar. SW does not stain in Melzer's. SH deep yellow to light brown, flared, funnel-shaped, rarely constricted, (6.3–)8.1–13.3(–16.8) µm wide at the spore base. SH wall with L1 (0.8–)1.0(–1.3) µm thick, and L2 (2.5–) 3.2(–3.8) µm thick. Pore occasionally open, but often closed by a membranous septum arising from SWL2.

7 Spores pale orange to brown, 52(–70) µm diam; sometimes ovoid to irregular; (25–)45–70(–140) × (25–)50–160 µm . . . . . ***Septoglomus nakheelum*** Al-Yahya'ei et al.

SWL1 semi-permanent, light yellow, (0.8–)1.4(–2.0) µm thick, slowly decomposing with time; in young and freshly matured spores the upper surface of this layer is covered frequently with small, local thickenings, (0.8–)1.1(–1.5) µm high, rarely is smooth. SWL2 grayish yellow to brown, (1.0–) 1.7(–3.5) µm thick. SW does not stain in Melzer's. SH pale orange to brown, flared to funnel-shaped, sometimes slightly constricted; (4.8–)7.2(–8.2) µm wide at the spore base. SH wall pale orange to brown; (1.8–)2.7(–4.0) µm thick at the spore base; pore (0.8–)2.6(–5.0) µm diam, often

closed by a curved septum arising from SWL 2; septum positioned up to 6.0 µm below the spore base.

8 Spores with three spore wall layers 9

8 Spores with four spore wall layers. . . . . ***Septoglomus turnauae*** Błaszk. et al.

Spores brownish orange to dark brown, singly formed in soil, (110–)115–135×125–140(–165) µm. SWL1 evanescent, hyaline, (1.0–)3.1(–5.0) µm thick. SWL2 permanent, light orange to brownish orange, (2.0–)5.1(–8.8) µm thick. SWL3 light orange to brownish orange, (3.8–)5.6(–9.0) µm thick. SWL4 semi-flexible, smooth, light orange to brownish orange, (0.8–)1.1(–1.4) µm thick. SWL1–4 do not stain in Melzer's. SH brownish orange to dark brown; cylindrical, sometimes slightly funnel-shaped or slightly constricted; (12.2–)16.7(–21.0) µm wide at the spore base. SH wall brownish orange to dark brown; (5.0–)7.7(–11.0) µm thick at the spore base; pore usually closed by a septum arising from SWL3–4. Pore (1.5–)2.2(–3.0) µm diam, narrowing with spore age due to thickening of SWL3.

9 Spores hyaline, white to yellow or orange 10

9 Spores brown to black 11

10 Spores hyaline to white, 44–97 × 46–94 µm . . . . . ***Septoglomus viscosum*** (T.H. Nicolson) C. Walker et al.

SWL1 semi-persistent, mucilaginous, viscose with age, up to 1 µm thick, with age becoming mucilaginous; SWL2 persistent, laminate, 1.0–3.0 µm thick, SWL3, semi-flexible, up to 1.0 µm thick. SH straight, cylindrical, persistent, concolorous with SW; pore regularly closed at spore base or at up to 10 µm distance from spore base by a straight septum.

10 Spores deep orange, to golden yellow, (70–)80–120 × (70–)110–150 µm . . . . . .***Septoglomus xanthium*** (Błaszk. et al.) Silva et al.

SWL1 permanent, flexible to semiflexible, hyaline, (0.7–)1.0(–1.5) µm thick, sometimes ballooning, and then extending up to 30 µm in PVLG. SWL2 yellowish white to golden yellow, (2.7–)5.8 (–8.8) µm thick. SWL3 flexible, smooth, hyaline, (0.5–)0.7(–1.3) µm thick. Layers do not stain in Melzer's. SH yellowish white to golden yellow, cylindrical or slightly flared, rarely constricted; (3.2–)7.1(–11.8) µm wide at the spore base. Pore occluded by a septum, (2.0–)2.9(–4.2) µm wide, continuous with SWL3 and occasionally also by a septum formed by SWL2, positioned up to 3.0–6.5 µm below the spore base.

11 Spores formed singly in soil, reddish brown to dark brown, (106–)108–127(–167) × (106–)135–167 µm . . . . . ***Septoglomus furcatum*** Błaszk. et al.

Spores rarely with two subtending hypha, located close together (within a maximum of 28 µm). SWL1 semi-permanent, hyaline to light orange, 1.3–2.5 µm thick, SWL2 semi-permanent, hyaline to golden yellow, (1.3–)1.9(–3.3) µm thick, SWL3 reddish brown to dark brown, 5.3–11.3 µm thick. Layers do not stain in Melzer's reagent. SH reddish brown to dark brown, cylindrical to slightly funnel-shaped, sometimes slightly constricted; (10.5–)17.9(–30.3) µm wide at the spore base; frequently with a forked branch formed 16–70 µm below the spore base. SH wall reddish brown to dark brown; (6.0–) 8.2(–12.0) µm thick at the spore base; continuous with SWL1–3, which extend far below the spore base. Pore (3.5–)4.3(–6.5) µm diam, narrowing with spore age due to thickening of wall layer 3 of the subtending hypha toward the center of its lumen, usually closed by a straight or curved septum, 2.0–3.8 µm thick, continuous with SWL3.

11 Spores dark brownish black to black, (95–)105–155(–175) × (90–)100–155(–170) µm . . . . . ***Septoglomus nigrum*** Oehl et al.

SWL1 hyaline to subhyaline, evanescent, 0.8–1.8 µm thick, SWL2 subhyaline to light brown, semi-persistent, 1.1–2.1 µm thick. Inner, structural layer (SWL3) dark brownish black to black, 3.8–7.1 µm thick. Sometimes, SWL1 stains pinkish to purple in Melzer's reagent. SH constricted to rarely cylindrical, 11.0–17.3 µm broad and 15–70 µm long, with a wall thickening toward the spore base, generally forming a plug-like, straight septum at the spore base, 2.0–5.1 µm thick. Pore (and the closing septum) at base 7.2–9.1 µm broad.

12 Spores brown to black brown, 150–330 µm . . . . . ***Septoglomus constrictum*** (Trappe) Sieverd. et al.

SWL1 hyaline to subhyaline, evanescent, 1.5–2.5 µm thick, SWL2 dark brown, 7–15 µm, persistent; hyphae regularly constricted at the spore base (10–22 µm at base, 15–30 µm in some distance to the base).

12 Spores dark reddish brown to reddish black, 137–175(–208) × 125–170(–204) µm, ... ***Septoglomus altomontanum*** Palenz. et al.

SWL1 subhyaline to dark yellow, 2.5–3.0 µm thick; SWL2 dark reddish brown to reddish black, 4.0–8.0 µm thick; SW not staining in Melzer's. SH regularly slightly lighter in color (dark yellow-brown to reddish brown) than the spores, cylindrical to sometimes somewhat funnel-shaped, often curved; often widest at the spore base and 20–35 µm from the spores, and then (15–)20–25(–31) µm wide; L1 of SH wall 2.0–3.0 µm, and L2 4.0–7.5 µm thick at the spore base, tapering to 0.5–1.0 and 1.5–2.5 µm towards the hyaline hyphal wall of the mycelia. Spore pore generally closed by a broad bridging septum arising from SWL2 at a short distance from the spore base.

Discussion

Septoglomus nigrum can easily be distinguished from all other species in the genus by the combination of spore color, size and the structure of the spore and subtending hyphal walls. The morphologically most similar species are *S. constrictum* (Trappe 1977) and *S. altomontanum* (Palenzuela et al. 2013). However, *S. constrictum* has larger (150–330 µm), brown to black brown spores, and *S. altomontanum* has stronger, cylindrical to funnel-shaped subtending hyphae, which are regularly slightly lighter in colour (dark yellow-brown to reddish brown) than the spores. Spores and subtending hyphae of *S. nigrum* are generally completely black, and the subtending hyphae at spore base are regularly constricted to rarely cylindrical. Phylogenetically, *S. nigrum* is closest to *S. altomontanum* and *S. africanum*, but rather distant to *S. constrictum*.

In the old genus *Glomus*, there are several other species whose dark brown to black spores might resemble *S. nigrum*, such as *Glomus ambisporum* and *Glomus tenebrosum* (Oehl et al. 2011). However, both these species usually form spores in sporocarps, which is not known for *Septoglomus* species. Moreover, *G. tenebrosum* forms larger spores (200–270 µm; Berch and Fortin 1983) than *S. nigrum*, and *G. ambisporum* forms triple-layered spores with a dark brown to black, laminate middle layer, and a membranous, thin inner-

most layer (Smith and Schenck 1985), while in *S. nigrum* the middle layer is subhyaline to light brown, semi-persistent, and the innermost layer is black and laminate.

Some *Septoglomus* spp. are not totally separated in the tree (*S. nakheelum*, *S. furcatum*, *S. constrictum* and some others). The LSU rDNA is known to be a good marker to identify species and classify them from genera to phyla (Moore et al. 2011), but in Glomeromycota a more variable region (ITS) produce better results to separate species. Unfortunately, it was not possible to obtain the ITS rDNA fragment of *S. nigrum*, but even with a more conserved marker (partial LSU rDNA), we demonstrated phylogenetically that this fungus is a new taxon.

The new species is quite frequent in Central European agricultural sites, such as extensively to intensively managed meadows and pastures, or cropping systems with plant cover almost all year long. The species is sensitive to periodic soil cultivation and when soils are left bare over months (Oehl et al. 2003), but seems to be relatively insensitive to high fertilization or nutrient availability levels (Table 1). Currently, it is difficult to evaluate the biogeographical distribution of *S. nigrum* on larger scales, concerning e.g. soil, vegetation, or climatic gradients, as in the past the new species, and several other *Septoglomus* spp. might have been often confused with *S. constrictum*. On the other hand, there was a fast progress on the taxonomy and systematic classification of this genus in the last decade (e.g. Błaszkowski et al. 2013a, b, 2014). This will certainly lead us to a better understanding of the geographical distribution and the functional importance of this genus, its type species *S. constrictum* and all the other members, including *S. nigrum*, in natural and man-made ecosystems around the globe.

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