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ORIGINAL ARTICLE

New aspects of the biology of *Mortierella alliacea*

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Abstract A fungal genotype related to *Mortierella alliacea* was detected by environmental PCR in samples of the mite species *Leptogamasus obesus* and *Oppiella subpectinata*, as well as in the soil body. The association may be epi- or endozoic. A genotypically corresponding strain was newly isolated from mites and examined to gain deeper insight into the biology of the scarcely known fungal species. Morphological and physiological traits, as well as molecular data confirmed an affiliation to *Mortierella alliacea* Linnemann. However, the strain showed minor morphological differences to the original description of *M. alliacea* (selected here as lectotype) and to the isolate CBS 894.68 (selected here as epitype) with regard to sporocystospore and chlamydospore morphology. Therefore, an emended species description is provided. The psychrotolerant fungus is able to grow at temperatures between 0 and 25 °C. Chitin degradation was not observed, and it lacked the capability to degrade starch, cellulose, lignin, and lipids. Proteolytic activity was only exhibited at 4 °C. Co-incubated mites were not affected by the fungus, indicating that the mites predominantly serve as vectors. The fungus' limited degradation capabilities suggest that it predominantly lives on readily accessible carbohydrates in soil.

Keywords Fungi · Mites · ITS rRNA gene · Lectotypification · *Leptogamasus obesus* · *Oppiella subpectinata*

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Introduction

The species of *Mortierella* (Mortierellaceae, Mortierellales, Mortierellomycotina, basal fungal lineages) are widely distributed and common inhabitants of soil, where they preferably colonize plant and animal debris (Gams 1977; Domsch et al. 2007). Molecular surveys obtained numerous sequences of the genus and these have accumulated in the international nucleotide sequence databases (www.insdc.org). The vast majority of these sequences, however, have not been assigned at species level for two reasons. First, type cultures and DNA sequences are lacking for many formally described taxa (Nagy et al. 2011). Second, the phylogenetic concept within Mortierellales is still rather inconsistent and controversially discussed. This makes a precise assignment of molecular data at species level rather difficult. As a consequence, discussions of mortierellalean fungi were frequently restricted to genus level and species-related traits are often neglected.

Little attention was paid to the ecological traits of *Mortierella* spp. in particular, because the species are generally considered to be saprotrophic soil inhabitants playing a minor role in soil metabolism (Domsch et al. 2007). However, some symbiotic interactions with other organisms have been reported. Best known are biology and life cycle of *M. wolfii*, infecting mammal tissues (e.g., Munday et al. 2006; Davies et al. 2010; Wada et al. 2011). In addition, facultative parasitism on water moulds was observed for *M. alpina* (Willoughby 1988). On the other hand, *Mortierella hypsicladia* itself is selectively attacked by the mycoparasite *Nothadelphia mortierellicola* (Degawa and Gams 2004). Associations of *Mortierella* with plants were occasionally reported from roots (Jiang et al. 2011), leaves (Peršoh 2013) and seeds (Ochora et al. 2001).

While these observations already indicate a more diverse ecological role of *Mortierella* spp. than usually assumed, the

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present study provides first insights into the biology of *M. alliacea*. In an ongoing molecular environmental study of soil mycota, we screened soil-inhabiting mites for associated fungi. We found several fungal genotypes assignable to different *Mortierella* species, one of which was *M. alliacea*. A newly isolated strain of this species is described by morphological, physiological and phylogenetic traits, which are compared to the phenotypic and genotypic characters of closely related strains.

Materials & methods

Sampling site and sample collection

The investigation site of about 4 m^2 in size is located in a mixed forest with predominant *Pinus sylvestris* on Hohe Warte hill close to the city of Bayreuth, Upper Franconia, Germany (49°58′16″ N, 11°34′51″ E, 460 m alt.). The understory is dominated by *Vaccinium myrtillus*, *Calluna vulgaris*, and two bryophyte species (*Polytrichum formosum* and *Pleurozium schreberi*). The soil can be characterized as a podsolized ranker being covered by an organic layer of 5–10 cm in depth.

Over a period of 18 months, four replicates of soil cores (250 cm^3 each) were monthly sampled from the uppermost 5 cm of the organic Oe layer, consisting of fragmented litter. The covering layer of fresh and undecomposed litter (Oi) was removed before. Three samples were used for mite extraction and a fourth to analyse the soil-inhabiting fungi. Mites were extracted by gradual heat treatment (Berlese 1905). Gamasid mites were identified using the key of Karg (1993), and oribatid mites were determined by F. Horak (Karlsruhe). Individuals of two frequent and ecologically divergent species, *Leptogamasus obesus* Holzmann (Gamasina) and *Oppiella subpectinata* Oudemans (Oribatida), were pooled into samples of five and ten, respectively, which served for DNA extraction (see Molecular analyses). The different sample sizes compensated for the different body sizes of the species and thus for the amount of potentially enclosed fungal material. Additional mites were extracted for the isolation of associated living fungi (see Fungal cultivation) and for experimental studies (see Exposure experiments). Springtails (Collembola) co-extracted with the mites served as food for *L. obesus* in the experimental approach.

Fungal cultivation

Mite individuals were washed in tubes containing sterile water using sterilized tweezers to remove adhering soil particles. To isolate internally and externally associated fungi, the mites were subsequently fragmented and dispersed on a Petri dish containing yeast malt agar (1 % malt extract, 0.4 % yeast extract, 0.4 % glucose, and 1.2 % agar) and antibiotics (0.1 % tetracycline added after autoclaving) to suppress bacterial growth. The agar plates were stored at 15 °C and screened daily. Emerging mycelia were removed and inoculated onto fresh agar plates. For light microscopic analyses, pure cultures were grown on soil extract agar (filtrate of a 1:1 mixture of soil and water, 3 % agar) under the same conditions. The strain morphologically and molecularly identified as *Mortierella alliacea* was deposited at Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (accession number: DSMZ 27011) and at Centraalbureau voor Schimmelcultures (CBS 134703). For the purpose of typification, a dried culture of CBS 894.68 (*Mortierella alliacea* Linnem.) on yeast malt agar was deposited at the Botanische Staatssammlung München (M-0276247).

Molecular analyses

DNA Isolation, amplification, and sequencing

Total DNA was extracted from mite samples (77 samples of *L. obesus* and 169 of *O. subpectinata*) and from fungal cultures using the Charge Switch® gDNA Plant Kit (Invitrogen) as recommended by the manufacturer, but using 0.2 μL tubes, and with all volumes reduced to 10 %. DNA from 18 soil samples (0.5 g) was isolated according to Peršoh et al. (2008). A part of the ITS rRNA gene (partial ITS*1*, *5.8S*, and partial ITS*2* region) was amplified in a total volume of 25 μL containing 0.1 μL Taq polymerase (5 U/μL, Invitrogen), 2.5 μL 10′ PCR buffer, 0.75 μL $MgCl_2$ (50 mM), 2.5 μL dNTP mix (2 mM) and 1.25 μL forward and reverse primers (10 μM) each, as well as 1 μL DNA extract. The newly designed primers fZy1V2 (5′- GCT GAC ATC GTC GTT CAC -3′) and rZy1V2 (5′- GCT CAA ATC CAA GTC TCT TAT GC -3′), both highly specific for DSMZ 27011 (*Mortierella alliacea*), were applied to selectively amplify fungal DNA. PCR products obtained from fungal cultures, as well as from mite and soil samples were sequenced using a Beckman-Coulter CEQ 8000 capillary sequencer. The ITS sequence of strain DSMZ 27011 was deposited in GenBank (www.ncbi.nlm.nih.gov) under accession number JX535352. Sequence and associated field data are also available in the Barcode of Life Data Systems (BOLD, www.barcodinglife.org, Ratnasingham and Hebert 2007) through the dataset DAFS.

Phylogenetic analysis

GenBank was searched for available ITS sequence data from verified strains of Mortierellales, mostly obtained in the course of phylogenetic studies (e.g., Hoffmann et al. 2011; Nagy et al. 2011; Schoch et al. 2012; Wagner et al. 2013). A

total of 277 sequences (90 different species comprising the genera *Dissophora*, *Gamsiella*, *Haplosporangium*, *Mortierella*, and *Umbelopsis*) were aligned together with the sequence of strain DSMZ 27011 (JX535352) and three identical sequences (GU324668, GU324669, GU324670) obtained from DNA extracts of mite samples in an ongoing study. Most likely phylogenetic trees were searched using the software package MEGA 5 (Tamura et al. 2011) and applying the GTR approximation of nucleotide substitution. To assess support of the respective branches, 1000 bootstrap replicates were calculated. Only unambiguously alignable positions (i.e. sequence parts bearing base patterns of recognizable similarity), corresponding to positions 22–42, 80–83, 104–118, 124–131, 133–297, 304–315, 335–345, and 347–353 of sequence JX535352 (strain DSMZ 27011), were included in the phylogenetic analyses. The whole alignment can be accessed from TreeBASE (http://treebase.org) under accession number 14593. A second phylogenetic analysis, comprising only the closest related sequences to JX535352, as revealed by the first calculation, included the positions 22–50, 52–97, 99–323, 326–358 (TreeBASE 14593).

Experimental approaches

Fungal growth rates

To assess temperature preferences, cultures of strain DSMZ 27011 were inoculated onto fresh yeast malt agar plates (see Fungal cultivation) and incubated in three replicates at 0, 4, 15, 20, and 25 °C, each. Photographs of the growing cultures were taken every 24 h for 10 days. To determine average growth rates, the diameters of the radiate colonies were measured with the software *ImageJ* (Rasband 1997–2008).

Substrate degradation

Degradation capability of strain DSMZ 27011 was tested for the following substrates: chitin, starch, cellulose, lignin, proteins, and lipids. Media including the corresponding reference substrates (Table 1) were prepared and modified according to Bradner et al. (1999), as well as Peterson et al. (2009) and inoculated with the fungus. Each of three replicates was incubated at 0, 4, 15, and 25 °C for 10 days. In case of chitin- and protein-enriched media clear zones around the fungal colonies should indicate successful degradation. Hyaline, i.e. colourless zones after staining were quantified for cellulose and starch media. Lignin degradation should be indicated by greenish blue colour shifts around the colonies after treatment. Crystalline coagulations were considered indicative for lipid degradation.

Exposure experiments

To assess the potential of strain DSMZ 27011 to infest living mites, 40 individuals of *L. obesus* were co-incubated with the fungus as described by Werner et al. (2012). The animals were checked daily for signs of fungal infection and dead individuals were immediately removed for microscopic analysis (see Light microscopic analyses). To test whether prey of *L. obesus* serves as vector, springtails were incubated for at least 2 days in the presence of the fungus and subsequently offered as food to *L. obesus*. Forty individuals of these mites were kept as described above and fed exclusively with springtails that were exposed to strain DSMZ 27011. An additional 40 mites served as control group and were not exposed to the fungus. Survival times of fungus-treated mites from the exposure experiments were compared to the life times of the control group using the nonparametric "Cox-Mantel test" implemented in STATISTICA *7* (StatSoft).

Light microscopic analyses

Mites from the exposure experiments had to be visually checked for fungal infestation. For this purpose, mites were briefly washed in a water drop to remove soil particles and transferred onto glass slides with a central spherical depression. The body surfaces were initially examined by light microscopy without further treatment. Subsequently, the cuticles were carefully perforated with an insect needle and the mite samples were stained with lactophenol cotton blue for a second examination. Culture material morphology of strain DSMZ 27011 and—for comparison—strain CBS 894.68 (oldest available strain assigned to *Mortierella alliacea* Linnemann 1953; collected 1958 by W. Gams) was examined under the microscope after staining with lactophenol cotton blue.

Results

Phylogenetic relationships

In the phylogenetic analysis comprising 277 sequences of verified *Mortierella* strains, the sequence of strain DSMZ 27011 (JX535352) and three corresponding sequences (GU324668, GU324669, GU324670), obtained from mite samples in the course of an ongoing study, clustered together with sequences belonging to *M. alliacea* (JX975990, JX976019), *M. pulchella* (JX976031, HQ630351, JX976082), *M. turficola* (HQ630350, JX975896, JX975919, JX975939, JX976040, JX976025), *M. fimbricystis* (GU559986), *M. elongatula* (HQ630349), and *M. cystojenkinii* (HQ630348) (79 % bootstrap support, Fig. 1). A second analysis (Fig. 2), restricted to these closely

Table 1 Composition of growth media and detection reagents used for fungal substrate degradation tests

Test substrate	Growth medium	Detection reagent
Chitin	Peptone 3.1 g; NaCl 1.9 g; colloidal chitin 2.5 g; agar 4.6 g; H_2O 400 mL	None
Starch	Peptone 3.1 g; NaCl 1.9 g; sol. starch 2.5 g; agar 4.6 g; H_2O 400 mL	Lugol's solution
Cellulose	Peptone 3.1 g; NaCl 1.9 g; carboxymethyl cellulose 2.5 g; agar 4.6 g; H_2O 400 mL	Congo red; 0,5 M HCl
Lignin	Yeast extract 1.6 g; malt extract 4.0 g; glucose 1.6 g; agar 4.8 g; H_2O 400 mL	K_2HPO_4 0.8 g; KCl 0.8 g; $MgSO_4 \cdot 7\ H_2O$ 0.8 g; agar 8.0 g; ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) 1 mM; H_2O 400 ml; pH 5
Peptides and protein	Nutrient broth 3.1 g Powdered milk 5.0 g; agar 4.6 g; H_2O 400 mL; pH 7.2	None
Lipid	Peptone 3.1 g; NaCl 1.9 g: Tween 80 4.0 mL; agar 4.6 g; H_2O 400 mL	None

related sequences, revealed strain DSMZ 27011 (JX535352) to form a well-supported (99 % bootstrap support) monophyletic group together with the other three sequences from mites (GU324668, GU324669, and GU324670) and two sequences belonging to *M. alliacea* (JX975990, JX976019).

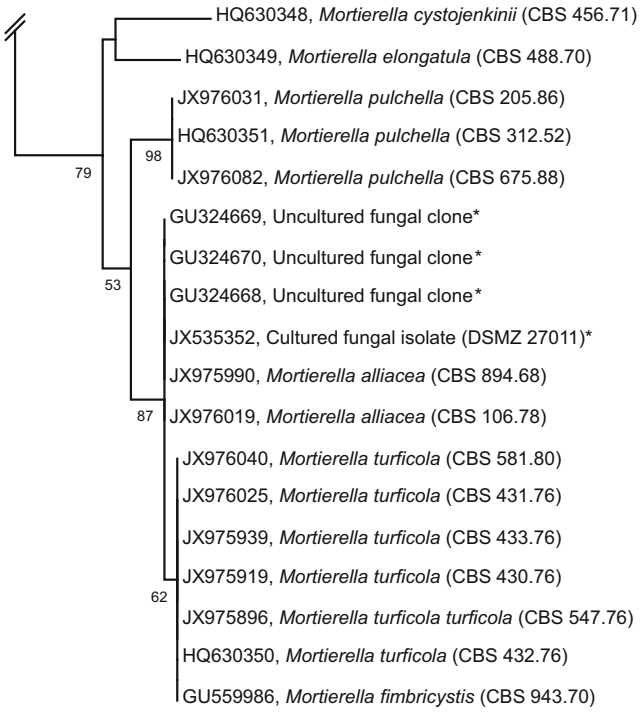

Fig. 1 Phylogenetic relationships among Mortierellales as inferred from ITS rRNA sequence data. Section ('//') around DSMZ 27011 of the most likely tree found by Mega 5, applying the GTR approximation of nucleotide substitution. Taxon names are preceded by the GenBank accession number. Support values from 1000 bootstrap replicates are noted below the respective branches. Sequences obtained in this study are marked with an *asterisk*

Morphology of strains DSMZ 27011 and CBS 894.68 (*M. alliacea*)

Growth of strain DSMZ 27011 was similar on yeast malt and soil extract agar at 15 °C. Initially, fungal hyphae expanded radially within the substrate without forming a noticeable aerial mycelium. The non-septate hyphae were comparatively thin and 1.5 to 5.0 μm in width (Fig. 3d). They showed mainly dichotomous to sympodial branchings. New hyphae were rapidly produced in the central area, growing radially in recognizable layers. After 3 days, aerial hyphae started to emerge from the older parts of the fungal colonies. On yeast malt agar, a white and dense cotton-like aerial mycelium developed (Fig. 3a). Arachnoid aerial hyphae (Fig. 3c) grew mostly straight and branched much less frequently than hyphae within the agar (Fig. 3d). All colonies began to emit a weak but noticeable garlic-like odour after 3 days. On soil extract agar, the development of the aerial mycelium was less intense, less pronounced and less dense. It also appeared more greyish in colour. Chlamydospores occurred first after 4 days on both media and were produced in high numbers in the following. Two types of chlamydospores were formed both intercalarily (Fig. 3b) and terminally (Fig. 3e): a smooth-walled type (Fig. 3b) and a fimbriate one with tubular appendages (Fig. 3e, h). All chlamydospores were globose to oval and elongate in shape. Smooth-walled chlamydospores measured between 28.5 and 31 μm diam., the fimbriate forms between 22 and 25 μm. The appendages were 1 to 2 μm wide and ranged from 16 to 19 μm in length. Young chlamydospores were nearly colourless and became slightly ochre-pigmented during maturation. Globose sporocysts, i.e. single-celled sporangia, occurred only sporadically after 2 weeks, but could be found regularly in old cultures (Fig. 3g). They were multisporic, colourless and measured between 9 and 19 μm diam, lacking a columella. Sporocystophores, i.e.

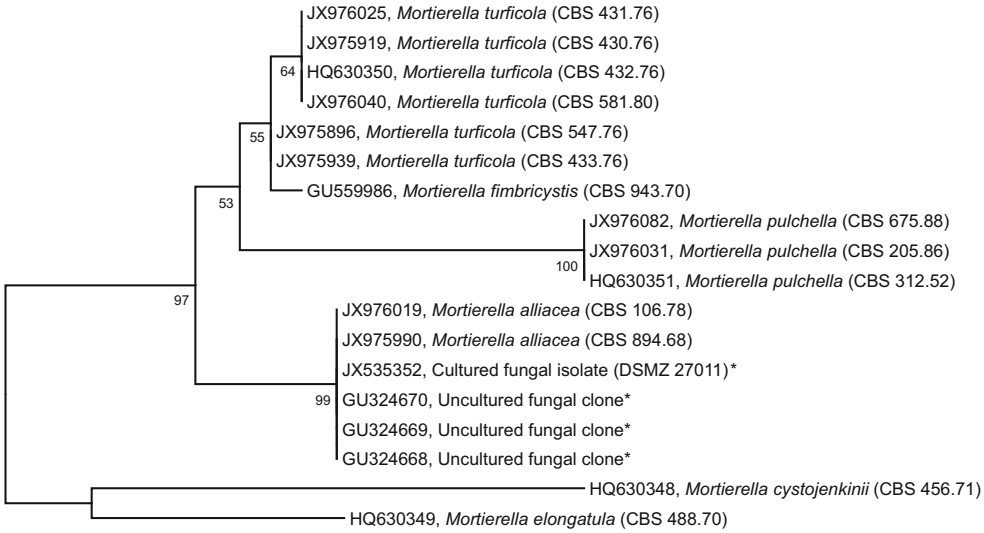

Fig. 2 Phylogenetic relationship among *Mortierella* spp. being closely related to strain DSMZ 27011 as inferred from ITS rRNA sequence data (cf. Fig. 1). Most likely tree found by Mega 5 applying the GTR approximation of nucleotide substitution. Taxon names are preceded by the GenBank accession number. Support values from 1000 bootstrap replicates are noted below the respective branches. Sequences obtained in this study are marked with an *asterisk*

sporangiophores, emerged from aerial mycelium, were often bent, up to 200 μm in length and narrowed from 3 to 5 μm at the base to a diameter of 1.5 μm directly below the sporangium. Rhizoids were absent. Sporocystospores (Fig. 3f) appeared smooth and ellipsoid, measuring 4.5 (3.5–6.0) μm in length and 3.0 (2.5–4.0) μm in width. A formation of zygospores was not observed.

Strain CBS 894.68 (*M. alliacea*) exhibited a quite similar morphology under identical growth conditions, except for two minor differences: First, its sporocystospores measuring 4.5 (3.5–6.0) × 2.5 (2.0–3.5) μm always appeared more elongate than those of DSMZ 27011. Second, only the fimbriate form of chlamydospores was observed, but not the smooth type.

Temperature dependence and degradation capabilities of strain DSMZ 27011

Strain DSMZ 27011 extended radially on average by 0.9 and 3.6 mm per day at 0 and 4 °C, respectively (Fig. 4). A white aerial mycelium of cotton-like appearance was formed after 10 days, but only at 4 °C, and then restricted to the central parts of the colonies. Reproductive structures (chlamydospores and sporocysts) were formed at 4 °C as described above, but with a delay of at least 1 week in comparison to higher temperatures. At 15 and 20 °C the colony diameter increased more rapidly, i.e. on average by 5.0 and 4.6 mm per day, respectively. The edges of the colonies were constantly smooth. Aerial hyphae were produced in high abundance and the dense mycelium appeared completely white and cotton-like after 8 days. All reproductive structures were formed as described above. At 25 °C the fungus grew initially 3.3 mm per day, but stagnated after 10 days. The aerial mycelium was comparatively sparse, lawn-like, and of slightly fawnish colour. Colony edges appeared irregular and frayed. Chlamydospores were sporadically formed, but sporangia remained completely absent.

Strain DSMZ 27011 did not degrade chitin, starch, cellulose, lignin, or lipids, regardless of the incubation temperatures (Table 2). Peptides and proteins were degraded only at 4 °C. It is surprising that *M. alliacea* could not degrade chitin. This may be due to absent growth factors (W. Gams, pers. comm).

Fungus-mite interactions

PCR with specific primers detected strain DSMZ 27011 in 26 out of 77 samples of *L. obesus* (34 %) collected at the forest site. From *O. subpectinata*, 54 out of 169 samples (32 %) were positive and nine out of 25 soil samples (36 %) yielded DSMZ 27011-specific PCR products. ITS sequences were identical for all sequenced PCR-products, regardless of their origin.

Fungus-exposed mites from the experiments ($n = 40$, mean survival time: 30 days) did not significantly differ from the control group ($n = 40$, mean survival time: 25 days) in their average life spans ($p = 0.491$, $t = -0.689$). Living mites of neither group showed signs of fungal infection, and dead mites were colonized only superficially. Mites fed fungus-exposed springtails survived slightly longer ($n = 40$, mean survival time: 36 days) than the control group, but the difference was not significant ($p = 0.162$, $t = 1.397$). Springtails grazing strain DSMZ 27011 colonies were also not affected by the fungus.

Taxonomy

Emended description of *M. alliacea*

Mortierella alliacea Linnem., Zentbl. Bakt. ParasitKde, Abt. II 107:225 (1953)

Lectotype (selected here): Original species description (p. 225–227) and drawings (figs. 1 and 2) in Linnemann (1953).

Epitype (selected here): M-0276247, dried culture of CBS 894.68, 1958 Tirol (Obergurgl, alpine raw humus soil), W. Gams.

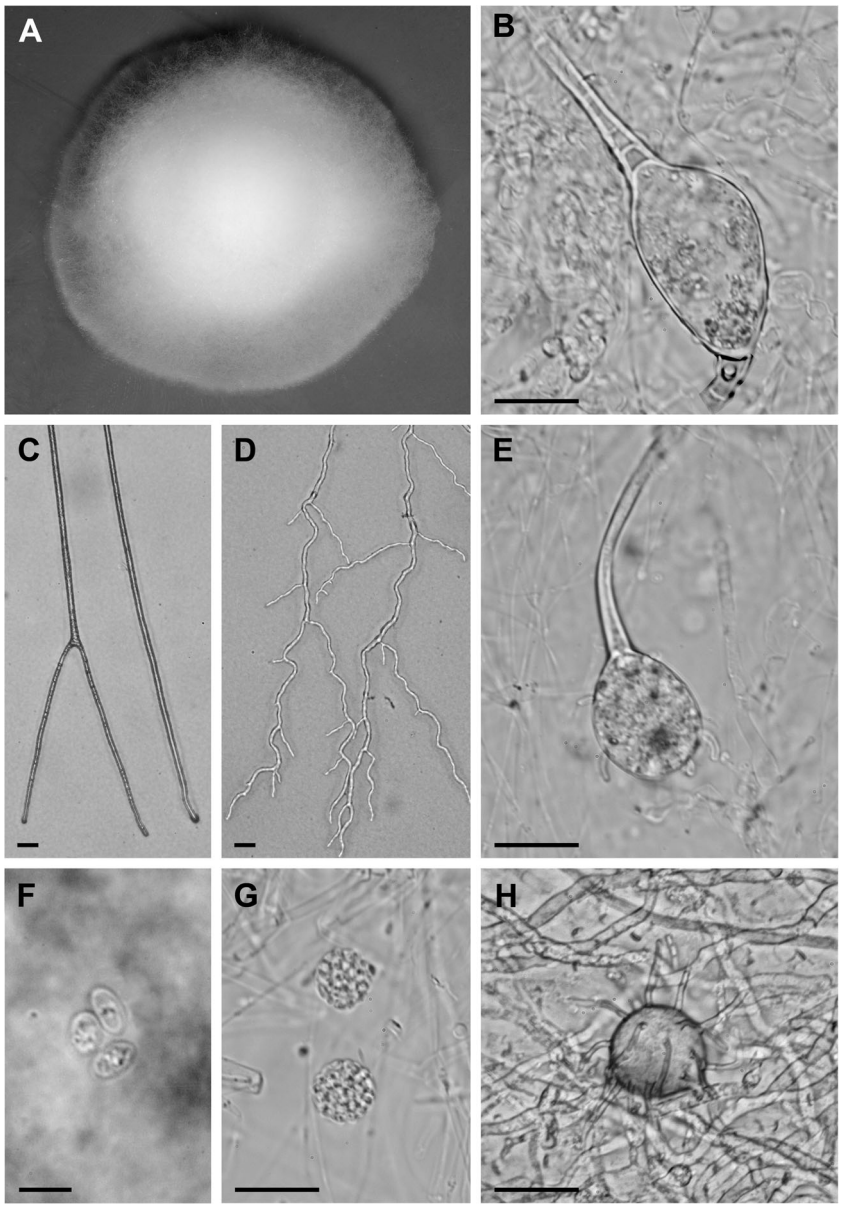

Fig. 3 Strain DSMZ 27011 on yeast malt agar. **a** Growing fungal colony, 8 d after inoculation. **b** Intercalarily formed chlamydospore (bright field, 400 ×). **c** Aerial hyphae (bright field, 100 ×) **d** Vegetative hyphae growing within substrate (bright field, 100 ×). **e** Young terminal chlamydospore with growing appendages (bright field, 400 ×). **f** Sporocystospores (bright field, 1000 ×). **g** Undispersed sporocysts on the agar surface or within the agar (bright field, 400 ×). **h** Fimbriate form of a fully developed chlamydospore (bright field, 400 ×). Scale bars: **b**, **c**, **d**, **e**, **g**, **h** 20 μm. **f** 5 μm

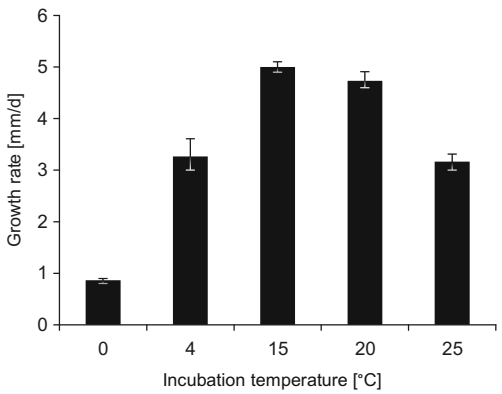

Fig. 4 Colony growth rates of strain DSMZ 27011 at different incubation temperatures. Mean growth rates (increase of colony diameter) from three replicates are given. *Whiskers* indicate minimum and maximum values

Description: Colonies growing moderately rapidly on 1 % yeast malt agar with 0.5 cm diametric increment per day at 15 °C. Emitting a weak garlic-like odour, typical for the genus. Aerial mycelium completely white and dense, in

Table 2 Substrate degradation by strain DSMZ 27011 at different incubation temperatures

	Chitin	Starch	Cellulose	Lignin	Peptides/Protein	Lipids
0 °C	–	–	–	–	–	–
4 °C	–	–	–	–	+	–
15 °C	–	–	–	–	–	–
20 °C	–	–	–	–	–	–
25 °C	–	–	–	–	–	–

appearance reminiscent of cotton. Vegetative hyphae non-septate, 1.5–5 μm wide, with dichotomous to sympodial branchings. Sporocystophores erect or bent, unbranched, arising from the aerial mycelium, up to ca. 200 μm long, from approx. 3–5 μm wide at the base narrowing to approx. 1.5 μm towards the tip. Rhizoid-like structures absent. Sporocysts multi-spored, hyaline and globose, 13 (9–19) μm diam., lacking a columella. Sporocystospores ellipsoid, hyaline, 4.5 (3.5–6) × 3 (2.5–4) μm, smooth-walled. Sexual reproduction and zygospore formation unknown. Two types of chlamydospores are extensively produced within aerial mycelium and substrate. 1) globose to elongate, smooth-walled, 28–31 diam; 2) globose to elongate, 22–25 × 22–25 μm, covered by tubular appendages of 16–19 × 1–2 μm. Both spore types emerge intercalarily and terminally, and become slightly ochre-pigmented during maturation.

The described fungus is known from a coniferous forest soil and can be regularly found in association with the soil-inhabiting mites *Leptogamasus obesus* Holzmann (Gamasina) and *Oppiella subpectinata* Oudemans (Oribatida). Cultures grow at least between 0 and 25 °C, under laboratory conditions.

Discussion

Phylogenetic position of strain DSMZ 27011

Nagy et al. (2011) analysed the resolution potential of the ITS rRNA gene among *Mortierella* spp. and concluded that most of the unassigned mortierellalean sequences can be ascribed to known species on the basis of reliable ITS rRNA sequence data from type cultures. Several other studies (e.g. Hoffmann et al. 2011; Wagner et al. 2013) demonstrated that the ITS region alone contributes valuable phylogenetic information (Petkovits et al. 2011), which is suitable for mapping relationships among zygomycetous fungi. Thus, the single-gene approach chosen here should be sufficient to resolve the species affiliation in question. Strain DSMZ 27011 obviously represents *M. alliacea*, because successive phylogenetic analyses (Figs. 1 and 2) revealed the culture-derived sequence JX535352 and its corresponding mite-derived sequences to cluster exclusively and well-supported with sequences originating from *M. alliacea*. However, for an appropriate identification according to traditional, morphology-based species concepts, morphological traits were analysed in addition. Congruency of the findings from these independent approaches thereby increased reliability of the results.

Morphological identification of strain DSMZ 27011 as *Mortierella alliacea*

A determination to species level could hardly be achieved by using the available identification keys (Zycha et al. 1969; Gams 1969, 1976, 1977) due to some misleading character states. To end up at *M. alliacea*, the key of Linnemann in Zycha et al. (1969) demands cylindrical sporocystospores being mostly twice as long as broad, which does not apply to strain DSMZ 27011. Gams (1969, 1977), on the other hand, specifies sporocystophores measuring less than 150 μm in length, which also does not apply. However, the most closely related species revealed by the molecular analyses beside *M. alliacea* can all be excluded with certitude. *M. pulchella* lacks chlamydospores (Gams 1976) in contrast to strain DSMZ 27011. Further differences are the branched sporocystophores and the development of sporocystospores, which clearly deviate in size and shape. *Mortierella fimbricystis*, again, differs from strain DSMZ 27011 by wider tips of the sporocystophores and larger sporocysts. Furthermore, aerial hyphae are lacking in strains of *M. fimbricystis*, the chlamydospores are larger and the covering appendages are by far shorter than those of strain DSMZ 27011. According to the descriptions by Gams and Hooghiemstra (1976), Zycha et al. (1969), and Yong (1930), *M. turficola* differs from strain DSMZ 27011 by clearly wider sporocystophores with basal rhizoids, globose and smaller sporocystospores being produced in much larger sporocysts, septate vegetative hyphae, and lacking chlamydospores.

Direct comparison of culture morphologies of strains DSMZ 27011 and CBS 894.68 (*M. alliacea*) suggests an affiliation to *M. alliacea*, in spite of the slight discrepancies concerning sporocystospore and chlamydospore morphology. As Linnemann (1953) concedes a pronounced intraspecific variability, this affiliation can be considered rather reliable. Therefore, we present the isolate DSMZ 27011 as a new strain of the species *M. alliacea*. As some minor discrepancies were found between the two compared isolates, an emended description of *M. alliacea* is given considering the traits of both strains and the additional features of the new strain. Because of the lack of a type specimen representing the descriptions by Linnemann (1953) we propose here the drawings accompanying the original species description to be designated as a **lectotype** (cf. McNeill et al. 2012). At the same time we propose M-0276247, a dried culture of the oldest available strain assigned to *M. alliacea* Linnem. (CBS 894.68, collected by W. Gams in 1958) to be designated as **epitype**, as it fits the original description best.

Ecology of *Mortierella alliacea*

The temperature-dependent growth profile of *M. alliacea* seems typical for a soil-dwelling fungus preferring moderate temperatures as they prevail most time of the year in its natural habitats. Strain DSMZ 27011 tolerates low temperatures near the freezing point, even though it grew more slowly at 4 and 0 °C. However, apart from a generally decelerated development no negative impact on colony formation or the

production of reproductive structures by low temperatures could be observed. At 25 °C, again, fungal growth already stagnated after a few days. No sporocycsts were formed, and the appearance of the colonies was atypical. Hence, the fungus seems to be unable to tolerate higher temperatures for a long period of time, although certain isolates of *M. alliacea* have been reported to grow even at 30 °C (Carreiro and Koske 1992). Like several other *Mortierella* species it is a typical psychrophile.

The poor spectrum of substrates degraded by strain DSMZ 27011 is in accordance with the generally limited substrate degradation ability of *Mortierella* spp. (Terashita et al. 1993; Schmidt et al. 2008). Some species, however, are supposed to be at least able to use protein, chitin, and facultatively cellulose (Jackson 1965; Terashita et al. 1993). The production of cold-active proteases by DSMZ 27011 indicates an adaption of the strain to low temperatures, which is quite common in the genus comprising many psychrophilic or at least psychrotolerant species (Gams 1963; Carreiro and Koske 1992; Kurek et al. 2007; Margesin et al. 2008).

The exposure experiments provided no evidence for an infective or pathogenic potential of strain DSMZ 27011 against mites or collembolans. This is in accordance with the absence of chitinolytic activity; a prerequisite for all entomopathogenic fungi, which have to penetrate the cuticles of their hosts (Humber 2008). The similar frequency of the fungus in samples from soil and mites also makes an obligate association between *M. alliacea* and mites appear unlikely. The ingestion of fungal material by *O. subpectinata* might explain the co-occurrence, but this applies not for the predatory mite *L. obesus* (Werner et al. 2012). As *M. alliacea* could easily be cultured from individuals of both species, an external adhesion of spores to the cuticle of the mites seems the most plausible kind of association. However, this could not be confirmed microscopically and if spores had been present, identification would still have been problematic as species of *Mortierella* are not discernable on the basis of spore characters alone (Gams 1969).

The findings suggest a relatively loose, most likely phoretic association between *M. alliacea* and mites. The mites may serve as facultative vectors for spore dispersal below the soil surface. Strong evidence for such an association is given by the way of conidia production among mortierellalean fungi. The comparatively small spores are borne from mucus-bearing heads and are, therefore, predestined for arthropod-mediated dispersal (Fletcher 1977). Maraun et al. (1998) already demonstrated an enrichment of *Mortierella* spp. in fungal communities of disturbed soils in the presence of oribatid mites. These mites could be effective vectors for *M. alliacea* to reach nutrient-rich soil compartments. The saprotrophic fungus colonizes the organic soil layers where it might take advantage of freeze-thaw-cycle-mediated plant root injuries (Kreyling et al. 2012) leading to the release of readily accessible carbohydrates (Schmidt et al. 2008). The soil fauna also tends to be particularly concentrated in the rhizosphere (Curl and Truelove 1986), which appears to be a suitable habitat for the psychrophilic *M. alliacea*, with its limited ability to degrade polymeric substances.

However, arthropod eggs and pupae are among the few substrates in soil, which are rich in sugars and proteins. These should be readily usable by fungi with such limited degradation capabilities like *M. alliacea*. Several quite common saprobic soil fungi were already shown to be capable of parasitizing nematode eggs (Błaszkowska et al. 2013). Therefore, it appears conceivable that the fungus–mite association is not limited to a phoretic one. As many arthropods hibernate as eggs or pupal stages, these may provide additional nutrient resources for soil-dwelling saprobes during winter. Insect eggs contain several types of non-polymeric carbohydrates like glucose, trehalose, mannitol, mannose, fructose, glycerol, and others (Quickenden 1970), and their shells consist of largely proteinaceous layers (Regier and Kafatos 1985). These compounds are also produced by hibernating arthropods as anti-freezing agents (Fields 1992). The psychrotolerant *M. alliacea* would be still able to degrade these substrates, when most of the other microorganisms are inhibited in their activity by low temperatures. Experimental studies and molecular surveys targeting its plausible resources are required to ascertain the actual nutritional sources of *M. alliacea*.

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