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Short communication

Mortierella sugadairana, a new homothallic species related to the firstly described heterothallic species in the genus

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ABSTRACT

A new species, *Mortierella sugadairana*, is described for a fungus forming homothallic zygospores with a club-shaped macrosuspensor and a microsuspensor originating from the macrosuspensor. The species was isolated from cool regions in Japan and morphologically and phylogenetically close to a heterothallic species *M. parvispora*, which is the first species reported as a heterothallic species in the genus. Mycelial growth of the species was limited at 30 °C, whereas two isolates of *M. parvispora* can grow. This may indicate that the species and *M. parvispora* adapted to different climates from a common ancestor involving differentiation of the manner of reproduction.

Recently, the subphylum Mortierellomycotina containing *Mortierella* and six other genera (Hoffmann, Voigt, & Kirk, 2011) was circumscribed as phylum Mucoromycota with other two subphyla, Mucoromycotina and Glomeromycotina (Spatafora et al., 2016). *Mortierella* is one of the largest genus in Mucoromycota, and they were morphologically classified into nine sections (Gams, 1977). However, this asexual morphology-based classification was not supported by a comprehensive phylogeny, and the genus *Mortierella* was phylogenetically separated into seven tentative groups mixed with four genera in Mortierellomycotina (Smith et al., 2013; Wagner et al., 2013).

In earlier years of taxonomic study of the genus *Mortierella*, the zygospore was only reported in homothallic species until Gams and Williams (1963) found a heterothallic zygospore formation in *M. parvispora* Linnem. After this case, homothallic and heterothallic zygospore formations were confirmed in 28 species including 13 homothallic (Brefeld, 1881; Dauphin, 1908; Degawa & Tokumasu, 1998a; Dixon-Stewart, 1932; Ellis, 1940; Gams, 1976; Gams & Hooghiemstra, 1976; Gams, Chien, & Domsch, 1972; Kuhlman, 1972; Watanabe, 1990; Watanabe, Watanabe, Fukatsu, & Kurane, 2001; van Tieghem, 1878) and 15 heterothallic species (Chien, Kuhlman, & Gams, 1974; Degawa & Tokumasu, 1997, 1998b; Gams & Williams, 1963; Gams et al., 1972; Kuhlman, 1972, 1975; Williams, Gray , & Hitchen, 1965) at present. Nearly 100 species have been described in *Mortierella* (Wagner et al., 2013), thus, information concerning zygospore morphologies of *Mortierella* is still limited.

In the course of the survey for the endofungal bacteria associated with *Mortierella*, we obtained three isolates of *M. parvispora*, SUT-174, YTM39 and YTM128, tentatively identified by a blastn search with a >99% similarity of the ITS1-5.8S-ITS2 (ITS) region (Takashima et al. in prep.). However, we observed that these putative *M. parvispora* isolates solely formed zygospores. As mentioned above, *M. parvispora* was known as the heterothallic species, suggesting these homothallic isolates could not be assigned to a known species. Here, we propose a new species, *M. sugadairana* Y. Takash., Degawa & K. Narisawa, sp. nov., based on the observations on the asexual and sexual morphologies of these isolates and phylogenetic analyses using ITS region and the partial 28S large subunit rDNA gene (LSU). We also observed mycelial growth of this species at 10, 20, and 30 °C to compare the mycelial growth with the closest species, *M. parvispora*.

Isolate SUT-174 was isolated by Tokumasu (2009) from Nagano in Japan, deposited to the NITE Biological Resource Center (NBRC), and distributed to us by the NBRC. Other two isolates YTM39 and

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YTM128 were isolated in the previous study from Hokkaido in Japan (Takashima et al. in prep.). Single-sporangiospore cultures of these isolates were incubated on $_{LC}$A (Miura's medium; 0.2 g yeast extract (Difco, Sparks, MD, USA), 1 g glucose (Wako Pure Chemical Industries, Osaka, Japan), 2 g $NaNO_3$ (Wako), 1 g KH_2PO_4 (Wako), 0.2 g KCl (Wako), 0.2 g $MgSO_4 \cdot 7H_2O$ (Wako), 15 g Bacto agar (Difco) in 1 L distilled water) (Miura & Kudo, 1970) at 18 °C for 5–7 d prior to zygospore induction. Three discs were cut out from the incubated mycelia using an autoclave-sterilized plastic straw (8 mm diam) as a substitute for a cork borer, and placed onto $_{LC}$A and hemp seed agar (HSA) (100 mL of hemp seed extract prepared by autoclave 10 g of hemp seed in 100 mL of distilled water, 15 g Bacto agar (Difco) in 1 L distilled water) incubated for 20–30 d in the dark at 18 °C which is the temperature used in the mating experiment for *M. parvispora* (Gams & Williams, 1963; Kuhlman, 1972). These cultures for zygospore induction were also used for the observation of asexual morphologies. We also tried to observe zygospores formed by heterothallic isolates of *M. parvispora* CBS 315.61 and CBS 316.61 to examine morphological differences of zygospores formed by a heterothallic and homothallic manners in the "parvispora-jenkinii complex" defined by Wagner et al. (2013). A disc was cut out from each culture and placed 1 cm apart on HSA, and incubated in the same condition mentioned above.

Morphological observation was carried out using a light microscope (BX51, Olympus Corp., Tokyo, Japan) equipped with a digital camera (DP25, Olympus Corp., Tokyo, Japan). The fungal materials were mounted in distilled water for zygospores and lactoglycerol for sporangiophores and sporangiospores. Additionally, mycelial growth of these three isolates and also *M. parvispora* CBS 315.61 and CBS 316.61 were measured for 5 d at 10, 20, and 30 °C on malt extract agar (MEA) (20 g malt extract (Difico), 20 g glucose (Wako), 1 g mycological peptone (Oxoid, Basingstoke, Hampshire, UK), 20 g Bacto agar (Difco) in 1 L distilled water). For the preparation of dried specimen, the type strain (SUT-174) was incubated on $_{LC}$A and HSA for 22 days, then inactivated by complete drying using a drying oven at 60 °C for 4 d and deposited in the Kanagawa Prefectural Museum of Natural History (KPM). Living cultures of isolates SUT-174, YTM39, and YTM128 were deposited to the NBRC.

In order to determine ITS region and the partial LSU sequences, template DNA of each isolate previously extracted following to Sato et al. (2010) was used. Then, 50 μL of PCR mixture was prepared and it contained 1 μL of template DNA, 2.5 μL of each primer solution of fungal universal primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) (White, Bruns, Lee, & Taylor, 1990) and LR5 (5′-TCCTGAGG-GAAACTTCG-3′) (Vilgalys & Hester, 1990), 4 μL of dNTPs, 0.15 μL of Ex Taq HS polymerase (Takara Bio Inc., Otsu, Japan), 5 μL of 10 × Taq Buffer, and 34.85 μL of MilliQ water. PCR amplification was performed as follows: initially 5 min at 95 °C; followed by 30 cycles of 95 °C for 30 s, 54 °C for 30 s, and 72 °C for 1.5 min, and a final extension step at 72 °C for 10 min using a thermal cycler. The PCR products were purified using polyethylene glycol followed by ethanol precipitation, and then the cycle sequence reaction was carried out using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturers' instructions by using PCR primers and adding sequencing primers, that is; ITS5, ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) (White et al., 1990), LR0R (5′-ACCCGCTGAACTTAAGC-3′, Vilgalys unpublished http://www.botany.duke.edu/fungi/mycolab), and LR5. Cycle sequencing products were purified by ethanol precipitation and sequenced using an Applied Biosystems 3130xl genetic analyzer (Applied Biosystems). Obtained sequences from each sequencing primer were assembled into a contig using GeneStudio Professional software version 2.2.0.0 (www.genestudio.com). Next, we conducted a phylogenetic analysis using the partial LSU region to determine the phylogenetic positions of our isolates among seven phylogenetic groups defined by Wagner et al. (2013). Subsequently, we conducted a phylogenetic analysis using the ITS region to show a species-level phylogenetic relationship of our isolates within the identified phylogenetic group based on the phylogenetic analysis using LSU. Both the partial LSU and ITS regions were aligned separately with retrieved sequences from GenBank listed in Supplementary Table S1 using MAFFT v7.212 (Katoh & Standley, 2013). The obtained alignment blocks were subject to Gblocks 0.91b (Castresana, 2000) to remove poorly aligned positions with the relaxed selection described in Talavera and Castresana (2007) setting with the following parameters (-t = d -b2 = 9 -b3 = 10 -b4 = 5 -b5 = h). After removing gaps, the alignment blocks were checked using MEGA 6.06 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) and positions containing gaps at both ends were removed. Model selection for maximum likelihood (ML) phylogeny was performed in the MEGA 6.06. The alignment blocks were used for a phylogenetic analysis with the ML method using the RAxML version 8.1.5 software (Stamatakis, 2014) under the GTRGAMMA model selected by the MEGA 6.06 and bootstrapping (1000 replicates) with the rapid bootstrap analysis option. The alignment block of the ITS region was also used for calculating proportions of nucleotide similarities of ITS sequences of three isolates of *M. sugadairana* to those of other isolates belong to the "parvispora-jenkinii complex" using the MEGA 6.06. Sequence data of isolates SUT-174, YTM39 and YTM128 were deposited in the Genbank (MF510830, LC219340 and LC219341, respectively). The alignments and trees are deposited to TreeBASE under http://purl.org/phylo/treebase/phylows/study/TB2:S21369.

1. Taxonomy

Mortierella sugadairana Y. Takash., Degawa & K. Narisawa, sp. nov. Figs. 1 and 2.

MycoBank no.: MB 822148.

Diagnosis: This new species is clearly distinguished from *M. parvispora* by homothallic zygospore formation bearing with a club-shaped macrosuspensor and a microsuspensor which is originated from the middle part of the macrosuspensor.

Type: Japan, Nagano prefecture, Sugadaira Research Station, Mountain Science Center University of Tsukuba, isolated from decayed twig of *Fagus crenata* Blume during an intensive investigation on the species diversity of microfungi in the center by Tokumasu (2009), dried fungal specimen (Holotype, KPM-NC0025509), ex-type strain (NBRC 104553).

Gene sequence ex-holotype: MF510830 (ITS + LSU).

Etymology: *Sugadaira*, the type locality of the fungus.

Colonies fast growing on $_{LC}$A, HSA and MEA at 18 °C and R.T. (ca. 23 °C), hyaline, broadly lobed, garlic-like odor faint. Sporulation good on $_{LC}$A and HSA at 18 °C and R.T. Sporangiophores arising from substrates and aerial hyphae with branching mesotonously at different levels, 150–1100 μm tall, 3–30 μm wide at base, a conspicuous rhizoid from a slightly swollen base when arising from substrates. Sporangia many-spored, leaving an inconspicuous collarette and a trace of a columella on dehiscence. Sporangiospores globose, smooth-walled, 2–6 μm diam. Chlamydospores absent. The species is homothallic. Zygospores formed buried in the agar medium (on $_{LC}$A and HSA) at 18 °C in dark, naked, smooth-walled, globose, 25–45 μm diam, wall 1.2–5.7 μm thick. Macrosuspensors club-shaped, thin-walled, 16–44 μm in wide at the thickest part of the tip. Microsuspensors, originated from the middle part of the macrosuspensor.

Distribution: Known from cool regions including the type locality (Sugadaira, Ueda-shi, Nagano pref.) and Hokkaido pref. (Ebetsu-shi and Obihiro-shi).

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