

Globisporangium oryzicola sp. nov., causing poor seedling establishment of directly seeded rice

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Abstract A new species, *Globisporangium oryzicola*, was isolated from directly seeded rice seedlings, and from soils of paddy fields and an uncultivated field. Despite their different origins, five of the seven isolates studied caused poor seedling establishment of rice in a laboratory inoculation experiment. The species is characterized by oogonia with smooth-walled or sometimes one projection, with one to two antheridia, and aplerotic oospores. Hyphal swellings were rarely observed. Phylogenetic analyses based on the internal transcribed spacer region of the ribosomal RNA gene and mitochondrial cytochrome c oxidase

subunit 1 and 2 genes confirmed that the species differed from other *Globisporangium* species. This novel species is described and illustrated in detail.

Keywords Molecular phylogeny · Oomycete · Paddy field · *Pythium* · Rice seedlings

Introduction

The genus *Pythium* Pringsh. was originally described by Pringsheim (1858), and subsequently there have been attempts to split the genus into several genera or subgenera based on morphological characteristics (Fischer 1892; Schröter 1893; Edson 1915). As molecular phylogenetic studies have advanced, new classifications of the genus *Pythium* have been proposed. Bala et al. (2010) established a new genus *Phytopythium* Abad, de Cock, Bala, Robideau and Lévesque comprising the *Pythium* species from clade K (Lévesque and de Cock 2004), which appear to be morphologically and phylogenetically distinct between *Pythium* and *Phytophthora*. In the same year, Uzuhashi et al. (2010) split the genus *Pythium* into five genera including four new genera, *Ovatisporangium*, *Globisporangium*, *Elongisporangium*, and *Pilasporangium*, based on sporangial morphology and the phylogeny of the D1–D2 region of the large subunit ribosomal RNA gene (LSU) and mitochondrial cytochrome oxidase II (*Cox2*) gene. The genus

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Ovatisporangium is now a synonym of the genus *Phytophthium*. The genus *Globisporangium* is characterized by globose hyphal swellings or sporangia, and it corresponds with the species of clades E–G, I and J presented by Lévesque and de Cock (2004).

Many species of the genus *Pythium* s. lat. (*Pythium* Pringsh.), which includes the genus *Globisporangium*, are known as pathogens of various plants, and they incite a wide range of disease symptoms such as pre- and post-emergence damping-off and seed and/or root rot. Some *Pythium* s. lat. species are known as causal agents of disease in rice (*Oryza sativa*) seedlings in many countries, especially *P. arrhenomanes*, which is one of the most important pathogens of rice (Van Buyten and Höfte 2013; Toda et al. 2015). On the other hand, many *Pythium* s. lat. species are also known as nonpathogenic saprophytes that colonize soil and water (van der Plaats-Niterink 1981; Uzuhashi et al. 2015).

A novel species of the genus *Globisporangium* was isolated from rice seedlings, and from soils of paddy fields and an uncultivated field. The species showed unique pathogenicity to the host plant and phylogeny of multi-gene sequence data. Here, we have characterized this new species.

Materials and methods

Isolation

Globisporangium oryzicola isolates were obtained from seedlings of directly seeded rice showing poor growth and from soil of a paddy field in Hiroshima Pref., Japan, and also from soil of an uncultivated field in Nagano Pref., Japan (Table 1). From the seedlings and soil of a paddy field, isolation was performed on *Pythium* selective nystatin-ampicillin-rifampicin-miconazole (NARM) medium (Morita and Tojo 2007). Discolored roots and shoots of rice seedlings (cv. Koshihikari) were washed in tap water to remove soil particles, placed on NARM, and incubated at 25 °C in darkness for 3 days. From the uncultivated field, *Globisporangium* isolate was obtained by a baiting technique using sterile green pepper seeds as described previously (Uzuhashi et al. 2009). A dried type-specimen was deposited at the Department of Botany, National Museum of Nature and Science, Tokyo (TNS), and living cultures were deposited at the

Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, the NARO Genebank-Microorganisms Section (MAFF), Genetic Resources Center, National Agriculture and Food Research Organization (NARO), Tsukuba, Ibaraki, Japan, the NITE, Biological Resource Center (NBRC), Kisarazu, Chiba, Japan, and Osaka Prefecture University (OPU), Sakai, Osaka, Japan.

Morphology and growth temperature

Colony patterns of all seven isolates were examined after incubation for 3 days at 25 °C on potato dextrose agar (PDA), potato carrot agar (PCA) prepared in accordance with the report by van der Plaats-Niterink (1981), and V8 juice agar (V8A) plates prepared as previously reported (Miller 1955). The morphology of the *Globisporangium* isolates was examined in grass blade water culture (van der Plaats-Niterink 1981). At least thirty hyphal swellings, oogonia, and oospores were measured for each isolate. To determine hyphal growth rates, the isolates were incubated on PCA at 0, 3, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, and 40 °C for 1–3 days. Hyphal growth was evaluated by visual measurement of the average increase in the colony along its longest diameter. The experiment was repeated two times by using a single plate per repetition.

DNA extraction and amplification

Genomic DNA of seven *Globisporangium* isolates was extracted in accordance with the protocol of Möller et al. (1992) with a modification to the mycelia grinding step. A piece of mycelium on PDA was placed in a 2 ml mastertube hard (BMS, Tokyo, Japan) with two metal beads and incubated at –30 °C until frozen. The frozen mycelia were crushed using a Shake master Neo (BMS) for 90 s at 15,000 rpm.

The internal transcribed spacer (ITS) region of the ribosomal RNA gene and mitochondrial cytochrome c oxidase 1 (*Cox1*) and 2 (*Cox2*) regions were amplified by PCR using the following primers; ITS5 and ITS4 (White et al. 1990) for ITS, OomCoxI-Levup and OomCoxI-Levlo (Robideau et al. 2011) for *Cox1*, and FM66 and FM58 (Martin 2000) for *Cox2*. PCR reaction volume was 25 µl, containing 2.5 µl 10 × ExTaq buffer (20 mM Mg²⁺), 2 µl dNTP mixture (2.5 mM each), 0.2 µM of each primer, 0.625

Table 1 Strains of *G. oryzae* isolated in this study

Strain	Other collection	Host or substrate	Locality of origin	Date of collection	GenBank accession no.		
					ITS	<i>Cox1</i>	<i>Cox2</i>
HT2-5 ^a	CBS 142206 MAFF 245646 NBRC 112448	Rice seedling	Higashihiroshima, Hiroshima Pref., Japan	June 2014	LC169733	LC169739	LC169746
HT42-1	MAFF 245721 NBRC 112449	Soil (paddy field)	Kure, Hiroshima Pref., Japan	July 2014	LC169734	LC169740	LC169747
HT42-2	MAFF 245722	Soil (paddy field)	Kure, Hiroshima Pref., Japan	July 2014	LC169735	LC169741	LC169748
HT44-1	MAFF 245723	Soil (paddy field)	Kure, Hiroshima Pref., Japan	July 2014	LC169736	LC169742	LC169749
HT44-2	MAFF 245724	Soil (paddy field)	Kure, Hiroshima Pref., Japan	July 2014	LC169737	LC169743	LC169750
HT45-2	MAFF 245725	Soil (paddy field)	Kure, Hiroshima Pref., Japan	July 2014	LC169738	LC169744	LC169751
UZ382	MAFF 241143 NBRC 112450	Soil (uncultivated field)	Nagano, Nagano Pref., Japan		AB468808	LC169745	AB468930

^a Ex-holotype

units Taq DNA polymerase (Takara Bio, Shiga, Japan), and 1 µl template DNA. The thermocycler program for amplification of the ITS region was 95 °C for 3 min followed by 35 cycles of 95 °C for 30 s, 55 °C for 1 min, 72 °C for 1 min, and a final extension was made at 72 °C for 10 min. The program for *Cox1* and *Cox2* were identical to that for ITS, except for a shorter annealing time of 30 s at 55 °C. All PCR products were purified using a MiniElute PCR Purification Kit (QIAGEN, Tokyo, Japan) in accordance with the manufacturer's instructions.

DNA sequencing and phylogenetic analyses

DNA sequencing reactions were performed using a BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan) with the same primers as in the initial PCR step. The products of the sequencing reactions were analyzed using an ABI Prism 3130×1 Genetic analyzer (Applied Biosystems). The sequences were aligned with relevant *Pythium* sequences obtained from the GenBank database using the ClustalW program included in MEGA version 7 (Kumar et al. 2016). The complete alignments were deposited in TreeBase as 19650 (<http://treebase.org/treebase-web/>). Phylogenetic analyses were conducted

using MEGA 7 with Neighbor-Joining (NJ) method of a distance matrix with Kimura 2-parameter model and minimal Maximum Likelihood (ML) method using Tamura-Nei model. The sequences of the ITS and *Cox1* regions were analyzed separately. Bootstrap values were obtained from 500 replicates.

Pathogenicity

The seven isolates for *G. oryzae* were used for pathogenicity analysis (Table 1). A CMA plug containing mycelium of each isolate was transferred to a 300 ml Erlenmeyer flask containing 1 g of autoclaved seeds of highland bent grass (*Agrostis castellana* Boiss. & Reut.), and 3 ml of distilled water. After 4 days of incubation at 25 °C in darkness, 10 ml of sterile distilled water was added. The water-soaked culture were further incubated for 7 days at 25 °C in darkness. The mycelium with the autoclaved seeds was macerated with approximately 5000 rpm for 1 min in sterile water using a juicer mixer (SML-G25, Sun Co Ltd., Osaka, Japan). The concentrations of propagules, mainly consisted of oospores, were determined using a plankton counting chamber (Matsunami Glass Industrial, Osaka, Japan) and adjusted to 10⁷ propagules/ml of the water. The 100 ml propagule

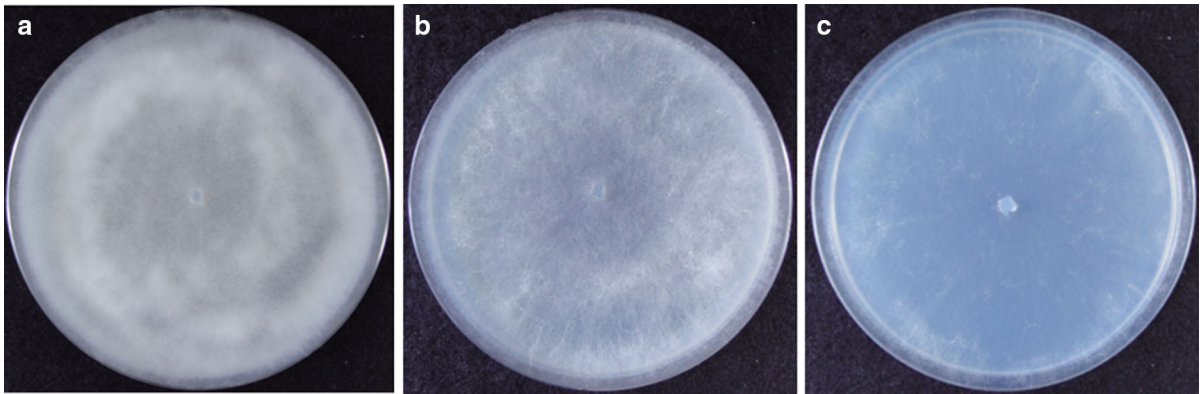


Fig. 1 Colony patterns of *G. oryzae* at 25 °C on: **a** PDA, **b** V8, **c** PCA

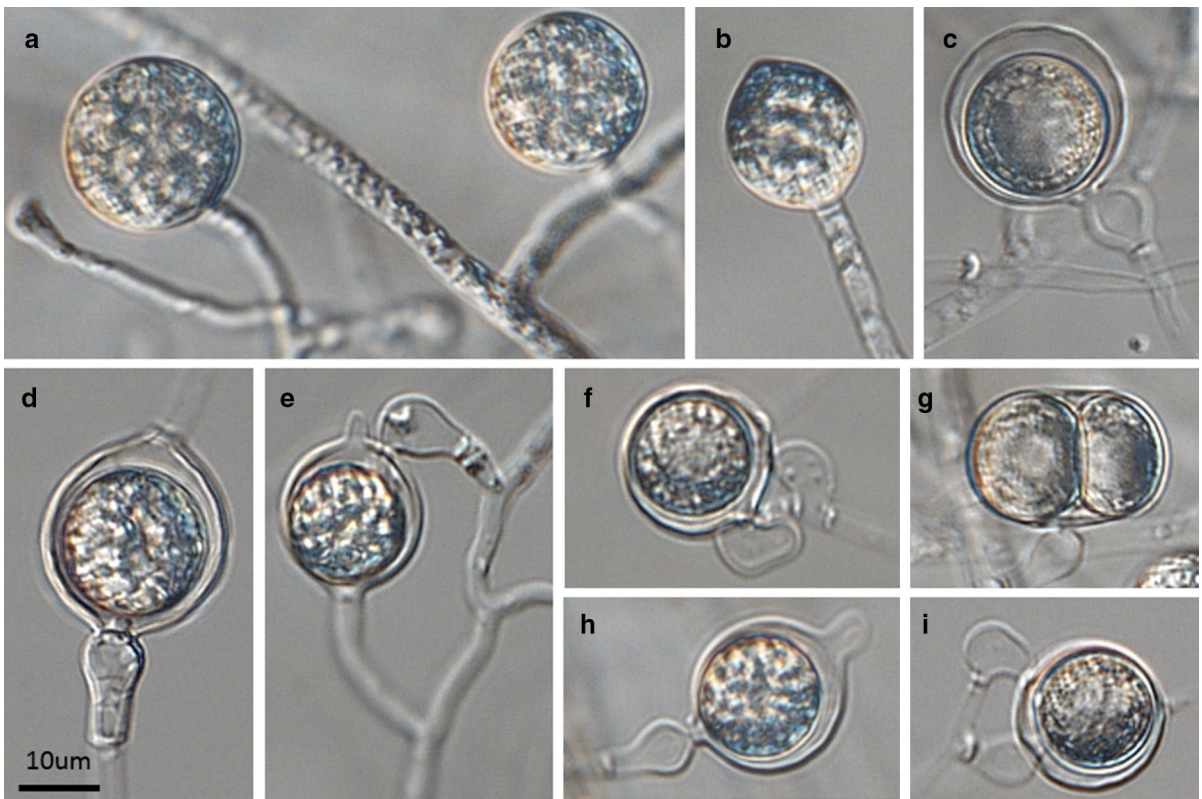


Fig. 2 Morphology of *G. oryzae*. **a** Globose, terminal hyphal swellings. **b** Terminal hyphal swelling. **c** Oogonium with an aplerotic oospore and a declinuous antheridium. **d** Oogonium with an aplerotic oospore and an antheridium. **e** Oogonium with a projection, and an aplerotic oospore, and a monoclinous antheridium. **f** Terminal oogonium with an aplerotic oospore,

and two monoclinous antheridia. **g** Terminal oogonium with two oospores. **h** Terminal oogonium with an aplerotic oospore, and a hypogynous antheridium. **i** Terminal oogonium with an aplerotic oospore, and two antheridia arising on stalks bifurcate near the oogonium. Scale bar 10 µm

suspension was mixed with 1 kg of a commercial rice nursery soil (Yanmar Sukoyakabaido, Yanmar Co. Ltd., Osaka, Japan) and adjusted to 10^6 propagules/g of dry soil. Then, 160 g of the *G. oryzae*-infested

soil was placed in a plastic pot (inner diameter 90 mm, inner depth 80 mm), and 20 germinated seeds of rice (*Oryza sativa*, cv. Koshihikari) were sown 5 mm deep in the soil. The soil was saturated with tap water,

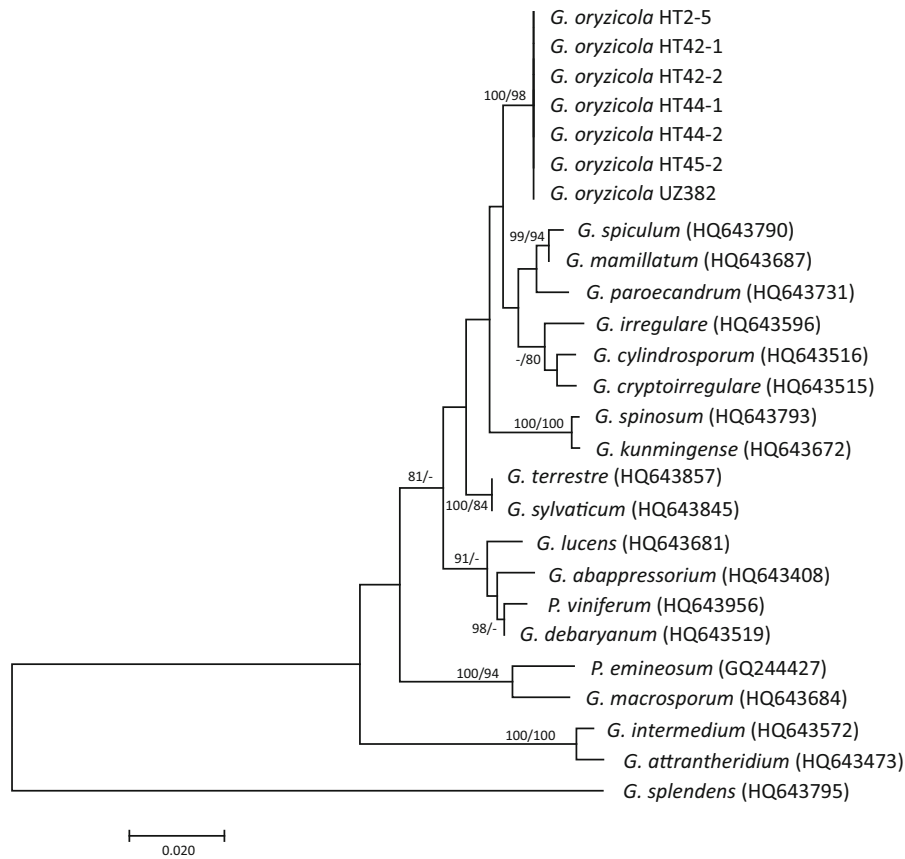


Fig. 3 Maximum-Likelihood (ML) tree based on the ITS sequence, showing the relationship between *G. oryzipicola* and other species in clade F (Lévesque and de Cock 2004).

G. splendens from clade I was used as an outgroup. Numbers along the nodes indicate bootstrap support values above 80% for ML/Neighbor-Joining, respectively

covered with a plastic bag, and kept in an incubator at 30 °C (day 12 h)/25 °C (night 12 h) with a light intensity of 73 mol/m²/s (measured at the plant level). The soil was irrigated daily with tap water. Plants with emerged second leaves was regarded as the seedling stand according to the anatomical monograph of rice seedlings (Hoshikawa 1989). The percentage emergence was recorded at 5 days after sowing. The infection of isolates was confirmed by reisolation on *Pythium* selective NARM medium. The experiments were repeated six times with one pot per treatment. Analysis of variance was conducted for the percentage emergence data of different treatments using JMP software (version 8; SAS Institute, Cary, NC, USA). Means of the data were compared using the least significant difference based on a Tukey–Kramer honestly significant different (HSD) test ($P < 0.05$).

Results

Morphology and growth temperature

All seven strains of *G. oryzipicola* showed similar colony patterns and growth temperature results. Colony patterns comprised cottony aerial mycelium with no special pattern on PDA, and some aerial mycelium with no special pattern on PCA and V8A (Fig. 1). To more clearly define the growth temperature, additional incubations were conducted at 4, 5, 6, 32, and 33 °C for just the HT2-5 strain. The strain was also able to grow at 32 °C, but no growth was observed at any of other temperatures mentioned.

The morphology of asexual and sexual structures were also similar among the 7 strains, although oogonia and oospores of HT42-1 were slightly smaller

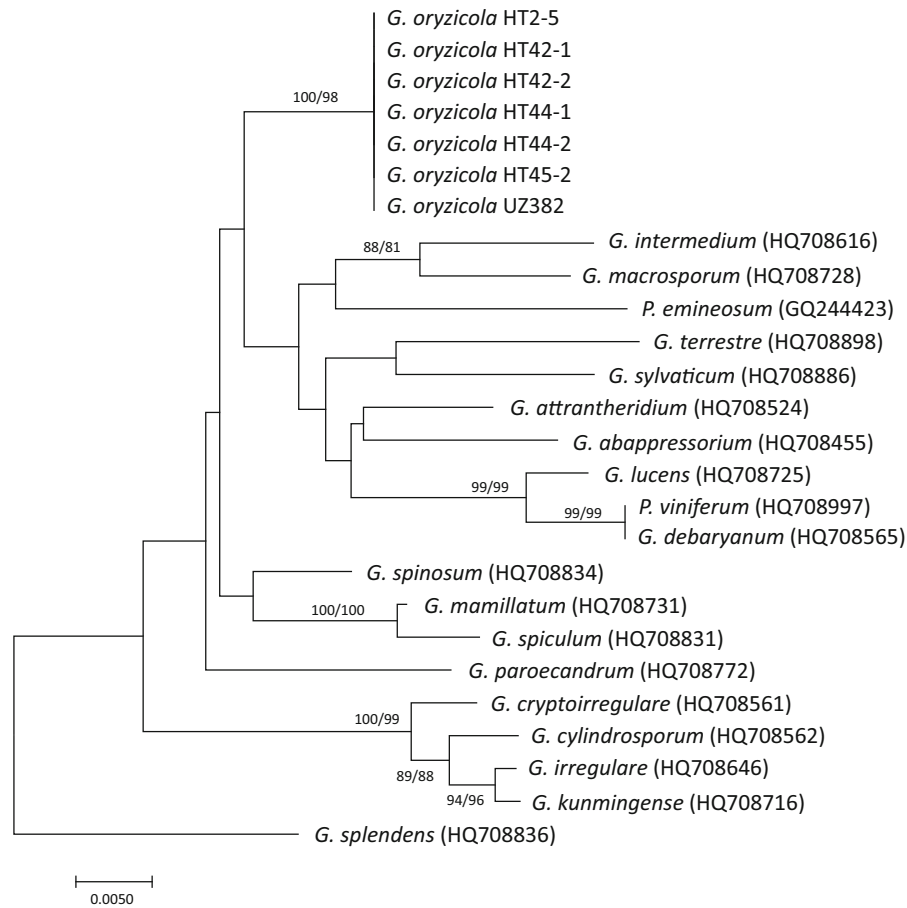


Fig. 4 Maximum-Likelihood (ML) tree based on the *Cox1* sequence, showing the relationship between *G. oryzipicola* and other species in clade F (Lévesque and de Cock 2004).

G. splendens from clade I was used as an outgroup. Numbers along the nodes indicate bootstrap support values above 80% for ML/Neighbor-Joining, respectively

than the others. Hyphal swellings were rarely observed, although they were sometimes difficult to distinguish from abortive oogonia. Zoospores were not observed in any of the strains. The sexual structures were abundantly produced in the water culture as well as in agar culture such as PCA. Oogonia were mostly smooth-walled, but sometimes had one projection (Fig. 2e, h). Antheridia were monoclinal or diclinal, produced one or two per oogonium, and sessile or with stalks, which sometimes bifurcated near the oogonium (Fig. 2i). Oospores were mostly one, but sometimes two per oogonium (Fig. 2g).

Phylogenetic analyses

The sequences of ITS, *Cox1*, and *Cox2* genes were identical among the isolated strains of *G. oryzipicola*.

The ITS and *Cox1* sequences of *G. oryzipicola* had 97% similarity with those of *G. paroecandrum*, and 98–99% similarity with those of *G. spinosum* as the highest one, respectively. Based on comparison with previous studies (e.g. Robideau et al. 2011; Ellis et al. 2012), the sequences of *G. oryzipicola* differ sufficiently from those of any other described *Pythium* s. lat. species. The *Cox2* sequence of *G. oryzipicola* showed 99% similarity with *P. spinosum* as the highest similarity. However, only a few *Cox2* sequences of clade F species have been deposited in the GenBank database. Therefore, a phylogenetic tree was not constructed based on this region.

In phylogenetic analyses based on the ITS and *Cox1* sequences, *G. oryzipicola* belonged to clade F, as described by Lévesque and de Cock (2004), in both of the phylogenetic trees (data not shown).

Table 2 Morphology and hyphal growth temperature of *G. oryzaicola* and morphologically and phylogenetically related species

	HT2-5 ^a	<i>G. paroeandrum</i> ^b	<i>G. spinosum</i> ^b	<i>G. irregulare</i> ^b	<i>G. cryptoirregulare</i> ^c	<i>G. ultimum</i> var. <i>ultimum</i> ^b
Cardinal temperature for hyphal growth (°C)	7–32	5–35	5–35	1–35	4–36	5–35
Daily growth at 25 °C on PCA (mm)	26.5	20–25	30–35	25	19 (on CMA) 27 (on PDA)	30
Width of hyphae (µm)	Up to 6	Up to 9	2.5–5(–7)	Up to 6	Up to 6	Up to 11
HS or Sp production ^d	HS: rarely observed	Sp: (Sub) Globose or ellipsoidal	HS: globose or limoniform, mostly smooth, occasionally with 1–2 digitate protuberances	HS: globose, obovate, limoniform or of irregular shape Sp: Globose (seldom produced)	Sp: globose	HS: globose
Diameter of HS or Sp (µm) ^d	13.1–21.8 (av. 17.8)	12–33	Up to 33	HS: Up to 25 Sp: 10–20	13–20 (av. 15.83)	20–25 (av. 29)
Position of HS or Sp ^d	Terminal	Intercalary or terminal	Terminal and Intercalary	Terminal and intercalary	Terminal and intercalary	Intercalary, sometimes terminal
Zoospore production	Not observed		Not observed	Produced	Often observed	Mostly not formed
Oogonium diameter (µm)	16.6–24.7 (av. 20.5)	(14–)17–24(–27) (av. 19)	(14–)17–21 (av. 18.5)	16–21 (av. 18.5)	14.5–18.6 (av. 16)	(14–)20–24(–25) (av. 21.5)
Position of oogonium	Terminal, occasionally intercalary	Intercalary, often in chains, rarely terminal	Terminal or intercalary	Intercalary, sometimes terminal	Intercalary, sometimes terminal	Terminal, sometimes intercalary
Oogonium ornamentation	Smooth, sometimes one finger-like projection	Smooth	With a varying number of blunt, digitate ornamentations	Smooth, or ornamented, mostly 0–5 projections of variable length per oogonium	Smooth and ornamented, mostly 0–5 projections of variable length per oogonium	Smooth
Oospore diameter (µm)	13.0–18.9 (av. 15.8)	(13–)15–21(–23) (av. 17)	(13–)15–19(–20) (av. 17.2)	15–18 (av. 15.9)	12–15 (av. 13.2)	(12–)17–20(–21) (av. 18)
Oospore wall thickness (µm)	Up to 1	1–1.5	Thin-walled	1–1.5	Up to 1.5	Often 2 or more
Plerotic or aperlotic of oospores	Aplerotic	Aplerotic	Plerotic, occasionally aperlotic	Mostly aperlotic, occasionally plerotic	Mostly aperlotic	Aplerotic
Number of oospores/oogonium	1 rarely 2	1	1	1	1	1
Number of antheridia/oogonium	1 or 2	1–2(–5)	1(–3)	1–2(–3)	1(–3)	1(–3)

Table 2 continued

	HT2-5 ^a	<i>G. paroecandrum</i> ^b	<i>G. spinosum</i> ^b	<i>G. irregulare</i> ^b	<i>G. cryptoirregulare</i> ^c	<i>G. ultimum</i> var. <i>ultimum</i> ^b
Monoclinous or diclinous of antheridium	Diclinous or monoclinous	Monoclinous, sometimes diclinous	Monoclinous, occasionally diclinous	Monoclinous, sometimes diclinous	Mostly monoclinous, sometimes diclinous	Mostly monoclinous
Antheridial cells and stalks	Stalked, sometimes branched, or sessile, sac-like, club-shaped	Sometimes sessile	Soon vanishing after fertilization, cells hardly inflated	Mostly stalked, sometimes branched, occasionally sessile, hypogynous	Stalked, not branched, occasionally sessile	Sac-like

^a Ex-holotype^b van der Plaats-Niterink (1981)^c Garzón et al. (2007)^d *HS* hyphal swellings; *Sp* sporangia**Table 3** Effects of *G. oryzae* isolates on seedling stand of rice

Strain	Seedling stand (%) ^a
HT2-5	60.8 ± 3.5 BC
HT42-1	42.5 ± 7.2 C
HT42-2	60.0 ± 4.0 C
HT44-1	83.3 ± 4.6 A
HT44-2	81.7 ± 4.1 AB
HT45-2	55.8 ± 5.4 C
UZ382	53.3 ± 5.4 C
Uninoculated	98.3 ± 1.1 A

^a Data represents mean ± standard errors. The experiment was repeated six times, with twenty plants per repetition. Values followed by the same letters in a column do not differ significantly among the *G. oryzae* isolates and the uninoculated control according to the Tukey–Kramer HSD test ($P < 0.05$)

Phylogenetic trees were constructed based on ITS and *Cox1* sequences separately with *G. oryzae* and species of clade F (Lévesque and de Cock 2004; Robideau et al. 2011), and *G. splendens* as the outgroup (Figs. 3, 4). Based either on the analysis of the ITS or *Cox1* sequences, the topologies of the trees were quite similar between ML and NJ analyses (Table 2).

Pathogenicity

Five *G. oryzae* strains (HT2-5, HT42-1, HT42-2, HT45-2, and UZ382) significantly ($P < 0.05$) reduced

seedling emergence of rice comparing with an uninoculated control (Table 3; Fig. 5). The other two strains (HT44-1 and HT44-2) had no significant effect on the seedling stands. *G. oryzae* was isolated from all the diseased plants.

Taxonomy

Globisporangium oryzae Uzuhashi and Tojo, sp. nov. (Figs 1, 2) MycoBank MB817839.

Etymology *oryzae* refers to the host from which it was isolated.

Colonies forming cottony aerial mycelium on PDA and some aerial mycelium with no special pattern on PCA and V8A. Daily growth at 25 °C on PCA 26.5 mm. Cardinal temperatures minimum 7 °C, optimum 25–28 °C, maximum 32 °C. Main hyphae up to 6 µm wide. Hyphal swellings rarely observed, terminal, globose or sub-globose, 13.1–21.8 µm (av. 17.8 µm) in diameter. Zoospores not observed. Oogonia produced in single culture, globose, smooth-walled of sometimes one finger-like projection, terminal, occasionally intercalary, 16.6–24.7 µm (av. 20.5 µm) in diameter. Antheridia sac-like, club-shaped, diclinous or monoclinous, one or two per oogonium, antheridial stalks sometimes branched. Oospores aplerotic, one or rarely two per oogonium, 13.0–18.9 µm (av. 15.8 µm) in diameter, thin-walled up to 1.0 µm.

Holotype Japan, Hiroshima, Higashihiroshima, in a rice seedling, June 2014, M. Tojo (Holotype, TNS-F-



Fig. 5 Seedling stands of rice in nursery soil infested with *G. oryzae* isolates HT2-5, HT42-1, HT42-2, HT44-1, HT44-2, HT45-2, and UZ382, and in uninoculated soil at 5 days after inoculation and sowing

66690; ex-type strain, HT2-5 = CBS 142206 = MAFF 245646 = NBRC 112448 = OPU 861).

Other material examined Japan, Hiroshima, Kure, from soil of a paddy field, July 2014, M. Tojo (HT42-1 = MAFF 245721 = NBRC 112449 = OPU 862, HT42-2 = MAFF 245722 = OPU 863, HT44-1 = MAFF 245723 = OPU 864, HT44-2 = MAFF 245724 = OPU 865, HT45-2 = MAFF 245725 = OPU 866) and Japan, Nagano, from uncultivated soil, S. Uzuhashi (UZ382 = MAFF 241143 = NBRC 112450 = OPU 867).

Discussion

Globisporangium oryzae is clearly distinct from other *Globisporangium* spp. by its phylogenetic relationship. On the other hand, there are no unique morphological characters that clearly distinguish *G. oryzae* from other species. *G. oryzae* belongs to clade F (Lévesque and de Cock 2004) in the phylogenetic analyses. Some species of this clade form ornamented oogonia, although the length, numbers, shapes, or frequency of projections are diverse among these species. *G. oryzae* mainly produced smooth-walled oogonia, but it also sometimes produced

oogonia with a projection. This feature is similar to those of *Globisporangium irregulare* and *G. cryptoirregulare* in this clade. However, both of these species sometimes produce oogonia with more than one projection (van der Plaats-Niterink 1981; Garzón et al. 2007), which has not been observed in *G. oryzae*. *G. irregulare* also differs from *G. oryzae* by producing plerotic oospores occasionally (Table 2). In any cases, *G. oryzae* has quite similar morphological characters with *G. irregulare* and *G. cryptoirregulare*. Molecular phylogenetic analyses would be the easiest way to distinguish *G. oryzae* from *G. irregulare* and *G. cryptoirregulare* (Figs. 3, 4). *G. oryzae* also morphologically resembles *G. ultimum* var. *ultimum* of clade I. For example, none of these species produce zoospores, and form smooth surface oogonia, aplerotic oospores, and sac-like antheridia. *G. oryzae* can be distinguished from *G. ultimum* var. *ultimum* by the smaller hyphal swellings, oogonia, and oospores (Table 2). In the BLAST searches, *G. oryzae* showed the closest homologies with *G. paroecandrum* and *G. spinosum* in the ITS and *Cox1* sequences, respectively. However, *G. oryzae* differs from *G. paroecandrum* by the smaller hyphal swellings, oogonia with a projection and stalked antheridia (Table 2). *G. spinosum* occasionally

produces the hyphal swellings with 1–2 digitate protuberances and ornamented oogonia, and its antheridia vanish after fertilization (Table 2), so *G. oryzae* is easily distinguished from *G. spinosum* by these features.

G. oryzae isolates were not only found in Hiroshima Pref., but also in Nagano Pref. Because the two areas are separated geographically by about 550 km, *G. oryzae* is considered to have a wide distribution throughout Japan.

The pathogenicity test demonstrated that *G. oryzae* is a potential rice pathogen, with varying levels of aggressiveness among the strains. The pathogenicity was not only found in strains from rice plants or rice paddy fields, but also in the strain UZ382, which was from an uncultivated field. Because of the various origins and pathogenicities, *G. oryzae* may act as an opportunistic pathogen of rice seedlings as well as being a saprophyte in nature. Its economic impact should be tested in further studies in actual farming conditions.

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