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Article

Pythium banihashemianum sp. nov. and *Globisporangium izadpanahii* sp. nov. Two New Oomycete Species from Rice Paddies in Iran

Fatemeh Salmaninezhad [1], Reza Mostowfizadeh-Ghalamfarsa [1] and Santa Olga Cacciola [2,*]

[1] Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, Iran 7144167186; f.Salmaninezhad@shirazu.ac.ir (F.S.); rmostofi@shirazu.ac.ir (R.M.-G)
[2] Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy 95123; olga.cacciola@unict.it (S.O.C)
* Correspondence: olga.cacciola@unict.it

Abstract: Investigation of oomycete diversity in rice paddies of Fars Province in Iran led to the identification of two new *Pythium sensu lato* (*s.l.*) species as *Globisporangium izadpanahii* sp. nov. and *Pythium banihashemianum* sp. nov. The identification was based on morphological and physiological features as well as the phylogenetic analysis of nuclear (ITS and *βtub*) and mitochondrial (*cox1* and *cox2*) loci using Bayesian inference and Maximum Likelihood. A major characteristic of *G. izadpanahii* was the production of globose hyphal swellings; this species did not produce vesicles and zoospores. The present paper describes formally these two new species and defines their phylogenetic relationships with other congeneric species. According to multiple gene genealogy analysis, *G. izadpanahii* grouped with other species of *Globisporangium* in the former clade G of *Pythium s.l.* and was closely related to both *G. nagaii* and the recently described *G. coniferarum.* The second species, designated *P. banihashemianum*, grouped with other species of *Pythium sensu stricto* in the former clade B of *Pythium s.l.* and according to the phylogenetic analysis shared an ancestor with *P. plurisporium.* In pathogenicity tests on rice seedlings, *P. banihashemianum* isolates were highly pathogenic causing severe root and crown rot, while *G. izadpanahii* isolates were not pathogenic.

Keywords: *Pythium*; *Oryza sativa;* multigene phylogenetic analysis; taxonomy; pathogenicity; root and crown rot

1. Introduction

Pythium sensu lato (*s.l.*) Pringsh. is a cosmopolitan, morphologically and genetically heterogeneous oomycete genus comprising more than 230 described species [1]. Several species of this genus have been reported as both facultative saprobes and plant, animal, and human pathogens [2–8], whereas many others are reported as exclusively saprobes or even beneficial antagonists of plant pathogens [9–15]. Plant pathogenic species of *Pythium s.l.* cause pre- and post-emergence damping-off as well as crown and root rot and may have a highly destructive impact on crops.

Around 20 years ago, in the light of advances in molecular biology techniques, the genus *Pythium s.l.* was re-examined and divided into 11 phylogenetic clades (from A to K), based on the analysis of ITS region of rDNA [16]. Since this early study it was clear that this genus was paraphyletic. Subsequently, multiple gene genealogy confirmed this assumption and *Pythium s.l.* was split into five distinct genera, including *Pythium sensu stricto* (hereafter referred to as *Pythium*), encompassing clades A, B, C, and D, *Elangisporangium*, corresponding to clade H, *Globisporangium,* encompassing clades E, F, G, and I, *Phytopythium* (syn. *Ovatisporangium*), corresponding to clade K, and *Pilasporangium,* the last one not coinciding with any of the 11 phylogenetic clades [17–19]. Each genus has its own unique morphological features, *i.e., Pythium* produces filamentous, filamentous inflated, or lobate sporangia, whereas *Globisporangium* species produce globose to subglobose sporangia, occasionally with internal proliferation [1,17,18]. Besides, *Phytopythium* species produce ovoid

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sporangia with internal or external proliferation, resembling sporangia of *Phytophthora* species, while *Elangisporangium* and *Pilasporangium* produce elongated sporangia and sporangia without proliferation, respectively [17,19].

Before the advent of molecular techniques, the identification of species of *Pythium s.l.* was problematic mainly due to pleomorphism of the sexual and asexual structures, the intraspecific phenotypic variability, inconsistency of isolates to form some of these structures *in vitro* and lack of a comprehensive, sound taxonomic framework [20–24]. Although molecular techniques along with phylogenetic analyses have substantially assisted in the identification of *Pythium s.l.* species, morphological traits maintain a fundamental taxonomic relevance. Moreover, it is generally recognized that more than one molecular marker is needed for distinguishing most genera and species of oomycetes [23,25–30].

Several species of *Pythium s.l.* are reported as rice seedling pathogens [31–43]. However, the diversity of *Pythium s.l.* populations in rice paddies has been little investigated worldwide.

More than 60 diverse taxa of *Pythium s.l.* have been reported from Iran [15,44]. In recent years, various cereal fields in Fars Province of Iran have been surveyed to isolate and identify *Pythium s.l.* species [43,45,46]. These studies revealed rice paddies are a favorable ecological niche for *Pythium s.l.* species. During the surveys of rice paddies, we recovered among isolates of various *Pythium s.l.* species two groups of isolates with distinctive characters that could not be assigned to any known species. Multi-locus phylogenetic inference indicated they were two new clearly distinct taxa, which were characterized and formally described as new species.

2. Materials and Methods

2.1. Isolation

During 2013 to 2015, samples were randomly collected from rhizosphere soil, water ponds and rice seedlings in diverse rice paddies of Fars Province, Iran. Geographic coordinates were recorded for each field by Global Positioning System (GPS) (Table 1). Samples were transported to the Mycology Laboratory of the Department of Plant Protection, Shiraz University, for isolation. Roots and basal stem of rice seedlings were washed with distilled water, blotted dry, cut into small segments (2 to 3 mm) and placed on the semi-selective medium for oomycetes CMA-PARP (Ground corn extract 40 g/L; agar 15 g/L; amended with 10 µg/mL pimaricin, 200 µg/mL ampicillin, 10 µg/mL rifampicin and 25 µg/mL PCNB) [47]. One hundred grams of each soil sample were placed in a plastic container and flooded with tap water to 1 cm above the soil surface [48]. Isolates were recovered from either soil or water samples by baiting with 5-mm surface sterilized leaf disks of bitter orange (*Citrus aurantium* L.) or 5 mm pieces of sterile meadow grass (*Poa annua* L.) at 25 ºC every 8 h for 48 h in total, and plating on CMA-PARP. Isolates were purified by hyphal tip method on water agar (WA, Agar 10 g/L) and stored on CMA (Ground corn extract 40 g/L; agar 15 g/L) slopes at 15 ºC.

Table 1. List of *Pythium sensu lato* isolates recovered from rice paddy fields of Fars Province of Iran with their GenBank accession numbers.

Species	Isolates	Date of collection	Location	Longitude	Latitude	Matrix	GenBank accession number			
							ITS[a]	*Btub*[b]	*cox*1[c]	*cox*2[d]
Pythium banihashemianum										
	068B1*†	Aug 2015	Kamfiruz	30°16.934'N	052°19.155'E	Rice root	KX228083	KX228113	OP321097	KX228120
	Th641†	Aug 2015	Persepolis	29°59.008'N	052°49.513'E	Rice soil	MK454538	MK540656	OP321102	MK455863
	Fk21†	Nov 2015	Fiurz Abad	28°51.407'N	052°30.666'E	Rice root	MK454539	MK540655	OP321098	MK455862
	048S1†	Nov 2015	Ramjard	30°02.780'N	052°49.513'E	Rice root	N/A	N/A	N/A	N/A
	038C3†	Nov 2015	Ramjard	30°07.234'N	052°32.983'E	Rice root	N/A	N/A	N/A	N/A
	033B7†	Nov 2015	Ramjard	30°07.274'N	052°32.946'E	Rice soil	N/A	N/A	N/A	N/A
	056S2†	May 2014	Kamfiruz	30°11.909'N	052°27.779'E	Rice soil	N/A	N/A	N/A	N/A
	K116-1†	Aug 2015	Kamfiruz	30°11.017'N	052°27.900'E	Rice soil	N/A	N/A	N/A	N/A
	Fs301†	Nov 2015	Fiurz Abad	28°49.735'N	052°29.149'E	Rice root	N/A	N/A	N/A	N/A

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F32-01†	May 2014	Fiurz Abad	28°49.989'N052°29.551'E	Rice soil	N/A	N/A	N/A	N/A
F201-3‡	May 2014	Fiurz Abad	28°51.587'N052°30.842'E	Rice soil	N/A	N/A	N/A	N/A
KC11**	Nov 2015	Ramjard	30°05.476'N052°35.563'E	Rice crown	KX228081	MK455866	OP321100	MK455858
KC5**	Aug 2014	Persepolis	29°58.892'N052°57.734'E	Rice soil	MK454707	MK455865	OP321099	MK455856
KCr09**	Aug 2014	Ramjard	30°05.901'N052°35.482'E	Rice root	MK454706	MK455864	OP321101	MK455857
G112-2**	May 2014	Kamfiruz	30°11.911'N052°27.777'E	Rice soil	N/A	N/A	N/A	N/A
K101-4**	May 2014	Kamfiruz	30°11.845'N052°27.787'E	Pond water	N/A	N/A	N/A	N/A
Globisporangium izadpanahii								
K330-7‡	Nov 2015	Firuz Abad	28°49.989'N052°29.551'E	Soil	MK454537	MK455869	OP321103	MK455859
KGr1	Nov 2015	Kamfiruz	29°58.823'N052°53.651'E	Rice crown	MK454535	MK455867	OP321105	MK455861
KB14	Nov 2015	Kamfiruz	30°19.236'N052°16.560'E	Pond water	MK454536	MK455868	OP321104	MK455860
Rfa01	Nov 2015	Ramjard	30°06.139'N052°26.892'E	Soil	N/A	N/A	N/A	N/A
KHa3	Nov 2015	Kamfiruz	30°18.134'N052°17.767'E	Rice root	N/A	N/A	N/A	N/A

[a]Internal transcribed spacers 1, 2 and 5.8S gene of rDNA. [b] β-tubulin. [c] cytochrome c oxidase subunit I. [d] cytochrome c oxidase subunit II. * = CBS 143876, Type species; ‡ = CBS 144006, Type species, † = Morphology Group I, ** = Morphology Group II.

2.2. Morphological characterization

In order to observe asexual reproductive structures (sporangia, vesicles and zoospores), isolates were transferred onto CMA containing sterile hemp (*Cannabis sativa* L.) seeds or turfgrass (*Poa* sp.) [49] for 24 h. Hemp seeds or turfgrass were then transferred to Petri dishes containing distilled water [50], sterile soil extract [51] or Schmitthenner solution [52] under fluorescent light for 48 h and were checked every 8 h for six times. Besides, sporangia formation was examined using French bean agar media (FBA, French bean extract 30 g/L; agar 15 g/L) [47] and sterile soil extract [53]. Sexual reproductive structures were obtained on hemp seed agar (HSA, ground hemp seed extract 60 g/L; agar 15 g/L) and carrot agar (CA, carrot extract 250 g/L; agar 15 g/L) incubated in darkness [49]. In order to examine the colony morphology, isolates were grown on CMA, HSA, CA, potato-dextrose agar (PDA, potato extract 300 g/L; dextrose 20 g/L; agar 15 g/L) and malt extract agar (MEA, 25 g/L; agar 15 g/L) [48]. Mycelium plugs (5 mm in diameter) from the edge of 3 d old cultures were placed in Petri dishes, each containing 20 mm of medium. The dishes were incubated at 25 ºC for 48 h. The effect of temperature on mycelium growth rate was tested on PDA with three replicate Petri dishes per isolate and per each tested temperature. Dishes were incubated at 0, 5, 10, 15, 20, 25, 30, 35 and 40 ºC.

2.3. DNA Extraction, PCR, Sequencing and Phylogenetic analyses

Mycelial DNA was extracted using the method described by Mirsoleimani and Mostowfizadeh-Ghalamfarsa (2013) [54]. Primers used for amplification and sequencing of nuclear (Internal transcribed spacers 1, 2 and 5.8S gene of rDNA= ITS; β-tubulin gene = *Btub*) as well as mitochondrial (cytochrome c oxidase subunit II= *cox*2) loci as well as the PCR conditions loci are reported in Table S1. PCR products were purified and sequenced with the primers used for amplification by a dye terminator cycle (Bioneer, Daejeon, South Korea). Sequences were deposited into GenBank. For low-quality ITS sequences, cloning was performed using Strata Cloning Kit (Agilent Technologies, Santa Clara, CA, USA) according to manufacturer's instruction [55].

The resulting sequences were edited and aligned by Geneious Prime 2022 [56] with subsequent visual adjustments. BLAST similarity searches were performed with blastn (for nucleotide-versus-nucleotide comparison) [57]. Partition homogeneity tests were conducted on combined nuclear and mitochondrial gene alignments by PAUP* 4.0a136 [58] using 100 replicates and a heuristic general search option. To reconstruct the phylogenetic trees, Bayesian inference analyses on individual and concatenated ITS, *Btub*, *cox*1, and *cox*2 loci were carried out with MrBayes 3.1 [59], as implemented in TrEase [60] running 10 M generations with the GTR Gamma + I substitution model and discarding 25% of the initial trees as burnin. In adition, Maximum Likelihood inference was done using RAxML as implemented in TrEase. All parameters were set to default. The robustness of the Maximum

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Likelihood trees was estimated by 1000 bootstraps. Phylogenetic trees were edited and displayed with Mega 11 [61].

2.4. Pathogenicity

The ability of isolates to cause seed rot, stunting, pre- and post-emergence damping off of rice seedlings was tested in pathogenicity assays. Inoculum was prepared according to the method described by Banihashemi (1989) [62], and Salmaninezhad and Mostowfizadeh-Ghalamfarsa (2019a) [43] using vermiculite amended with 120 mL/L hemp seed extract (extract of 60 g boiled hemp seeds), colonized by the mycelium.

For pre-emergence damping-off tests, rice seeds were washed and planted in pots containing sandy loam soil (500 mL) infested with 10 mL inoculum. Control seeds were planted in pots containing sandy loam soil (500 mL) mixed with 10 ml of sterile vermiculite amended with hemp seed extract. For post-emergence damping-off tests, 20 d old seedlings were transplanted into pots containing sandy loam soil (500 mL) infested with 10 mL of inoculum. Control seedlings were transplanted into pots containing sandy loam soil (500 mL) mixed with 10 ml of sterile vermiculite amended with hemp seed extract. Symptoms were scored two weeks later. Reisolation were performed from both symptomatic and control seedlings using CMA-PARP medium, according to the method described by Afeck et al. (1990)[63].

3. Results

3.1. Pythium s.l. isolates

Overall, 1169 isolates of *Pythium s.l.* were recovered from rice paddies of Fars Province during the survey. Among them, two groups of isolates with distinctive morphological characteristics were selected for further characterization in this study. Isolates of the first group (16 isolates) produced filamentous to slightly inflated sporangia which released zoospores in aqueous medium. Isolates of the other group (five isolates) produced globose to subglobose hyphal swellings and were not able to produce zoospores. None of these groups of isolates corresponded to the already described species, according to identification keys of Van der Plaäts-Niterink [2] and Dick [64].

3.2. Phylogenetic analyses

Isolates within each group had identical sequences of nuclear and mitochondrial loci. The ITS sequences of isolates of the first group showed 99% similarity with *P. plurisporium* Abad, Shew & L. T. Lucas and two other undescribed *Pythium* species. The ITS sequences of isolates of the second group showed 87 to 88% similarity with *G. coniferarum* Salmaninezhad & Mostowf., three undescribed *Pythium* species, and *G. nagaii* (Ito & Tokun) Uzuhashi, Tojo & Kakish. The final alignment length was 877 bp for ITS, 393 bp for *cox*1, 488 bp for *cox*2, 459 bp for *Btub*, and 2045 bp for combined gene regions for *Pythium* sp.; and 1317 bp for ITS, 393 bp for *cox*1, 484 bp for *cox*2, 480 bp for *Btub*, and 2709 bp for combined gene regions for *Globisporangium* sp. In all-genes phylogenetic trees (Figures 1 and 2; Figures S1 and S2), each group of isolates formed a well-supported monophyletic group, which substantiates the conclusion the two groups were novel species. Bayesian posterior probability was 1.00 for each new lineage in the combined tree and ranged from 0.93 to 1.00 across nuclear and mitochondrial gene trees (Figures S3–S8). The two novel species were designated *Pythium banihashemianum* sp. nov. and *Globisporangium izadpanahii* sp. nov., respectively. *Pythium banihashemianum* sp. nov. was located in Clade B of ITS phylogenetic tree and was related to *P. plurisporium*, *P. kashmirense* Paul, *P. afertile* Kanouse & Humphery, *P. rhizo-oryzae* Paul, *P. graminicola* Subraman, *P. vanterpoolii* Kouyeas & Kouyeas, and *P. torulosum* Cocker & Patt. *Globisporangium izadpanahii* sp. nov. was located in clade G of the ITS phylogenetic tree and was related to *G. coniferarum* Salmaninezhad & Mostowf, *G. nagaii* S. Ito & Tokun., *G. okanoganense* (Lipps) Uzuhashi, Tojo & Kakish, *G. paddicum* (Hirane) Uzuhashi, Tojo & Kakish, *G. iwayamai* (Ito) Uzuhashi, Tojo & Kakish, *G. canariense* (Paul) Uzuhashi, Tojo & Kakish, *G. violae* (Chesters & Hickman) Uzuhashi, Tojo

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& Kakish, and *G. cederbergense* (Bahramisharif, Botha & Lamprecht) Nguyen & Spies. The position of each new species was consistent in all phylogenetic trees.

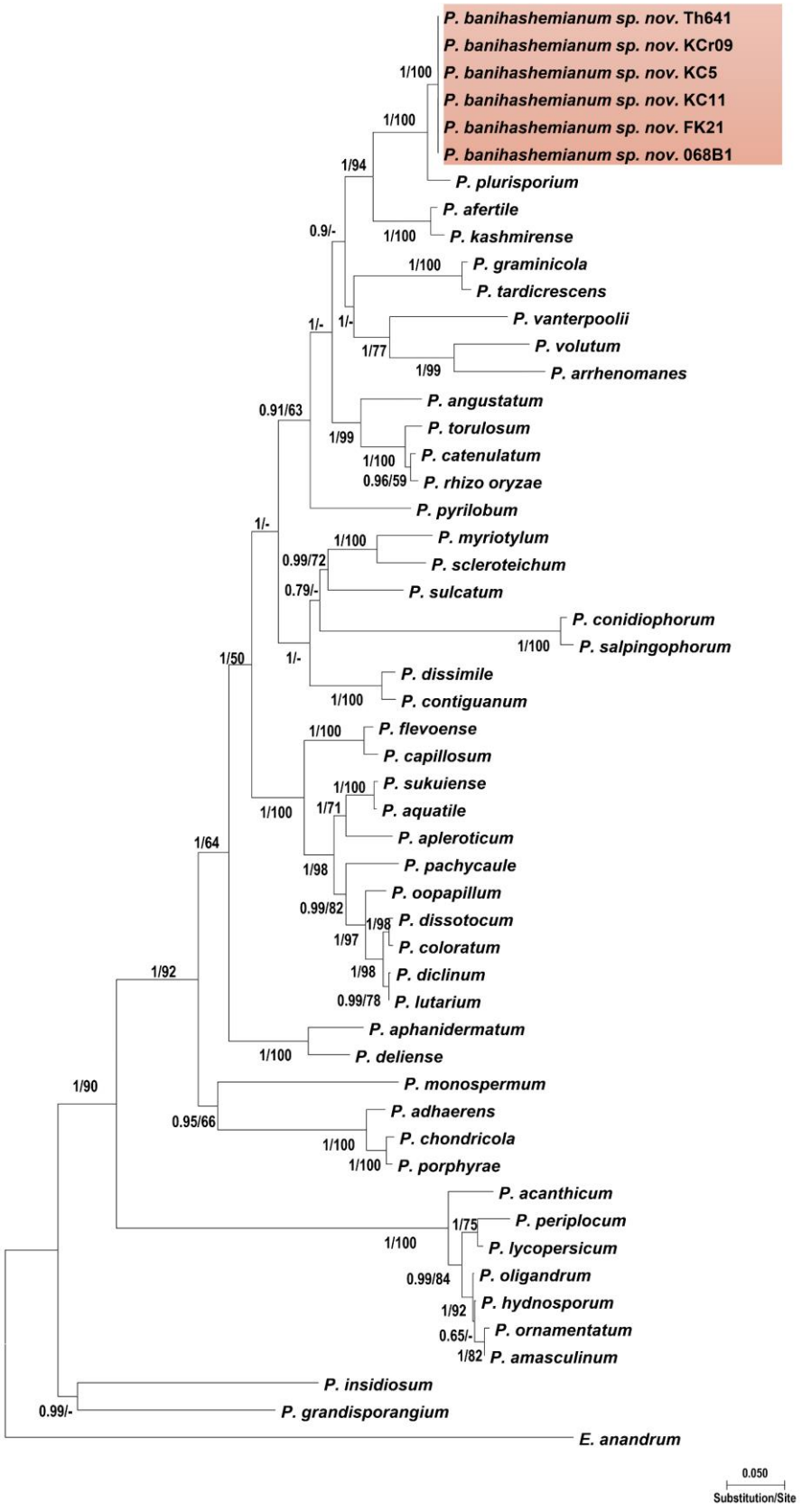

Figure 1. Phylogenetic relationships of *Pythium banihashemianum* from rice paddies of Fars Province among 46 *Pythium sensu stricto* species based on the analysis of multigene genealogies of nuclear (ITS and *Btub*) and mitochondrial (*cox1* and *cox2*) sequences in Maximum Likelihood tree. Numbers on

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branches represent posterior probability based on Bayesian analysis and the bootstrap support based on Maximum Likelihood, respectively.

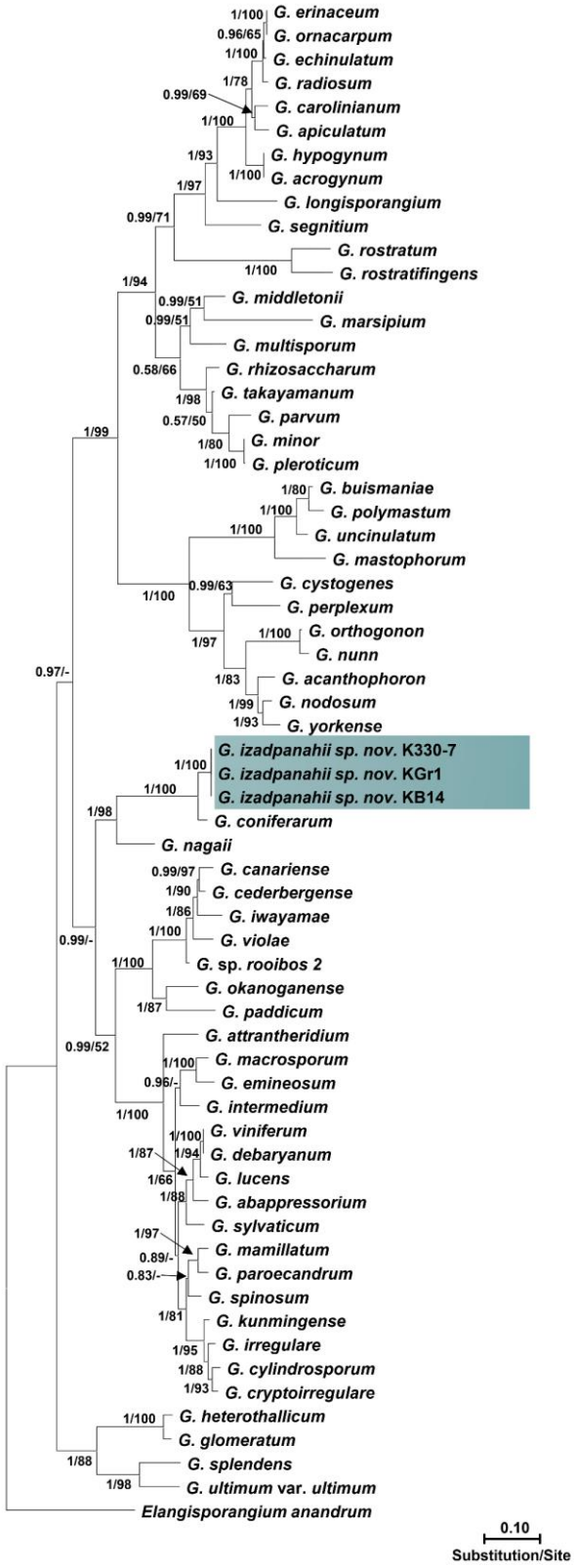

Figure 2. Phylogenetic relationships of *Globisporangium izadpanahii* from rice paddies of Fars Province among 46 *Globisporangium* species based on the analysis of multigene genealogies of nuclear (ITS and *Btub*) and mitochondrial (*cox1* and *cox2*) sequences in Maximum Likelihood tree. Numbers on branches represent posterior probability based on Bayesian analysis and the bootstrap support based on Maximum Likelihood, respectively.

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3.3. Pathogenicity

In pathogenicity tests (Table 2), *P. banihashemianum* sp. nov. was pathogenic on rice. The isolates of these species caused pre- and post-emergence damping-off, crown rot (Figure 3), seed rot, and a severe decrease in growth rate. They were re-isolated from symptomatic seedlings. Conversely control seedlings did not show any symptom. Isolates of *G. izadpanahii* sp. nov. did not induce any disease in seeds or rice seedlings and could not be reisolated from the roots and crowns of tested plants (Table 2).

Table 2. Pathogenicity results of the *Pythium sensu lato* species examined in this study.

Species	Isolate code	Pathogenicity on rice	Symptom					
			Post-emergence damping-off (%)	Pre-emergence damping-off (%)	Seed rot (%)	Stunting (%)	No growth (%)	Host tissue colonization*
Pythium banihashemianum								
	068B1**	+	80	70	80	-	60	+
	Fk21**	+	70	60	90	-	70	+
	Th641**	+	90	60	40	-	50	+
	KC11†	+	70	90	80	-	80	+
	KC5†	+	80	60	60	-	70	+
	KCr09†	+	90	50	70	-	50	+
Globisporangium izadpanahii								
	K330-7	-	-	-	-	-	-	-
	KB14	-	-	-	-	-	-	-
	KGr1	-	-	-	-	-	-	-

*(+) positive and (-) negative results, **= Morphology Group I, † = Morphology Group II.

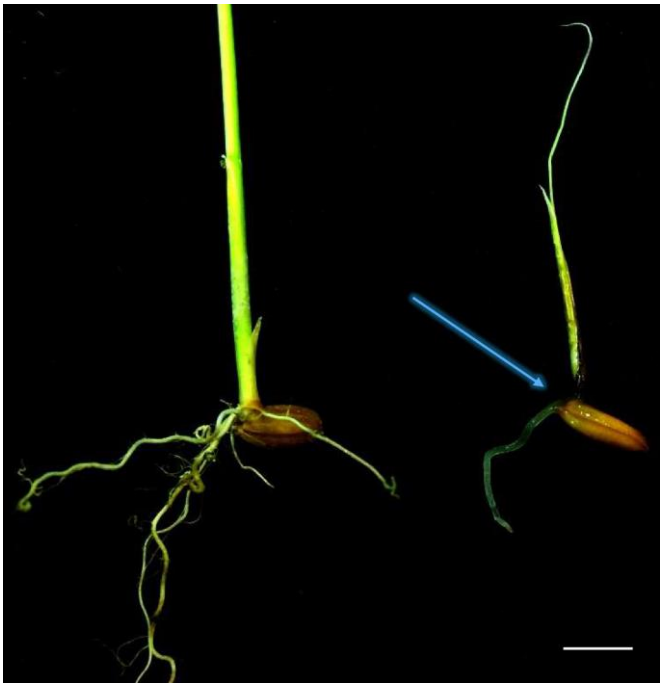

Figure 3. Pathogenicity tests on roots and crown of rice (*Oryzae sativa*). Root and crown rot caused by *Pythium banihashemianum* (068B1) (left: control; right: infected crown and roots). Bar = 1 cm.

3.4. Taxonomy

Pythium banihashemianum Mostowf. & Salmanin. ***sp. nov.*** (Figures 1, 4, 6 and 7)
MycoBank: MB824523

Typification: IRAN, Fars province: Kamfiruz (30°16.934'N−052°19.155'E), from roots of *Oryza sativa*, 16 Aug 2015, F. Salmaninezhad 068B1 (**holotype** CBS 143876, living culture preserved in a

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metabolically inactive state at Westerdijk Fungal Biodiversity Institute). GenBank: ITS = KX228083; *βtub* = KX228113; *cox*1 = OP321097; *cox*2 = KX228120.

Etymology: After Prof. Ziaeddin Banihashemi, who is a pioneer in oomycete studies in Iran.

Two different group of isolates are identified based on their morphological characteristics.

Group I: Colonies on PDA and HSA show a rosette pattern, on CA show an intermediate pattern and on MEA and CMA show chrysanthemum and radial pattern, respectively (Figure 4a). *Main hypha:* 3.1–4.5 (av. 3.5) µm width. *Sporangia*: not observed on solid media but produce abundantly in aqueous medium containing sterile hemp seeds, filamentous, slightly inflated to rarely dendroid (Figure 5e). *Zoospores*: released through a discharge tube 50-110 µm long. *Hyphal swellings:* not present. *Oogonia*: smooth, rarely globose [29.8–37.4 (av. 33.3) µm] (Figure 5), ovoid, jug shaped, sometimes without any specific shape (Figure 5m), mostly (more than 80%) with two adjacent projections. *Oogonial projections*: 0.5–0.9 (av. 0.7) µm long (Figure 5h). *Antheridia*: 4–8 per oogonium, clavate and crook–necked, making apical or lateral contact, paragynous, monoclinous and diclinous with very long stalk which mostly encircle around oogonia (Figure 5k). Each oogonium contains more than one oospore (up to 3). *Oospores*: aplerotic, globose to subglobose, 28.1–35.5 (av. 32.5) µm diam., with a wall of 1.4–2 (av. 1.7) µm thick. Oospore formation is specific, oogonium stalk initially swells, leading to the first oospore formation. Subsequently, the terminal section of oogonium swells, and the oospore moves into this section resulting in the formation of a second oospore in the oogonium swollen stalk (Figure 5i). Morphometric characteristics are shown in Table 3. Colonies on PDA have an average radial growth rate of 2.5 mm d^{-1} at 10 °C, 5 mm d^{-1} at 15 °C, 7 mm d^{-1} at 20 °C, 10 mm d^{-1} at 25 °C and 30 °C, 11 mm d^{-1} at 35 °C, 1 mm d^{-1} at 40 °C, no growth occurred at 5 °C. *Cardinal temperatures*: minimum 10 °C, optimum 35 °C, and maximum 40 °C (Figure 6).

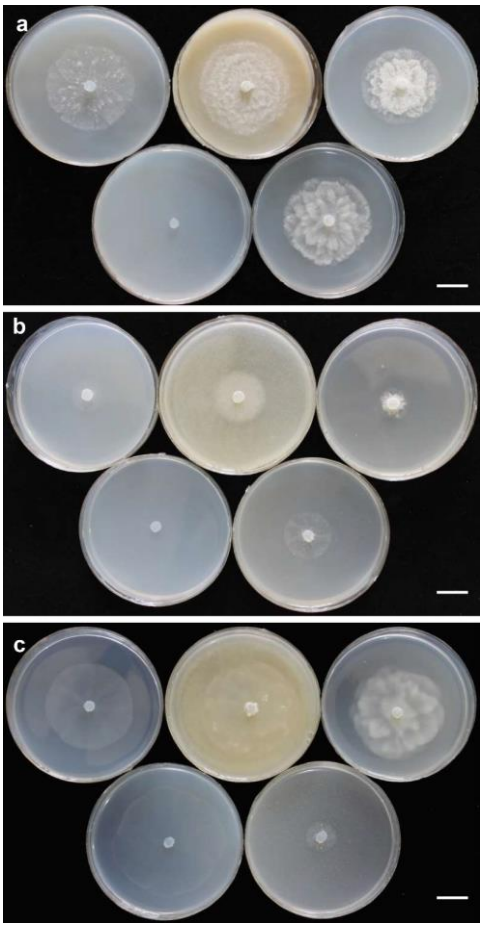

Figure 4. Colony morphology of *Pythium banihashemianum* Group I isolate 068B1 (a), *P. banihashemianum* Group II, isolate KC5 (b), and *Globispirangium izadpanahii* isolate K330-7 (c) after 24 h on various media at 25 °C; top (from left to right): carrot agar, malt extract agar and potato-dextrose agar; bottom (from left to right): cornmeal agar and hemp seed agar. Bar = 1 cm.

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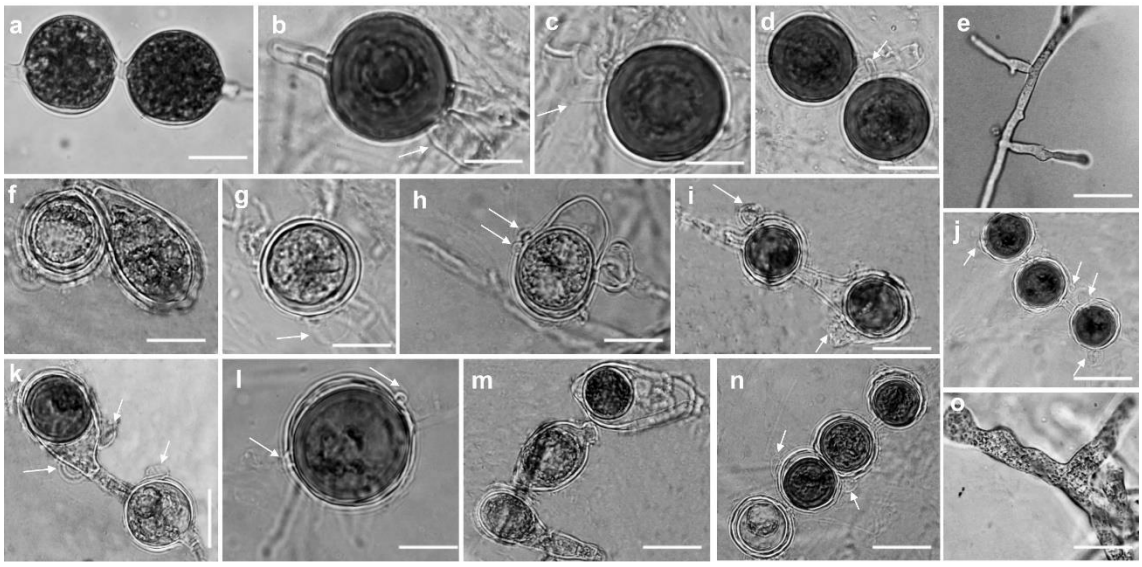

Figure 5. Morphological structures of *Globisporangium izadpanahii* (a-d) and *Pythium banihashemianum* (e-o). a: hyphal swellings; b: intercalary oogonium with a single clubbed shape antheridium; c: smooth oogonium with paragynous antheridium; d: perfectly plerotic oospore with a long papilla; e: filamentous sporangium (Group I); f: smooth-walled ovoid oospores (Group I); g: aplerotic oospore with a single antheridium (Group II); h: formation of oospore in oogonium with two papillae (Group I); i: oogonium with two oospores (final formation of oospores in a single oogonium) (Group I); j: catenulate oospores with two paragynous antheridia (arrows) per oogonium (Group II); k: aplerotic catenulate oospores with two monoclinous antheridia (arrows) per oogonium (Group II); l: oogonium with single oospore with two symmetrical papilla (Group II); m: aplerotic oospores with no specific shape (Group I); n: catenulate oospores with both mono- and diclinous antheridia (Group II); o: slightly inflated filamentous sporangium. Bars: = 10 µm, except for e and o where Bar = 20 µm.

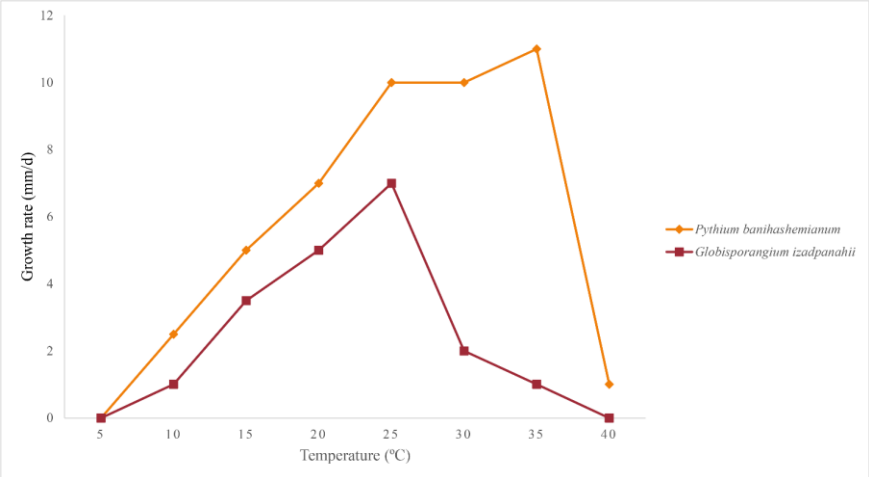

Figure 6. Average radial growth rate of *Pythium banihashemianum* (16 isolates), and *Globisporangium izadpanahii* (five isolates) on potato-dextrose agar at different temperatures.

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Table 3. Morphological characters and dimensions (µm) of *Pythium sensu lato* species acquired from rice paddies of Fars Province, Iran.

Species	Isolate	Sporangium/hyphal swelling			Main hyphae		Oogonium			Oospore				Antheridium		
		Shape	Average	Range	Average	Range	Shape	Average	Range	Type	Average	Range	Wall	Shape	Average	Range
Pythium banihashemianum																
	068B1*	Filamentous, slightly inflated	Variable	Variable	3.5±0.5	3.1–4.5	Globose to subglobose	33.3±3.3	29.8–37.4	Aplerotic	32.5±1.3	28.1–35.5	1.7±0.7	Crook-necked	6.1 × 16.2	6.0 × 15.9–6.4 × 16.7
	Fk21*	Filamentous, slightly inflated	Variable	Variable	3.7±0.5	4.0–4.9	Globose to subglobose	35.7±1.7	30.5–38.4	Aplerotic	30.9±0.6	23.6–41.7	1.3±0.5	Crook-necked	5.9 × 16.3	4.8 × 15.7–6.7 × 16.9
	Th261*	Filamentous, slightly inflated	Variable	Variable	3.5±1.1	3.3–4.1	Globose to subglobose	34.5±0.8	30.7–37.0	Aplerotic	31.8±0.7	24.8–42.6	1.4±0.7	Crook-necked	6.0 × 15.7	4.9 × 13.7–6.7 × 16.3
	Fs301*	Filamentous, slightly inflated	Variable	Variable	3.6±1.0	2.9–3.8	Globose to subglobose	34.0±0.7	28.5–35.1	Aplerotic	31.5±0.5	27.8–41.0	1.6±1.0	Crook-necked	6.1 × 16.7	5.9 × 14.0–6.7 × 17.6
	F32-01*	Filamentous, slightly inflated	Variable	Variable	3.0±0.1	3.2–4.5	Globose to subglobose	33.3±0.9	30.1–38.0	Aplerotic	30.8±1.7	26.9–40.6	1.4±0.5	Crook-necked	5.7 × 15.9	4.5 × 13.0–6.6 × 16.3
	F201-3*	Filamentous, slightly inflated	Variable	Variable	3.5±1.4	3.1–4.3	Globose to subglobose	35.1±1.8	32.2–36.3	Aplerotic	31.0±0.8	25.7–41.0	1.5±1.7	Crook-necked	5.6 × 14.4	4.4 × 13.9–6.6 × 16.0
	048S1*	Filamentous, slightly inflated	Variable	Variable	3.3±1.0	3.0–3.8	Globose to subglobose	37.1±1.0	33.2–39.0	Aplerotic	32.8±0.1	24.6–42.7	1.3±0.9	Crook-necked	6.2 × 16.3	5.9 × 14.7–6.5 × 16.8
	038C3*	Filamentous, slightly inflated	Variable	Variable	3.2±1.3	4.3–4.5	Globose to subglobose	37.5±0.5	35.0–39.3	Aplerotic	30.6±0.9	25.8–41.6	1.5±0.6	Crook-necked	6.0 × 15.0	4.8 × 13.3–6.7 × 16.0
	033B7*	Filamentous, slightly inflated	Variable	Variable	3.5±0.5	4.0–4.7	Globose to subglobose	36.9±0.2	31.5–37.7	Aplerotic	31.7±1.4	26.6–40.9	1.6±1.1	Crook-necked	6.1 × 15.5	5.9 × 14.7–6.7 × 16.3

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056S2*	Filamentous, slightly inflated	Variable	Variable	3.7±1.5	3.1–4.4	Globose to subglobose	35.7±0.4	31.9–37.0	Aplerotic	31.5±0.4	24.6–42.2	1.4±0.8	Crook-necked	5.9 × 14.8	4.9 × 13.7–6.7 × 16.6
G112-2**	Filamentous, slightly inflated	Variable	Variable	3.5±0.8	2.8–4.0	Globose to subglobose	34.7±0.7	30.7–36.2	Aplerotic	32.8±0.5	23.8–41.0	1.5±1.3	Crook-necked	6.3 × 16.9	5.5 × 14.9–6.8 × 17.6
K101-4**	Filamentous, slightly inflated	Variable	Variable	3.9±0.3	3.6–4.2	Globose to subglobose	35.9±1.0	32.1–37.4	Aplerotic	31.6±1.0	25.0–40.9	1.7±0.1	Crook-necked	6.4 × 17.0	6.0 × 15.7–7.2 × 18.3
KC11**	Filamentous, slightly inflated	Variable	Variable	3.1±0.7	2.7–4.0	Globose	38.8±1.3	28.1–40.5	Aplerotic	32.4±0.6	26.7–36.4	1.5±0.5	Crook-necked	7.1 × 13.7	6.4 × 13.0–7.7 × 14.0
KC5**	Filamentous, slightly inflated	Variable	Variable	3.0±0.5	2.8–4.4	Globose	36.9±1.2	30.0–37.1	Aplerotic	33.9±0.7	27.5–34.2	1.4±0.2	Crook-necked	7.5 × 14.5	6.0 × 14.2–8.0 × 16.0
KCr09**	Filamentous, slightly inflated	Variable	Variable	3.1±1.1	2.9–4.2	Globose	37.0±0.2	29.8–38.7	Aplerotic	35.2±0.5	30.5–36.2	1.7±0.5	Crook-necked	7.7 × 13.9	6.0 × 13.9–8.3 × 15.7
Globisporangium izadpanahii															
K330-7	Globose	13.2±0.5	12.9–13.7	4.3±0.5	4.0–4.8	Globose	61.7±0.5	60.0–63.9	Plerotic	61.7±0.5	60.0–63.9	9.2±0.2	Clavate	25.4±0.4	21.9–28.4
KB14	Globose	12.9±0.2	12.0–13.5	4.0±1.5	3.9–5.0	Globose	61.5±1.7	60.5–62.4	Plerotic	61.5±1.7	60.5–62.4	7.3±0.5	Clavate	24.2±1.0	20.7–28.0
KGr1	Globose	13.0±0.7	12.7–13.8	4.7±1.1	4.5–5.7	Globose	60.6±0.8	59.7–62.8	Plerotic	60.6±0.8	59.7–62.8	8.4±0.7	Clavate	27.1±0.5	23.1–29.5
Rfa01	Globose	12.9±1.5	12.5–13.7	4.5±1.0	4.3–5.6	Globose	61.0±0.6	59.5–62.9	Plerotic	61.0±0.6	59.5–62.9	8.6±0.6	Clavate	27.2±1.1	24.5–30.0
KHa3	Globose	13.1±0.8	12.4–13.3	4.3±0.9	4.0–5.5	Globose	60.9±0.8	58.9–62.7	Plerotic	60.9±0.8	58.9–62.7	9.0±0.3	Clavate	25.5±0.5	22.9–29.0

*= Morphology Group I, **= Morphology Group II.

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Group II: Colonies show a radial pattern on CA and HSA, a uniform pattern on CMA, an intermediate pattern on PDA and no specific pattern on MEA (Figure 4b). *Main hyphae*: 2.7–4.0 (av. 3.1) µm in width. *Sporangia*: filamentous and inflated, never observed on solid media and produced abundantly on aqueous medium with sterile hemp seeds. (Figure 5o). *Zoospores*: released after 12 h from 61–115 µm long discharge tubes. The mycelium grows easily on HSA and CA, producing abundant oogonia, antheridia and oospores. Two kinds of oospore formation are observed: single (Figures 5g, and l) and catenulate (Figures 5j and n). *Oogonia*: smooth, globose 28.1-40.5 (av. 38.3) µm, terminal, mostly with more than one oospore (up to 5 with catenulate formation) (Figure 5n). More than 80% of the oogonia contain two papillae on both sides which are 1.1–2.3 (av. 1.7) µm long (Figure 5l). *Antheridia*: 1–2 per oogonia with catenulate oospores (Figure 5j) and rarely (less than 5%) up to 4 per oogonia with a single oospore, crook–necked, making apical contact, paragynous, mostly monoclinous, rarely diclinous. *Oospores*: globose, aplerotic, smooth, most (more than 90%) catenulate, 26.7–36.4 (av. 32.4) µm diam., with a wall of 0.8–3.0 (av. 1.5) µm thick. Morphometrical results are shown in Table 3. Colonies on PDA have an average radial growth rate of 1 mm d^{-1} at 5 °C, 2 mm d^{-1} at 10 °C, 3 mm d^{-1} at 15 °C, 5 mm d^{-1} at 20 °C, 7 mm d^{-1} at 25 °C, 12 mm d^{-1} at 30 °C, 9 mm d^{-1} at 35 °C and 1 mm d^{-1} at 40 °C. *Cardinal temperatures*: minimum 5 °C, optimum 30 °C, and maximum 40 °C (Figure 6).

Other specimens examined: IRAN. Fars province: Kamfiruz (30°11.017'N–052°27.900'E), from rhizosphere of *Oryzae sativa*, 16 Aug 2015, F. Salmaninezhad K116-1. IRAN, Fars province: Kamfiruz (30°11.845'N–052°27.787'E), from pond water of paddy fields, 20 May 2014, F. Salmaninezhad K101-4. IRAN, Fars province: Kamfiruz (30°11.911'N–052°27.777'E), from rhizosphere of *O. sativa*, 20 May 2014, F. Salmaninezhad G112-2. IRAN, Fars province: Kamfiruz (30°11.909'N–052°27.779'E), from the soil of paddy fields, 20 May 2014, F. Salmaninezhad 056S2. IRAN, FARS province: Ramjard (30°07.274'N–052°32.946'E), from rhizosphere of *O. sativa*, 9 Nov 2015, F. Salmaninezhad 033B7. IRAN, Fars province: Ramjard (30°07.234'N–052°32.983'E), from roots of *O. sativa*, 9 Nov 2015, F. Salmaninezhad 038C3. IRAN, Fars province: Ramjard (30°02.780'N–052°49.513'E), from the roots of *O. sativa*, 9 Nov 2015, F. Salmaninezhad 048S1. IRAN, Fars province: Firuz Abad (28°51.587'N–052°30.842'E), from rhizosphere of *O. sativa*, 20 May 2014, F. Salmaninezhad F201-3. IRAN, Fars province: Firuz Abad (28°51.407'N–052°30.666'E), from *O. sativa* roots, 9 Nov 2015, F. Salmaninezhad Fk21. GenBank: ITS = MK454539; *βtub* = MK540655; *cox*1= OP321098; *cox*2 = MK455862. IRAN, Fars province: Firuz Abad (28°49.735'N–052°29.149'E), from *O. sativa* roots, 9 Nov 2015, F. Salmaninezhad Fs301. IRAN, Fars province: Firuz Abad (28°49.989'N–052°29.551'E), from rhizosphere of *O. sativa*, 20 May 2014, F. Salmaninezhad F32-01. IRAN, Fars province: Persepolis (29°59.008'N–052°49.513'E), from rhizosphere of *O. sativa*, 16 Aug 2015, F. Salmaninezhad Th641. GenBank: ITS = MK454538; *βtub* = MK540656; *cox*1= OP321102; *cox*2 = MK455863. IRAN, Fars province: Persepolis (29°58.892'N–052°57.734'E), from rhizosphere of *O. sativa*, 16 Aug 2014, F. Salmaninezhad KC5. GenBank: ITS = MK454707; *βtub* = MK455865; *cox*1=OP321099; *cox*2 = MK455856. IRAN, Fars province: Ramjard (30°05.901'N–052°35.482'E), from *O. sativa* roots, 16 Aug 2014, F. Salmaninezhad KCr09. GenBank: ITS = MK454706; *βtub* = MK455864; *cox*1=OP321101; *cox*2 = MK455857. IRAN, Fars province: Ramjard (30°05.476'N–052°35.563'E), from *O. sativa* crown, 9 Nov 2015, F. Salmaninezhad KC11 (CBS 143875). MB824524. GenBank: ITS = KX228081; *βtub* = MK455866; *cox*1= OP321100; *cox*2 = MK455858.

Notes: This species belongs to the clade B of the ITS phylogenetic tree *sensu* Lévesque and de Cock [16] and is closely related to *P. plurisporium* (Figure 1). *Pythium banihashemianum* sp. nov. differs from all other *Pythium* species from clade B by its high-temperature tolerance, amorphous oogonia with more than one oospore, and from *P. plurisporium* by producing a high proportion of papillate oogonia, containing more than one papilla in most oogonia, the presence of two papillae on oogonia, the special formation of oospores, and its unique sequences of mitochondrial and nuclear genes. Adjacent papillae were abundant in isolate 068B1. Isolates were recovered from rice paddies in the north and northwestern regions of Fars Province of Iran.

Globisporangium izadpanahii Salmanin. & Mostowf. ***sp. nov.*** (Figures 2, 4, 5 and 7)

MycoBank: MB824525

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Typification: IRAN, Fars province: Firuz Abad (28°49.989'N−052°29.551'E), from rhizosphere of *Oryzae sativa* nursery, 9 Nov 2015, F. Salmaninezhad K330-7 (**holotype** CBS 144006, living culture preserved in a metabolically inactive state at Westerdijk Fungal Biodiversity Institute). GenBank: ITS = MK454537; *βtub* = MK455869; *cox*1 = OP321103, *cox*2 = MK455859.

Etymology: After Prof. Keramatollah Izadpanah, who is a leading phytopathologist in Iran.

Colonies on PDA and MEA show a rosette pattern and on HSA, CMA and CA a radial pattern (Figure 4c). *Sporangia and zoospores*: not produced. *Hyphal swelling*: terminal or intercalary, formed in aqueous medium after one week, 12.9–13.7 (av. 13.2) µm in diam, never observed on solid media (Figure 5a). *Main hyphae*: 4.0–4.8 (av. 4.3) µm in width. *Oogonia*: globose, smooth, terminal or intercalary, 62.0–63.9 (av. 63.0) µm diam (Figures 5b, c, and d), most contain a needle shaped papilla up to 0.8–3.1 (av. 1.0 µm) long (Figure 5d). *Antheridia:* just one per oogonium, crook–necked, elongated, and clavate, mostly monoclinous, rarely diclinous, making apical contact with oogonium, paragynous and sometimes hypogynous (Figures 5b and c). *Oospores*: globose, perfectly plerotic, with a wall which is up to av. 9.2 µm thick. Morphometric characteristics are shown in Table 3. Colonies on PDA have an average radial growth rate of 1 mm d^{-1} at 10 °C, 3.5 mm d^{-1} at 15 °C, 5 mm d^{-1} at 20 °C, 7 mm d^{-1} at 25 C, 2 mm d^{-1} at 30 °C, 1 mm d^{-1} at 35 °C and no growth at 5 °C and 40 °C. *Cardinal temperatures*: minimum 10 °C, optimum 25 °C, and 35 °C (Figure 6).

Other specimens examined: IRAN, Fars province: Kamfiruz (29°58.823'N−052°53.651'E), from *Oryzae sativa* crown, 9 Nov 2015, F. Salmaninezhad KGr1. GenBank: ITS = MK454535; *βtub* = MK455867; *cox*1=OP321105; *cox*2 = MK455861. IRAN, Fars province: Ramjard (30°06.139'N−052°26.892'E), from rhizosphere of *Oryzae sativa*, 9 Nov 2015, F. Salmaninezhad Rfa01. IRAN, Fars province: Kamfiruz (30°18.134'N−052°17.767'E), from rhizosphere of *O. sativa*, 9 Nov 2015, F. Salmaninezhad KHa3. IRAN, Fars province: Kamfiruz (30°19.236'N−052°16.560'E), from pond water of paddy fields, 9 Nov 2015, F. Salmaninezhad KB14. GenBank: ITS = MK454536; *βtub* = MK455868; *cox*1=OP321104; *cox*2 = MK455860.

Notes: This species belongs to the clade G of the ITS phylogenetic tree *sensu* Lévesque and de Cock [16] in the vicinity of *G. coniferarum* and *G. nagaii* (Figure 1). *Globisporangium izadpanahii* sp. nov. does not form sporangia and zoospores under standard conditions tested including different temperatures. However, the formation of hyphal swellings in aqueous medium after one week, the unique type of oogonia with a long needle-shaped papilla, strictly plerotic oospores, special and unique growth pattern on various media, and especially, the presence of an elongated clavate antheridium differentiated this species from other known *Globisporangium* species. Additionally, the unique sequences of mitochondrial and nuclear genes separated *G. izadpanahii* sp. nov. from other species. Isolates were recovered from rice paddies in the northwestern and southwestern regions of Fars Province of Iran.

4. Discussion

This study is part of a larger project aimed at investigating the diversity of *Pythium s.l.* populations in rice paddies of Fars Province in Iran. Among more than a thousand *Pythium s.l.* isolates recovered, 16 already known species and three new *Pythium* species, *P. heteroogonium*, *P. longipapillum* and *P. oryzicollum*, had been previously identified on the basis of morphological and molecular traits [43,46,65]. In the present paper, two groups of isolates from the same large set of isolates recovered from rice paddies, showing distinctive morphological characters and forming two separate well-supported monophyletic lineages, were characterized and formally described as new species, *P. banihashemianum* and *G. izadpanahii*, respectively. The species diversity of *Pythium s.l.* in rice paddies of Fars Province [43,46] indicates the aquatic environment of this peculiar type of managed ecosystems offers a favorable ecological niche to these oomycetes.

According to phylogenetic analysis, *Pythium banihashemianum* sp. nov. grouped within the clade B of the ITS phylogenetic tree of *Pythium sensu stricto* but in a separate lineage from other known species. The closest relatives of this species are *P. plurisporium*, *P. kashmirense* and *P. afertile*. The isolates assigned to *P. banihashemianum* sp. nov. were in turn split into two diverse morphotypes. Both groups produced filamentous-type sporangia. However, the sporangia produced by the first

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morphotype (Group I) were mostly dendroid while isolates of the second morphotype (Group II) produced mostly inflated sporangia. In contrast to Group I, which produced one to three oospores in a single oogonium, Group II produced mostly one oospore per oogonium. Besides, the existence of asymmetrical oogonia with papillae separates morphotype I from morphotype II, which formed catenulated globous oogonia. The number of antheridia per oogonium was up to eight in Group I and occasionally up to four in Group II. So great intraspecific morphological variability is a rare, yet interesting phenomenon that was previously reported for *P. plurisporium*, a closely related species to *P. banihashemianum* [66]. *Pythium plurisporium* isolates form two different types of oogonia, with a single oospore or with more than one oospore [66,67]. While both *P. banihashemianum* sp. nov. and *P. plurisporium* have more than one oospore per oogonium, the number of oospores per oogonium in *P. banihashemianum* sp. nov. (up to at most 3) is less than in *P. plurisporium* (up to 6) [66,67]. Moreover, unique formation of oospores in *P. banihashemianum* sp. nov. clearly separates it from *P. plurisporium* and any other *Pythium* species described so far.

The existence of sexual structures in *P. banihashemianum* sp. nov. clearly separates it from *P. afertile,* which does not reproduce sexually. *Pythium kashmirense* and morphotype II of *P. banihashemianum* sp. nov. have sporangial type in common. A difference between the two species is that in *P. kashmirense* antheridial filaments coil around the oogonial stalks [68]. Although ITS and *βtub* phylogenetic trees could not differentiate *P. banihashemianum* sp. nov. from *P. plurisporium*, mitochondrial loci phylogeny clearly separated them from each other, showing *P. banihashemianum* sp. nov. is a new distinct species. Consistently with Robideau *et al.* [23] and Hyde *et al.* [1], more than one gene phylogeny was needed to separate *P. banihashemianum* sp. nov. from *P. plurisporium*.

Globisporangium izadpanahii sp. nov. was a sister taxon to *G. coniferarum* Salmanin. & Mostowf. and *G. nagaii*. This species formed a monophyletic separate lineage in all phylogenetic trees and was located in clade G of *Pythium s.l.* Although polytomy was observed in *cox*1 loci analyses, *G. izadpanahii* sp. nov. location in clade G was highly supported by analyzing other loci. Polytomy in *cox*1 analyses was also observed previously in *Pythium s.l.* [1,23]. Specific type of antheridia, and the absence of sporangia, vesicle or zoospore formation separated *G. izadpanahii* sp. nov. from other described species. There are fundamental morphological differences between *G. izadpanahii* sp. nov. and its sister species *G. coniferarum* and *G. nagaii.* In contrast to *G. izadpanahi* sp. nov., *G. coniferarum* produces ovoid to ellipsoid sporangia with vesicles and zoospores [55]. Furthermore, one of the key characteristics of *G. coniferarum* is the production of abundant chlamydospores and different shapes of oogonia (from globose to ovoid and ellipsoid) [55], while in *G. izadpanahii* sp. nov. isolates chlamydospores and oogonia were exclusively globose. Production of terminal, ovoid to pyriform and proliferating sporangia in *G. nagaii* [2] also separates it from *G. izadpanahii* sp. nov. Besides, in *G. nagaii* oogonia are terminal and globose, with aplerotic oospores, and the antheridia disappear soon after fertilization [2]. Such characters have never been observed in *G. izadpanahii* sp. nov. isolates. Even though *G. izadpanahii* sp. nov. is a member of clade G, other members of this clade show no similar morphological characteristics to this species.

The presence of multiple divergent copies of the ITS region is a well-known phenomenon among *Pythium s.l.* species and was reported previously for *G. coniferarum* [55]. Analyzing *Globisporangium izadpanahii* sp. nov. ITS sequences of the clones showed that the ITS region had many insertions, which led to the overlapping of the direct sequences, disruption of the electropherograms and consequently low-quality sequences. Furthermore, *G. izadpanhaii* sp. nov. isolates also showed intraspecific ITS sequence heterogeneity. Using the resulting contigs of ITS clones, we showed that *G. izadpanahii* is a new species located in a separate lineage close to *G. nagaii.* Other loci sequences (i.e., *Btub, cox*1, and *cox*2) showed a very high quality and strongly supported the separation of this new species.

The two novel species described in this study differed from each other in some characteristics, such as cardinal temperatures for growth and pathogenicity on rice, which have ecological implications. *Pythium banihashemianum* sp. nov. was a severe pathogen of rice seedlings, causing pre- and post-emergence damping-off, as well as root, crown, and seed rot. *Globisporangium izadpanahii*

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sp. nov. isolates did not cause any symptom on rice seedlings, and were not able to colonize root and crown tissues.

5. Conclusions

The description of two novel species, *P. banihashemianum* and *G. izadpanahii*, in addition to those identified previously, contributes to the advancement of the systematics of genera segregated from the *Pythium s.l.* complex. This study confirms that rice paddies are a wide repository of diversity of these oomycetes. In pathogenicity tests on rice seedlings, *P. banihashemianum* was proved to be an aggressive pathogen while *G. izadpanahii* was not pathogenic, indicating species of *Pythium s.l.* may have multiple and different ecological roles in these agricultural ecosystems. The agronomic, phytopathological and taxonomic relevance of unveiling the diversity of *Pythium s.l.* populations in managed ecosystems would encourage extending the study to other geographic areas and diverse cereal crops.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Phylogenetic relationships of *Pythium banihashemianum* from rice paddy fields of Iran among 60 *Pythium s.s.* species based on Maximum Likelihood of internal transcribed spacers 1, 2 and 5.8S gene of rDNA sequences. Numbers above the branches represent posterior probability based on Bayesian analysis, bootstrap support based on Maximum Likelihood analyses; Figure S2: Phylogenetic relationships of *Globisporangium izadpanahii* (from rice paddy fields of Iran among 75 *Globisporangium* species based on Maximum Likelihood analysis of internal transcribed spacers 1, 2 and 5.8S gene of rDNA sequences. Numbers above the branches represent posterior probability based on Bayesian analysis, bootstrap support based on Maximum Likelihood analyses; Figure S3: Phylogenetic relationships of *Pythium banihashemianum* from rice paddy fields of Iran among 58 *Pythium s.s.* species based on Maximum Likelihood analysis of cytochrome c oxidase subunit I sequences. Numbers above the branches represent posterior probability based on Bayesian analysis, bootstrap support based on Maximum Likelihood analyses; Figure S4: Phylogenetic relationships of *Globisporangium izadpanahii* from rice paddy fields of Iran among 61 Globisporangium species based on Maximum Likelihood analysis of cytochrome c oxidase subunit I sequences. Numbers above the branches represent posterior probability based on Bayesian analysis, bootstrap support based on Maximum Likelihood analyses; Figure S5: Phylogenetic relationships of *Pythium banihashemianum* from rice paddy fields of Iran among 54 *Pythium s.s.* species based on Maximum Likelihood analysis of cytochrome c oxidase subunit II sequences. Numbers above the branches represent posterior probability based on Bayesian analysis, bootstrap support based on Maximum Likelihood analyses; Figure S6: Phylogenetic relationships of *Globisporangium izadpanahii* from rice paddy fields of Iran among 75 *Globisporangium* species based on Maximum Likelihood analysis of cytochrome c oxidase subunit II sequences. Numbers above the branches represent posterior probability based on Bayesian analysis, bootstrap support based on Maximum Likelihood analyses; Figure S7: Phylogenetic relationships of *Pythium banihashemianum* from rice paddy fields of Iran among 33 *Pythium s.s.* species based on Maximum Likelihood analysis of Beta tubulin sequences. Numbers above the branches represent posterior probability based on Bayesian analysis, bootstrap support based on Maximum Likelihood analyses; Figure S8: Phylogenetic relationships of *Globisporangium izadpanahii* from rice paddy fields of Iran among 38 *Globisporangium* species based on Maximum Likelihood analysis of Beta tubulin sequences. Numbers above the branches represent posterior probability based on Bayesian analysis, bootstrap support based on Maximum Likelihood analyses; Table S1: List of primers used in this study with their PCR conditions; Table S2: *Globisporangium* spp. isolate codes and GeneBank accession numbers for phylogenetic analyses comparison; Table S3: *Pythium sensu stricto* isolate codes and GenBank accession numbers for phylogenetic analyses comparison; Table S4: Base pair differences across *Btub*, ITS, *cox*1, and *cox*2 sequences showing the inter- and intraspecific variation of *Globisporangium izadpanahii* (IZA) and other related species, including *G. coniferarum* (CON), *G. nagaii* (NAG), *G. violae* (VIO), *G. okanoganense* (OKA), *G. canariense* (CAN), *G. monoclinum* (MON), and *G. iwayamae* (IWA); Table S5: Base pair differences across *Btub*, ITS, *cox*1, and *cox*2 sequences showing the inter- and intraspecific variation of *Pythium banihashemianum* (BAN) and other related species, including *P. plurisporium* (PLU), *P. afertile* (AFE) and *P. kashmirense* (KAS); Table S6: Morphological comparison of the species described in this study with their related species

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, F.S. and R.M.-G; methodology, F.S. and R.M.-G; software, F.S. and R.M.-G; validation, F.S and R.M.-G; formal analysis, F.S.; investigation, F.S.; resources, R.M.-G and S.O.C.; data curation, F.S.; writing—original draft preparation, F.S.;

writing—review and editing, F.S., R.M.-G. and S.O.C.; visualization, F.S.; supervision, R.M.-G.; project administration, R.M.-G.; funding acquisition, R.M.-G. and S.O.C. All authors have read and agreed to the published version of the manuscript.

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