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Morphological and molecular taxonomy of *Pythium monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., and *P. iranense*, sp. nov., from Iran

Farzaneh BADALI, Masoud ABRINBANA & Jafar ABDOLLAHZADEH

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Morphological and molecular taxonomy of *Pythium monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., and *P. iranense*, sp. nov., from Iran

Farzaneh BADALI
Department of Plant Protection, Faculty of Agriculture and Natural Resources, Urmia University, P.O. Box 165, Urmia (Iran)

Masoud ABRINBANA
Department of Plant Protection, Faculty of Agriculture and Natural Resources, Urmia University, P.O. Box 165, Urmia (Iran)
m.abrinbana@urmia.ac.ir (corresponding author)

Jafar ABDOLLAHZADEH
Department of Plant Protection, Faculty of Agriculture, University of Kurdistan, P.O. Box 416, Sanandaj (Iran)

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ABSTRACT
Two new species of *Pythium* Pringsheim isolated from soils of northwestern regions in Iran, are described: *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., and *P. iranense*, sp. nov. Both species are morphologically distinct from all known species. *Pythium monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., is morphologically characterized by the presence of a swollen element in oogonial stalks below terminal oogonia, mostly monoclinous antheridia originating at various distances from the oogonia that occasionally arising from inflated part of oogonial stalk and, rarely production of peanut-shaped and double oospores. *Pythium iranense*, sp. nov., is distinguished from other species of the genus by the production of smooth-walled oogonia that occasionally have 1-3 papillae, globose to rarely elongated oogonia, 1-2(-5) antheridia per oogonium or crowd of antheridia around oogonia, 1-2 rarely 3-4 oospores per oogonium, rarely peanut-shaped oospores and immature oospores provided with 1-6 projections. Phylogenetic relationships of the isolates belonging to these new species with other related species were investigated using internal transcribed spacers of rRNA genes and partial cytochrome *c* oxidase subunit I sequence data. The phylogenetic analyses separated the two species from closely related species and placed *P. iranense*, sp. nov., in clade J1 but the clade of *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., was unknown.

KEY WORDS
Oomycota, *Peronosporomycetes*, phylogeny, Pythiaceae, new species.

RÉSUMÉ
Taxonomie morphologique et moléculaire de Pythium monoclinum *Abrinbana, Abdollahz. & Badali, sp. nov., et* P. iranense*, sp. nov., d'Iran.*
Deux nouvelles espèces de *Pythium* Pringsheim isolées de sols des régions du nord-ouest de l'Iran sont décrites : *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., et *P. iranense*, sp. nov. Les deux taxons sont morphologiquement distincts de toutes les espèces connues. *Pythium monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., est caractérisé morphologiquement par la présence

d'un élément gonflé dans les tiges oogoniales au-dessous de l'oogone terminale, principalement des anthéridies monocliniques provenant de diverses distances de l'oogone, provenant parfois d'une partie gonflée de la tige oogoniale et produisant rarement des oospores en forme de cacahouètes doubles. *Pythium iranense*, sp. nov., se distingue des autres espèces du genre par la production d'oogonies à parois lisses, qui ont parfois 1-3 papilles, globuleuses à rares, et 1-2(-5) anthididies par oogonium ou une foule d'anthéridies autour de l'ogogonia, 1-2 rarement 3-4 oospores par oogone, rarement des oospores en forme d'arachide et des oospores immatures pourvues de 1-6 projections. Les relations phylogénétiques des isolats appartenant à ces nouveaux taxons avec d'autres espèces apparentées ont été étudiées à l'aide de données de séquences internes transcrites internes et de données de séquences partielles de la sous-unité I de cytochrome c oxydase. Les analyses phylogénétiques ont séparé les deux espèces des espèces étroitement apparentées et ont placé *P. iranense*, sp. nov., dans le clade J1, mais le clade de *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., était inconnu.

MOTS CLÉS
Oomycota,
Peronosporomycetes,
phylogénie,
Pythiaceae,
espèces nouvelles.

INTRODUCTION

Pythium Pringsheim is a ubiquitous genus occupying various ecological niches. Most species of the genus are common saprobes in soil and other environments but some of them are mycoparasites or pathogens of plants, insects and mammals (van der Plaats-Niterink 1981; de Cock *et al.* 1987; Su *et al.* 2001; Schroeder *et al.* 2013). Plant pathogenic species have a devastating impact on field and greenhouse grown crops, however, mycoparasitic species can stimulate plant growth, induce resistance to biotic stresses and act as biological control agents to protect crops against fungi and fungus-like plant pathogens (van der Plaats-Niterink 1981; Schroeder *et al.* 2013).

Identification and taxonomy of *Pythium* species has been mainly based on morphology (e.g. van der Plaats-Niterink 1981; Dick 1990). However, morphological overlap among different species, intraspecific variation and absence of sexual structures in some species are the major limitations in morphological identification of the members of the genus that may lead to misidentification (van der Plaats-Niterink 1981; Martin 2000; Dick 2001). Therefore, traditional morphology-based taxonomy is increasingly being supplemented with DNA sequence data for accurate identification and species delimitation in *Pythium*. Early studies on taxonomy and phylogeny of the genus have employed different genomic regions sequences (Briard *et al.* 1995; Cooke *et al.* 2000; Martin 2000; Villa *et al.* 2006; Bahramisharif *et al.* 2013), however, the nuclear marker internal transcribed spacers regions of rRNA genes (ITS1-5.8S-ITS2 = ITS) was used in most studies to support the description of novel species in the genus (Paul 2002a, b; Mathew *et al.* 2003). Furthermore, a combination of sequence from this genomic region with partial sequence of the mitochondrial gene cytochrome *c* oxidase subunit I (*coxI*) are now used as DNA barcodes for accurate identification of oomycetes including most species of *Pythium* (Robideau *et al.* 2011). Despite being suggested as barcode, *coxI* has also been used as a useful complementary molecular marker in taxonomy of *Pythium* (Bala *et al.* 2010a; Long *et al.* 2012, 2014; Abrinbana *et al.* 2016).

The first comprehensive phylogenetic analysis of *Pythium* using 28S and ITS sequences divided the genus into 11 clades (A-K) (Lévesque & de Cock 2004). Further studies using 18S, 28S and *coxI* sequence data showed that species belonging to clade K are phylogenetically and morphologically intermediate between *Pythium* and *Phytophthora* and, therefore, members of this clade were transferred to *Phytopythium* Abad, de Cock, Bala, Robideau, Lodhi & Lévesque (Bala *et al.* 2010b; de Cock *et al.* 2015). In a phylogenetic reevaluation of *Pythium* and related oomycete taxa using *coxII* and D1/D2 region of 28S sequences, the genus was divided into five genera corresponding to the sporangium morphology (Uzuhashi *et al.* 2010). Although few studies adopted the proposed taxonomy (Uzuhashi *et al.* 2017), but these names have not been widely accepted because of the lack of bootstrap support for some proposed genera and the placement of species of other oomycete genera within some of the *Pythium* clades.

During a study on the systematics of *Pythium* species in soils of East and West Azarbaijan provinces located in northwest of Iran, two new species were isolated that had unique characters. Therefore, this study was aimed to characterize representative isolates of these species using morphology and phylogenetic analyses of ITS and partial *coxI* sequence data.

MATERIAL AND METHODS

Isolation

Pythium strains were isolated from l samples collected from different locations in East and West Azarbaijan provinces during 2012-13 (Table 1). Isolation was performed using boiled hemp-seed halves as bait and culturing the baits on selective medium as described before (Abrinbana *et al.* 2016). Representative isolates were deposited in the culture collections of the Iranian Research Institute of Plant Protection (IRAN, Tehran, Iran) and Westerdijk Fungal Biodiversity Institute (CBS, Utrecht, The Netherlands) and dried holotype specimens were deposited in the herbarium of the former institution.

Morphology and growth

Morphology of *Pythium* species were characterized in water cultures prepared following the methods of Abrinbana *et al.* (2016). Plates were incubated at 15, 20 and 25°C under cool

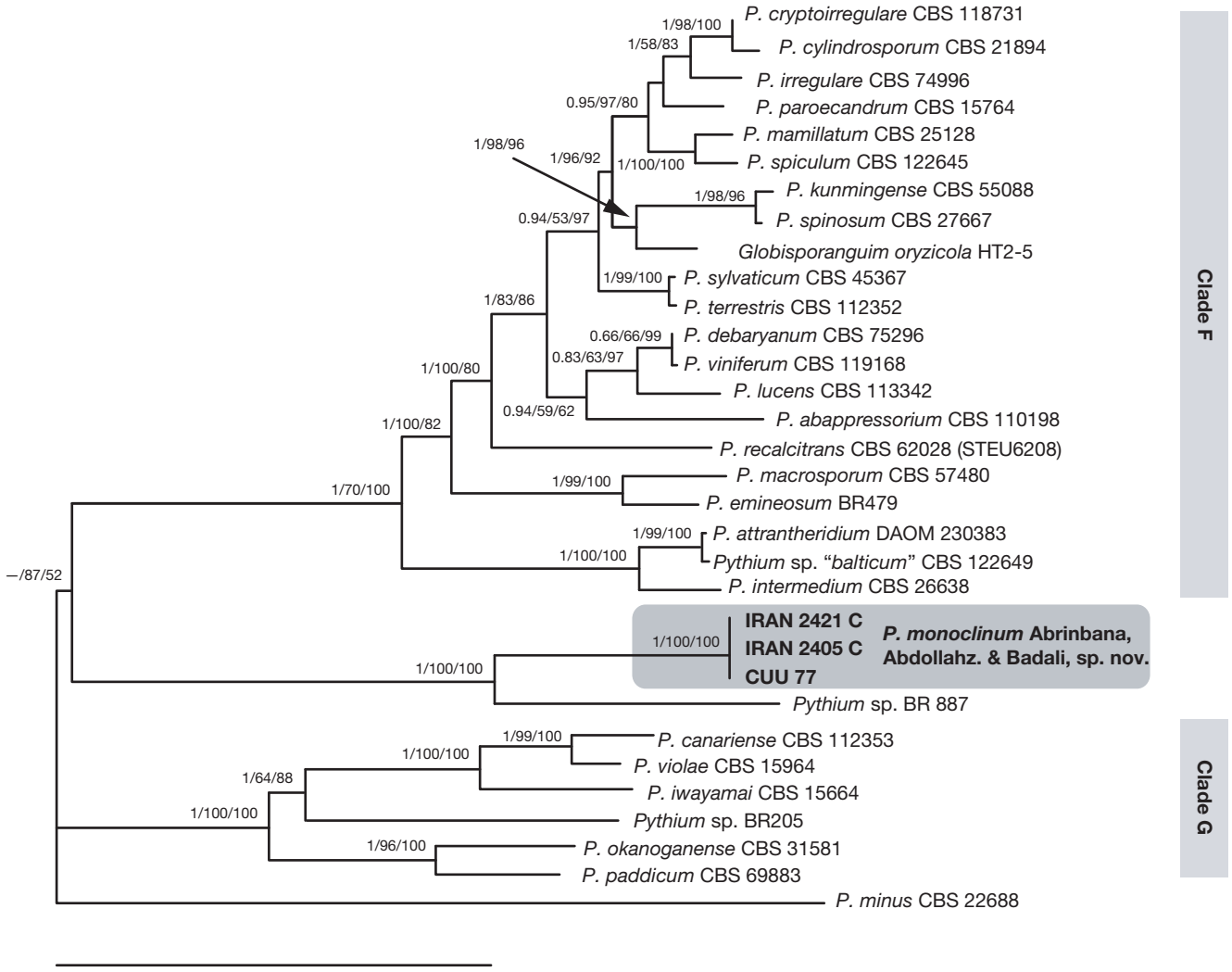

FIG. 1.— Neighbor-joining tree of *Pythium* Pringsheim clades F and G species inferred from complete ITS region sequences. Bayesian/maximum parsimony/neighbor-joining posterior probabilities and bootstrap support values are given at the nodes. – indicates support <50% for a particular clade. *Pythium minus* Ali-Shtayeh (CBS 22688) from clade E was used as outgroup. Scale bar: 0.1 substitutions.

TABLE 1.— *Pythium* Pringsheim isolates sequenced in this study.

					GenBank accession numbers	
Species	**Strain**	**Substrate**	**Location**	**Collector**	**ITS**	***Cox1***
P. monoclinum Abrinbana, Abdollahz. & Badali, sp. nov.	IRAN 2421 C	Uncultivated soil	East Azarbaijan, Islami Island	F. Badali	MH203014	MG182702
	IRAN 2405 C	Uncultivated soil	East Azarbaijan, Islami Island	F. Badali	MH203015	MG182703
	CUU 77	Uncultivated soil	East Azarbaijan, Islami Island	F. Badali	MH203016	MG182704
P. iranense Badali, Abrinbana & Abdollahz., sp. nov.	IRAN 2386 C	Soil under *Prunus armeniaca*	West Azarbaijan, Maku	M. Abrinbana	MG182709	MG182705
	IRAN 2387 C	Soil under *Prunus armeniaca*	West Azarbaijan, Maku	M. Abrinbana	MG182710	MG182706

white fluorescent light in alternating 12 h light-dark condition for 15 d. Cultures were examined daily and the shape, size and position of hyphal swellings, oogonia, antheridia and oospores were determined for each species. The means were calculated from measurement of at least 20 reproductive structures. Morphological characters were compared to those for species described in the monograph of *Pythium* (van der Plaats-Niterink 1981) and other original descriptions

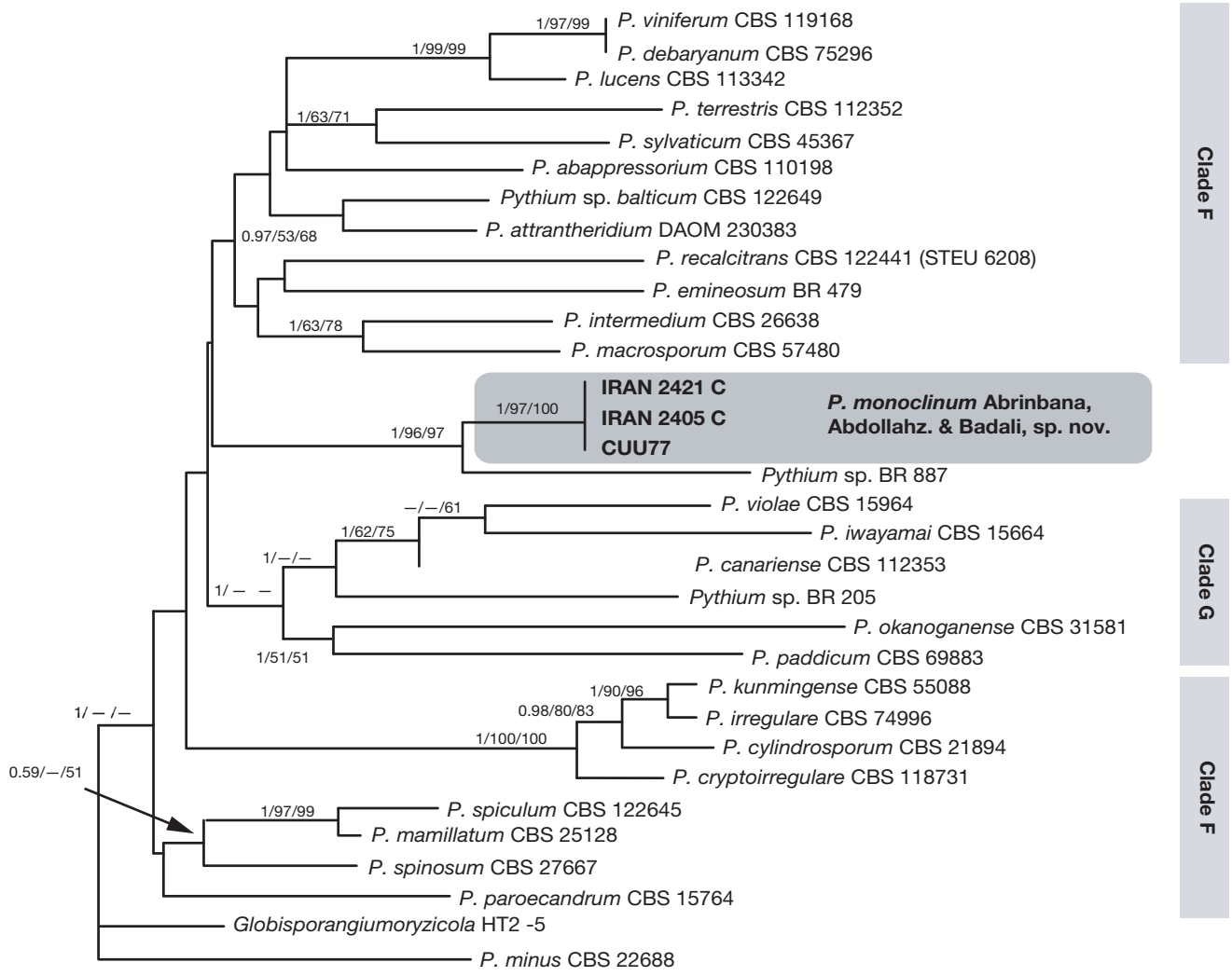

Fig. 2.— Neighbor-joining tree of *Pythium* Pringsheim clades F and G species inferred from partial *coxI* sequences. Bayesian/maximum parsimony/neighbor-joining posterior probabilities and bootstrap support values are given at the nodes. – indicates support <50% for a particular clade. *Pythium minus* Ali-Shtayeh (CBS 22688) from clade E was used as outgroup. Scale bar: 0.01 substitutions.

of phylogenetically or morphologically related species (Lifshitz *et al.* 1984; Paul 2002a, b; Mathew *et al.* 2003; McLeod *et al.* 2009; Bala *et al.* 2010a; Long *et al.* 2012).

Growth rates of isolates were measured on potato carrot agar (PCA) (van der Plaats-Niterink 1981) at 5-40°C with intervals of 5°C in the dark. After 24 and 48 h, radial growth was measured for each isolate with three replicates. When growth stopped, the cultures were returned to 25°C to check if the growth resumed and the culture is still viable.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from mycelium grown in potato dextrose broth using the methods of Raeder & Broda (1985) as modified by Abdollahzadeh *et al.* (2009). Amplifications and sequencing of ITS were performed using the primer pairs ITS1 and ITS4 (White *et al.* 1990). Partial *coxI* was amplified and sequenced with primers OomCoxI-Levup and Fm85mod (Robideau *et al.* 2011). The PCR conditions described by Abrinbana *et al.* (2016) and Robideau *et al.* (2011) were followed for amplification of ITS and *coxI*, respectively. PCR products were purified and sequenced by Macrogen (Republic of Korea).

Phylogenetic analyses

All sequences were manually checked and edited with BioEdit 7.5.0.3 (Hall 2006). The available ITS and *coxI* sequences of type or authentic strains of all known species in clades E2, F, G and J1 were retrieved from GenBank. Furthermore, the ITS and *coxI* sequences for representatives of neighboring clades including *P. minus* Ali-Shtayeh strain CBS 22688 from clade E and *Phytopythium litorale* (Nechw.) Abad, de Cock, Bala, Robideau, Lodhi & Lévesque strain CBS 118360 (Lévesque & de Cock 2004; de Cock *et al.* 2015) were obtained from GenBank and were used as outgroup for the clades. Sequences were aligned with MAFFT 7 (Katoh & Standley 2013). Alignments were checked and manual adjustments were made where necessary. To check the possibility of combining the ITS and *coxI* datasets, a partition homogeneity test (PHT) (Farris *et al.* 1995; Huelsenbeck *et al.* 1996) was conducted in PAUP 4.0b10 (Swofford

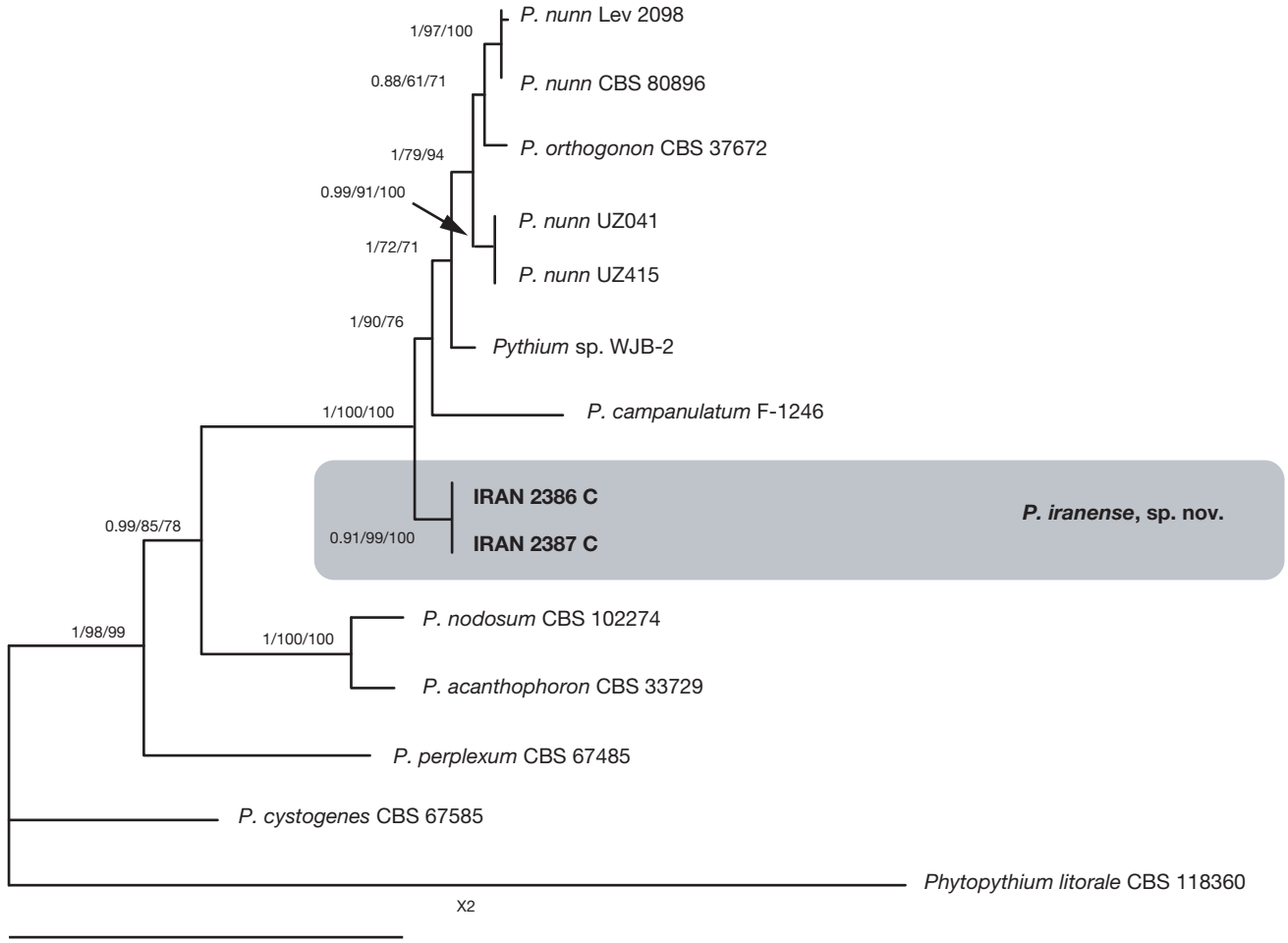

Fig. 3.— Neighbor-joining tree of *Pythium* Pringsheim clade J1 species inferred from complete ITS region sequences. Bayesian/maximum parsimony/neighbor-joining posterior probabilities and bootstrap support values are given at the nodes. – indicates support <50% for a particular clade. *Phytopythium litorale* (Nechw.) Abad, de Cock, Bala, Robideau, Lodhi & Lévesque (CBS 118360) was used as outgroup. Scale bar: 0.1 substitutions.

2003). Phylogenetic analyses were carried out with PAUP for neighbor-joining (NJ) and maximum parsimony (MP) and MrBayes 3.2.1 (Ronquist *et al.* 2012) for Bayesian inference (BI) analyses as described before (Abdollahzadeh *et al.* 2010, 2014). New sequences were deposited in GenBank (Table 1) and the alignments in TreeBASE (S26985).

RESULTS

Morphology and growth

All studied isolates produced hyphal swellings, oogonia, antheridia and oospores. Sporangia and zoospores not observed. Growth rates were measured for all isolates. Based on morphology, the isolates were separated into two distinct species different from all known species (Tables 2; 3). None grew or were viable at 40°C. Among the studied isolates, IRAN 2405 C, IRAN 2421 and CUU 77 were capable of growth at 5°C.

Sequences and phylogenetic analyses

BLAST analyses of ITS and partial *coxI* sequences and preliminary NJ analyses placed the isolates IRAN 2386 C and IRAN 2387 C in the first subclade of clade J (J1) proposed by Lévesque & de Cock (2004), however, the three isolates IRAN 2405 C, IRAN 2421 and CUU 77 belonged to none of the known *Pythium* clades although they were phylogenetically related to species in clades G and F (data not shown). The partition homogeneity test in PAUP was significant (P = 0.001) indicating that the ITS and *coxI* datasets were not congruent and produced trees with different topologies. Thus, the analyses were performed for ITS and *coxI* datasets individually.

The ITS sequences dataset for clades F and G contained 1155 characters including gaps. The phylogenetic analysis revealed that 672 characters were parsimony informative, 386 were constant and 97 were variable and parsimony-uninformative. One most parsimonious tree of 2082 steps was inferred from the heuristic search (CI = 0.67, HI = 0.33, RI = 0.84). The NJ tree obtained from ITS sequences is shown in Figure 1 with posterior probabilities for BI and bootstrap support values for MP and NJ (BI/MP/NJ).

The aligned *coxI* dataset of clades F and G comprised 671 characters, of which 554 were constant and 40 were variable and parsimony-uninformative. A heuristic search of the

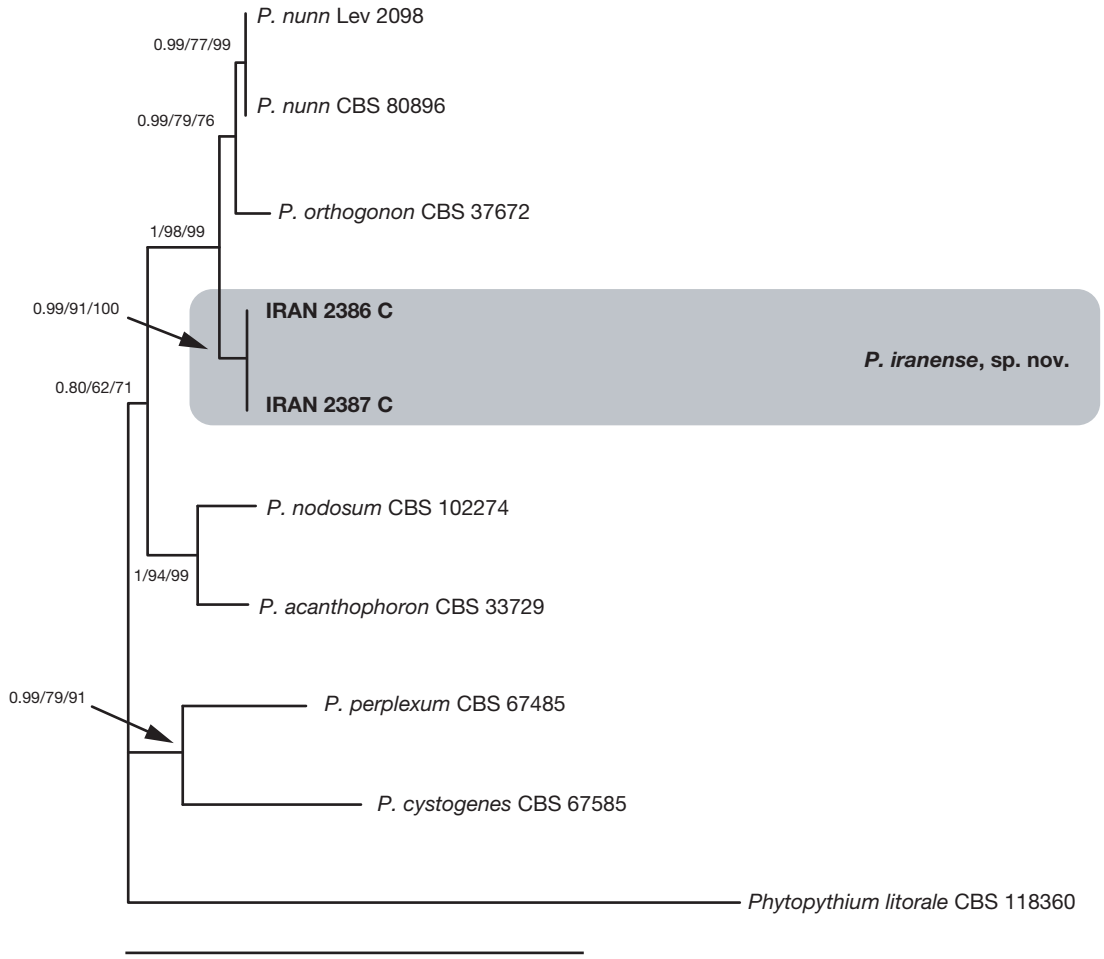

Fig. 4.— Neighbor-joining tree of *Pythium* Pringsheim clade J1 species inferred from partial *coxI* sequences. Bayesian/maximum parsimony/neighbor-joining posterior probabilities and bootstrap support values are given at the nodes. – indicates support <50% for a particular clade. *Phytopythium litorale* (Nechw.) Abad, de Cock, Bala, Robideau, Lodhi & Lévesque (CBS 118360) was used as outgroup. Scale bar: 0.1 substitutions.

remaining 77 parsimony informative characters resulted in one most parsimonious tree of 323 steps (CI = 0.48, HI = 0.52, RI = 0.61). The NJ tree obtained from *coxI* sequences is shown in Figure 2 with posterior probabilities for BI and bootstrap support values for MP and NJ (BI/MP/NJ).

The ITS sequences dataset for clade J1 contained 1036 characters including gaps. The phylogenetic analysis indicated that 178 characters were parsimony informative, 564 were constant and 294 were variable and parsimony-uninformative. One most parsimonious tree of 655 steps was inferred from the heuristic search (CI = 0.86, HI = 0.13, RI = 0.79). The NJ tree obtained from ITS sequences is shown in Figure 3 with posterior probabilities for BI and bootstrap support values for MP and NJ (BI/MP/NJ).

Phylogenetic analysis of 678 characters in the aligned *coxI* dataset of clade J1 showed that 43 characters were parsimony informative, 525 were constant and 110 were variable and parsimony-uninformative. One most parsimonious tree of 190 steps was inferred from the heuristic search (CI = 0.89, HI = 0.11, RI = 0.77). The NJ tree obtained from *coxI* sequences is shown in Figure 4 with posterior probabilities for BI and bootstrap support values for MP and NJ (BI/MP/NJ).

Phylogenetic analyses indicated the position of the studied isolates and placed them in two distinct monophyletic groups representatives of two new species. The species in unknown clade but phylogenetically related to clades F and G, and species within clade J1 are described below as *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., and *P. iranense*, sp. nov., respectively.

Based on phylogenetic analyses of ITS sequences, *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., was clearly differentiated from any other species (Fig. 1). The ITS region of this species was 907 bp long and was identical among the three isolates. In the ITS phylogeny, *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., was related to *Pythium* sp. BR887 (GI323302076). In the phylogenetic analyses based on partial *coxI* sequences, *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., isolates were distinct from other species (Fig. 2). The partial *coxI* gene region was 671 bp long and 100% similar among the three examined isolates. In the *coxI* phylogeny, *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., was related to *Pythium* sp. BR887 (Fig. 2).

Based on the ITS phylogeny, *P. iranense*, sp. nov., isolates were distinct from other species in clade J1 (Fig. 3).

The ITS region of this species was 871 bp long with 100% similarity between the two examined isolates. *Pythium* sp. WJB-2 (GI237784574), *P. campanulatum* Mathew, Singh & Paul, *P. orthogonon* Ahrens and *P. nunn* Lifsh., Stangh. & Baker were the most closely related taxa. In phylogenetic analyses based on partial *coxI* sequence, isolates of this species were differentiated from other studied species (Fig. 4). The partial *coxI* gene region was 678 bp long and identical between the two isolates. In the *coxI* phylogeny, *P. iranense*, sp. nov., was closely related to *P. orthogonon* and *P. nunn*.

Based on morphology and phylogenetic analyses, two identified taxa are distinguished from all other known taxa and described as new species.

Taxonomy

Family Pythiaceae J.Schröter
Genus *Phytopythium* Abad, de Cock, Bala, Robideau, Lodhi & Lévesque

Pythium monoclinum Abrinbana, Abdollahz. & Badali, sp. nov.
(Fig. 5)

MycoBank. — MB 823189.

Typus. — **Iran**. East Azarbaijan Province, Islami Island, from uncultivated soil; 8.V.2013; coll. F. Badali, IRAN 16695 F as dried culture in Iranian Research Institute of Plant Protection, ex-type culture (IRAN 2421 C).

Additional specimens examined. — **Iran**. East Azarbaijan Province, Islami Island, from uncultivated soil; 8.V.2013; coll. F. Badali, living culture in Iranian Research Institute of Plant Protection (IRAN 2405 C) and Westerdijk Fungal Biodiversity Institute (CBS 143584). **Iran**. East Azarbaijan Province: Islami Island, from uncultivated soil; 8.V.2013; coll. F. Badali, living culture in Collection of Urmia University (CUU 77).

Etymology. — Name refers to the mostly monoclinous antheridia.

Description

Colonies
On PCA submerged with a radiate pattern and on CMA partially submerged without a special pattern. Main *hyphae* up to 5 µm diam.

Sporangia and zoospores
Not observed.

Hyphal swellings
Globose, occasionally subglobose, terminal or intercalary, 15-26 µm diam. (av. 21.65 µm).

Oogonia
Smooth-walled, globose, subglobose, rarely elongated, mostly terminal, occasionally subterminal or intercalary, rarely in chains, sometimes oogonial stalks consisting a swollen element below the terminal oogonia, globose oogonia 16-26 µm diam. (av. 20.94 µm), elongated oogonia up to 35 µm long.

Antheridia
1-2 per oogonium, mostly monoclinous, originating at various distances from the oogonia, occasionally arising from inflated part of oogonial stalk located immediately below oogonium, rarely diclinous, occasionally sessile, antheridial stalks mostly bending towards oogonia. Antheridial cells clavate, swollen, elongated, sometimes with constriction, 10-22 × 5-11 µm (av. 14.92 × 6.62 µm).

Oospores
Globose, subglobose, rarely peanut-shaped, plerotic, rarely aplerotic, usually one but rarely two per oogonium, globose oospores 10-24 µm diam. (av. 18.71 µm), elongated oospores up to 32 µm long, the oospore wall up to 2 µm thick.

Daily growth
On PCA 3 mm at 5°C, 6 mm at 10°C, 9 mm at 15°C, 11 mm at 20°C, 14 mm at 25°C, 10 mm at 30°C, 7 mm at 35 and 0 at 40°C. The isolates were not viable at 40°C.

Notes

Differs from other known *Pythium* species by presence of globose to elongated smooth-walled oogonia, a swollen element in oogonial stalks below terminal oogonia, mostly monoclinous antheridia that occasionally arising from swollen part of oogonial stalk and their stalk mostly bending towards oogonia, (sub) globose or peanut-shaped oospores, mostly plerotic oospores and 1-2 oospores per oogonium.

Pythium iranense Badali, Abrinbana & Abdollahz., sp. nov.
(Fig. 6)

MycoBank. — MB 823190.

Typus. — **Iran**. West Azarbaijan Province, Maku, from soil under *Prunus armeniaca*, 10.V.2013, coll. M. Abrinbana, IRAN 16697 F as dried culture in Iranian Research Institute of Plant Protection, ex-type culture (IRAN 2386 C).

Additional specimen examined. — **Iran**. West Azarbaijan Province, Maku, from soil under *Prunus armeniaca*, 10.V.2013, coll. M. Abrinbana, living culture in Iranian Research Institute of Plant Protection (IRAN 2387 C) and Westerdijk Fungal Biodiversity Institute (CBS 143585).

Etymology. — Name refers to Iran where it was first found.

Description

Colonies
On PCA partially submerged with a narrow chrysanthemum pattern and on CMA submerged with a narrow chrysanthemum pattern. Main *hyphae* up to 5 µm diam.

Sporangia and zoospores
Not observed.

Hyphal swellings
Very rarely produced, globose, terminal or intercalary, 10-25 µm diam. (av. 15.08 µm).

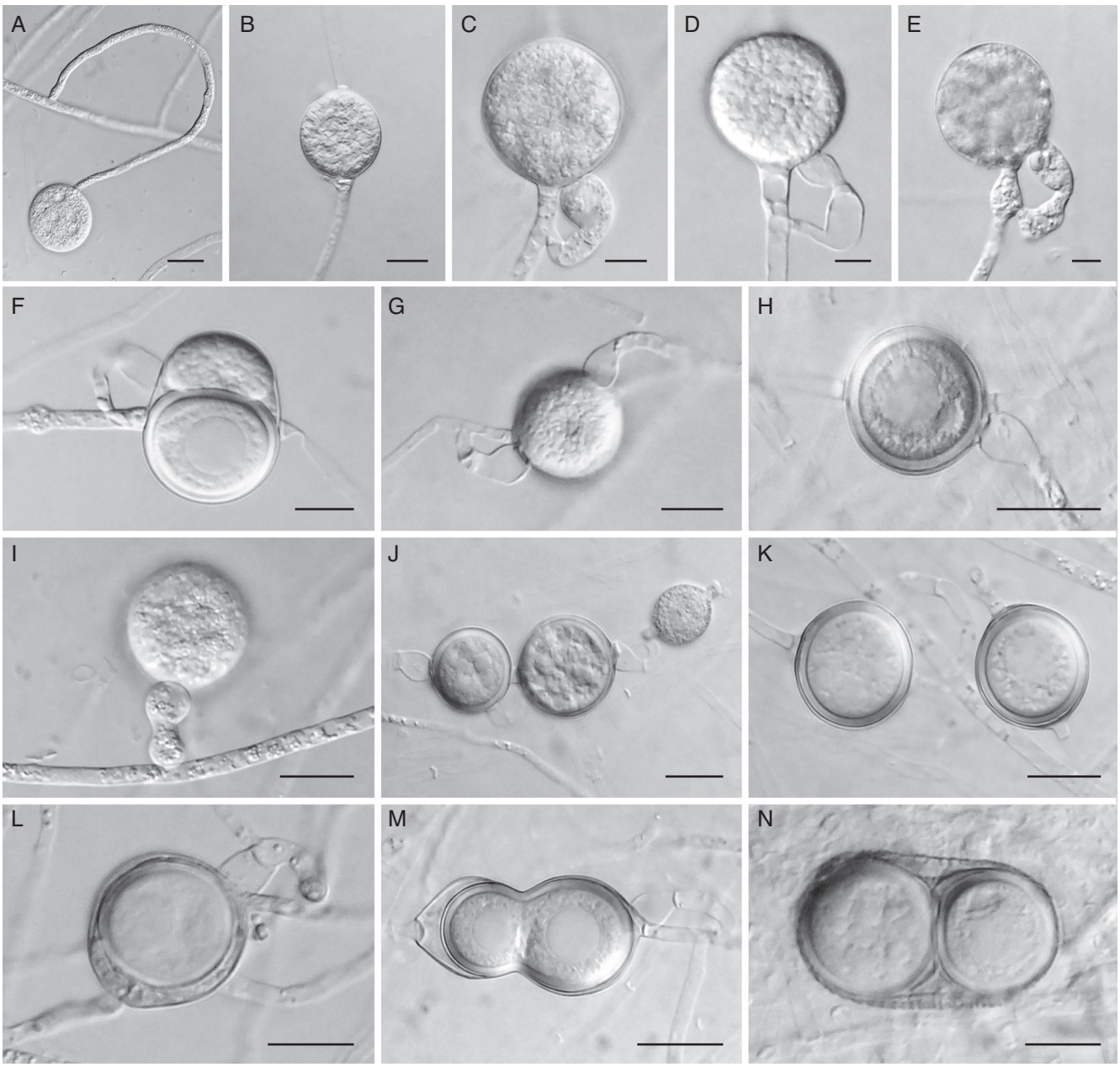

Fig. 5. — *Pythium monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov. (IRAN 16695 F, holotype): **A**, terminal hyphal swelling; **B**, intercalary hyphal swelling; **C**, **D**, monoclinous antheridia with inflated or non-inflated antheridial stalks and antheridial cells attached to oogonia; **E**, monoclinous antheridium originated from swollen part of oogonial stalk; **F**, monoclinous antheridium and intercalary oogonium with two oospores; **G**, intercalary oogonium provided with two ono-clinous antheridia; **H**, diclinous antheridium and intercalary oogonium; **I**, sessile diclinous antheridium with constriction attached to oogonium; **J**, chain of ogonia and hyphal swelling; **K**, plerotic oospores; **L**, aplerotic oospore; **M**, peanut-shaped oospore; **N**, double oospore. Scale bars: A, 10 µm; B-E, 5 µm F-N, 10 µm.

Oogonia

Smooth-walled, occasionally with 1-3 papillae, globose, rarely elongated, terminal, subterminal, intercalary, sometimes lateral, globose oogonia 12-24 µm diam. (av. 19.65 µm), elongated oogonia up to 38 µm long.

Antheridia

1-2(-5) per oogonium, rarely several antheridia crowding around oogonia, monoclinous or diclinous, occasionally sessile. Antheridial stalks straight, sometimes crooked or wavy. Antheridial cells clavate, swollen, elongated, crook-necked, bell-shaped, occasionally with constriction, 9-21 × 4-11µm (av. 13.69 × 6.92 µm).

Oospores

Globose, occasionally subglobose, rarely peanut-shaped, immature oospores sometimes provided with 1-6 projections, aplerotic, occasionally plerotic, usually one, at times two, rarely 3-4 per oogonium, globose oospores 13-22 µm diam. (av. 18.04 µm), elongated oospores up 30 µm long, the oospore wall up to 2 µm thick.

Daily growth

On PCA 0 mm at 5°C, 4 mm at 10°C, 6 mm at 15°C, 9 mm at 20°C, 13 mm at 25°C, 15 mm at 30°C, 14 mm at 35°C and 0 mm at 40°C. The isolates were viable at 5°C but not at 40°C.

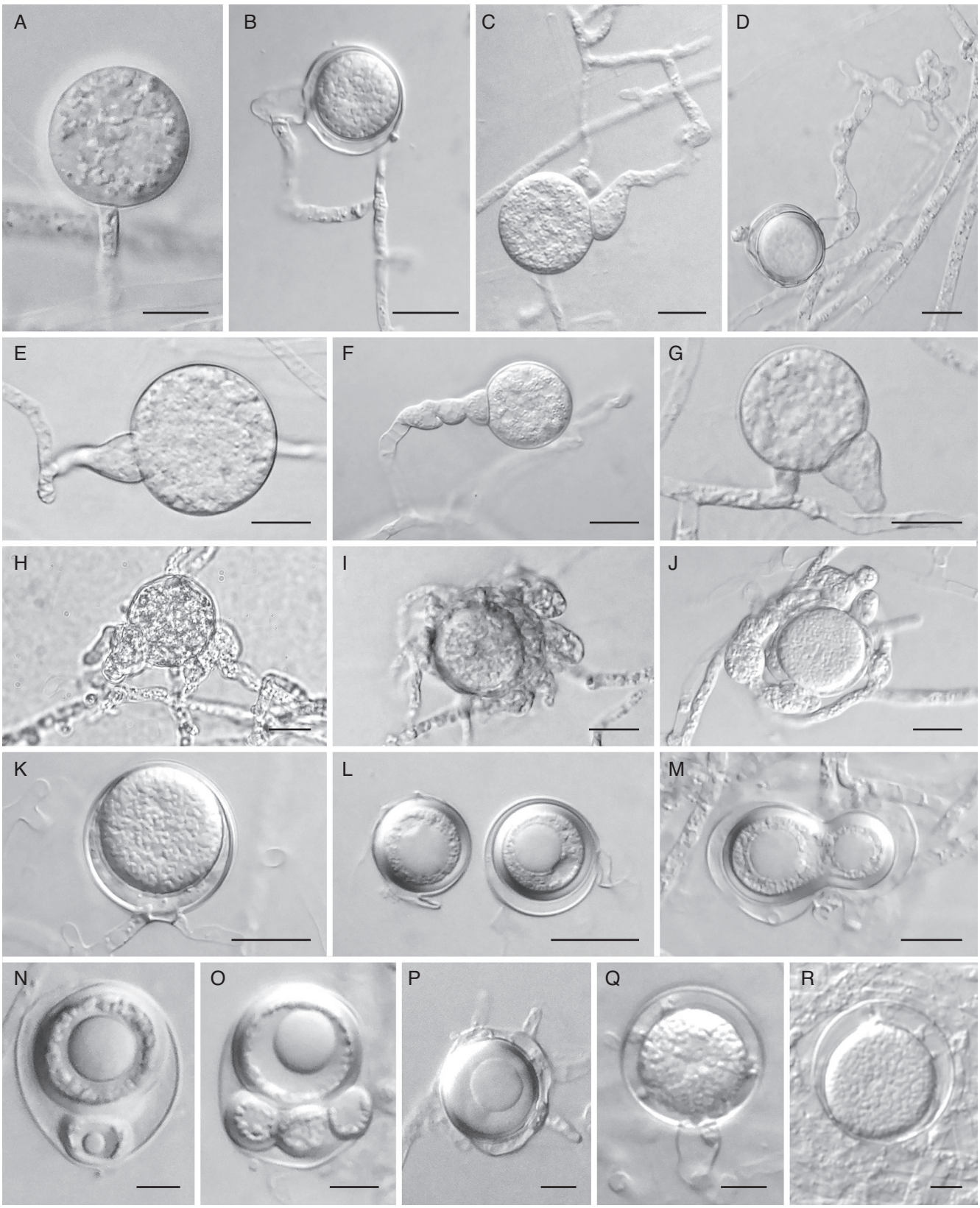

Fig. 6.— *Pythium iranense* Badali, Abrinbana & Abdollahz., sp. nov. (IRAN 16697 F, holotype): **A**, terminal hyphal swelling; **B**, monoclinous antheridium and terminal oogonium; **C**, two diclinous antheridia attached to oogonium; **D**, antheridium with wavy stalk and oogonium; **E**, sessile diclinous antheridium and terminal oogonium; **F**, antheridial cell with constriction; **G**, bell-shaped sessile monoclinous antheridium and terminal oogonium on short side branch; **H**, antheridia attached to oogonium; **I**, **J**, crowd of antheridia around oogonia; **K**, lateral oogonium; **L**, plerotic and aplerotic oospores; **M**, peanut-shaped oospore; **N**, double oospore; **O**, quadruple oospore; **P**, oogonium with three papilla; **Q**, **R**, immature oospores with projections. Scale bars: A-M, 10 µm; N-R, 5 µm.

TABLE 2.— Morphological differences between *Pythium monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., and morphologically similar species *P. emineosum* Bala, de Cock & Lévesque.

Morphological character	***P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov.**	***P. emineosum* Bala, de Cock & Lévesque**
Colony pattern on PCA	Submerged, radiate	Broad chrysanthemum
Hyphae	Up to 5 µm	Up to 7 µm
Sporangia	Not observed	Globose, terminal or intercalary, 11-25 µm in diam.
Hyphal swellings	Globose, occasionally subglobose, terminal or intercalary, 15-26 µm diam. (av. 21.65 µm)	Not observed
Antheridia	1-2 per oogonium, mostly monoclinous, occasionally arising from inflated part of oogonial stalk, rarely diclinous, occasionally sessile, antheridial cells clavate, swollen, elongated, occasionally with constriction, 10-22 × 5-11 µm (av. 14.92 × 6.62 µm)	1-3 per oogonium, monoclinous, diclinous, hypogynous, occasionally sessile, lateral, intercalary
Oogonia	Smooth-walled, globose, subglobose, rarely elongated, mostly terminal, occasionally subterminal or intercalary, rarely in chain, sometimes oogonial stalks consisting of a swollen element below the terminal oogonia, 16-26 µm diam. (av. 20.94 µm), elongated up to 35 µm	Smooth-walled, globose, cylindrical or peanut-shaped, intercalary or occasionally terminal, 13.6-28.3 µm (av. 19.9 µm) diam.
Oospores	Globose, subglobose, rarely peanut-shaped, plerotic, rarely aplerotic, single, rarely double, globose (10-24 µm diam. (av. 18.71 µm), elongated up 32 µm long, wall up to 2 µm thick	Globose, elongated, peanut-shaped, plerotic or aplerotic, single or double, 11.9-24.4 µm (av. 18 µm) in diam, wall 0.4-1.2 µm thick
Growth rate on PCA at 25°C	14 mm	15 mm
References	This study	Bala *et al.* 2010a

NOTES

Differs from other known *Pythium* species by presence of antheridial complex around the oogonia, various shapes of antheridial cells, smooth-walled oogonia with papillae, ornamented oospores, (sub)globose or peanut-shaped oospores and 1-4 oospores per oogonium.

DISCUSSION

Phylogenetic analyses based on complete ITS and partial *coxI* sequences supported the phylogenetic position of *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., and *P. iranense*, sp. nov., and their recognition as new species. Morphology of the two species correlates well with sequence data because both taxa have unique characters distinguishing them from all known species.

Phylogenetic analyses of ITS and partial *coxI* sequences confirmed the position of *P. iranense*, sp. nov., within J1, however, *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., and *Pythium* sp. BR887 that grouped into a highly supported clade, did not fit into known *Pythium* clades proposed by Lévesque & de Cock (2004). In the ITS phylogeny of oomycetes presented by Robideau *et al.* (2011, Supporting Information), *Pythium* sp. BR887 has also not been clustered within any of the *Pythium* clades. Therefore, *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., may not belong to any known *Pythium* clades but its confirmation needs further investigation using sequence data from additional genomic regions.

The combination of morphological features of *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., is unique in the genus, but it shares some characters with *P. emineosum* Bala, de Cock & Lévesque (Bala *et al.* 2010a) (Table 2). The latter species belongs to clade F but *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., was not closely related to this or other species in clade F. Furthermore, *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., differs morphologically from *P. emineosum* by its lower number of antheridia per oogonium (1-2 vs 1-3 per oogonium), mostly terminal and occasionally subterminal oogonia, a swollen element in oogonial stalks below terminal oogonia and lack of hypogynous antheridia (Table 2).

The phylogenetic analyses of ITS and *coxI* sequences indicated that *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., is related to *Pythium* sp. BR887. Since the latter is an undescribed species, comparison of morphological characters of *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., with this species is impossible.

Distinctive characters of *P. iranense*, sp. nov., are the presence of antheridial complex around the oogonia, various shapes of antheridial cells, smooth-walled oogonia with papilla, ornamented oospores, peanut-shaped oospores and 1-4 oospores per oogonium. This striking combination of morphological characters has not been reported in other *Pythium* species. Nevertheless, this species is similar to *P. segnitium* Paul (Paul 2002b) and *P. baisense* Long, Wei & Guo (Long *et al.* 2012) in having antheridial or sterile hyphal complex around the oogonia and peanut-shaped oospores in smooth-walled oogonia. However, *P. iranense*, sp. nov., differs from these species by the presence of crooked antheridial stalks and cells, the papilla on oogonia, the projections on oospores, the higher number of oospores per oogonium (1-4 vs 1-2 per oogonium) and the absence of zoospores. It is also distinguished from *P. segnitium* by the possession of aplerotic oospores and the lack of hypogynous antheridia and from *P. baisense* by the occasional production of plerotic oospores.

TABLE 3.— Morphological differences between *Pythium iranense* Badali, Abrinbana & Abdollahz., sp. nov., and closely related species from clade J1.

Morphological character	***P. iranense* Badali, Abrinbana & Abdollahz., sp. nov.**	***P. campanulatum* Mathew, Singh & Paul**	***P. orthogonon* Ahrens**	***P. nunn* Lifsh., Stangh. & Baker**	***Pythium* Pringsheim sp. WJB-2**
Colony pattern on PCA	Partially submerged, narrow chrysanthemum	Submerged, broad chrysanthemum	Indistinct radiate	Not reported	Not reported
Hyphae	Up to 5 µm	Up to 6 µm	Up to 5 µm	Up to 6.5	Not reported
Sporangia	Not observed	Not formed	Globose, subglobose, terminal, rarely intercalary, 12-20 µm (av. 17 µm) in diam.	Not formed	Globose, terminal, av. 18 µm in diam.
Hyphal swellings	Very rarely produced, globose, terminal or intercalary, 10-25 µm diam. (av. 15.08 µm)	Globose, ovoid to somewhat elongated, terminal or intercalary, 11-25 µm (av. 18 µm) in diam.	Not formed	Globose, oval or lemon, 12-22.5 µm (av. 16.2 µm) in diam.	Globose, ellipsoid or obpyriform, terminal or intercalary, av. 16 µm in diam.
Antheridia	1-2(-5) per oogonium, rarely several antheridia crowding around oogonia, monoclinous or diclinous, occasionally sessile, antheridial stalks straight, sometimes crooked or wavy, antheridial cells clavate, swollen, elongated, crook-necked, bell-shaped, occasionally with constriction, 9-21 × 4-11µm (av. 13.69 × 6.92 µm)	1-5 per oogonium, monoclinous or diclinous, antheridia surround the oogonia forming a complicated knot around it, antheridial cells campanulate, at times elongated	1(-2) per oogonium, monoclinous or diclinous, antheridial cells often crook-necked	1-5 per oogonium, monoclinous or diclinous, antheridial stalks inflated or crooked, antheridial cells lobulate or club shaped	1-2 per oogonium, monoclinous, antheridial stalks unbranched, uninflated, originate short distance from oogonia, antheridial cells inflated, elongated
Oogonia	Smooth-walled, occasionally with 1-3 papillae, globose, rarely elongated, terminal, subterminal, intercalary, sometimes lateral, 12-24 µm diam. (av. 19.65 µm), elongated up to 38 µm	Smooth-walled, globose, mostly terminal, 13-27 µm (av. 20.6 µm) diam.	Smooth-walled, globose, terminal or unilaterally intercalary, 17-20(-21) µm (av. 18 µm) in diam.	Smooth-walled, globose, terminal, 18.5-23 µm (av. 20.8 µm) diam.	Smooth-walled, terminal, intercalary, av. 18 µm in diam.
Oospores	Globose, occasionally subglobose, rarely peanut-shaped, immature oospores sometimes with 1-6 projections, aplerotic, occasionally plerotic, single, occasionally double, rarely triple or quadruple, globose (13-22 µm diam. (av. 18.04 µm), elongated up 30 µm long, wall up to 2 µm thick	Globose, usually aplerotic, at times plerotic, single, occasionally double, 11-20 µm (av. 16.8 µm) in diam., wall 1-2 µm thick	Globose, plerotic, single, wall 1.5-3 µm thick	Globose, aplerotic, single, 18-20.5 µm (av. 19.2 µm) in diam., wall up to 2 µm thick	Plerotic or nearly so, av. 17 µm in diam., wall 2 µm thick
Growth rate on PCA at 25°C	13 mm	15 mm	12 mm	Not reported	Not reported
References	This study	Mathew *et al.* 2003	van der Plaats-Niterink 1981	Lifshitz *et al*. 1984	McLeod *et al.* 2009

Pythium iranense, sp. nov., differs morphologically from the phylogenetically related species such as *P. campanulatum* (Mathew *et al.* 2003), *P. orthogonon* (van der Plaats-Niterink 1981), *P. nunn* (Lifshitz *et al.* 1984) and *Pythium* sp. WJB-2 (GI237784574) by the production of papilla on oogonia, elongated oogonia, peanut-shaped oospores, oospores with projections, and higher number of oospores per oogonium (Table 3). Furthermore, *P. iranense*, sp. nov., possesses several other characters that distinguish it from the closely related species. Whereas this species produce lateral oogonia and antheridial crowd around oogonia, these features have only been reported in *P. orthogonon* and *P. campanulatum*, respectively, and hence, it can be distinguished from the related

taxa that lack these characters. Furthermore, *P. iranense*, sp. nov., with mostly aplerotic oospores differs from *P. orthogonon* and *Pythium* sp. WJB-2 with plerotic oospores. Other characters that distinguish *P. iranense*, sp. nov., are asexual reproduction type and structures. This species produce globose or subglobose hyphal swellings and lack the zoospores, whereas, *P. orthogonon* forms sporangia and zoospores, and the other related taxa produce various shapes of hyphal swellings.

The ITS and *coxI* sequences of *P. monoclinum* Abrinbana, Abdollahz. & Badali, sp. nov., and *P. iranense*, sp. nov., were highly discriminative and can be used as DNA barcode for their identification and differentiation from other previously described *Pythium* species.

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