Morphological and molecular identification of newly recovered *Pythium* species, *P. abappressorium* and *P. spinosum* from Iran, and evaluation of their pathogenicity on cucumber seedlings

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Abstract: During a survey on the biodiversity of the genus *Pythium* in northern Iran, one isolate of *P. abappressorium* and one isolate of *P. spinosum* were recovered from the rhizosphere of *Urtica dioica* in Abesh-Ahmad region (39° 02′ 33″ N, 47° 18′5 9″E) of Ardebil province and from the rhizosphere of *Cynodon dactylon* in Hashtroud region (37° 28′ 40″ N, 47° 03′ 03″ E) of East-Azarbaijan province, Iran. Based on combination of cultural, morphological, cardinal growth rate, sequence data from ITS-rDNA and pathogenicity assay, the isolates were identified as *P. abappressorium* and *P. spinosum*. Phylogenetic analyses of the ITS-rDNA sequences clustered our isolates with representative sequences for the species isolates from GenBank. The species represent new records for the mycobiota of Iran. With this paper, we provide full illustration for these species and further discuss their phylogeny and morphology with closely related species and pathogenicity on cucumber (*Cucumis sativus*) seedlings.

Zusammenfassung: Im Laufe einer Untersuchung zur Biodiversität der Gattung *Pythium* im nördlichen Iran wurden aus der Rhizosphäre von *Urtica dioica* in Abesh-Ahmad (39° 02′ 33″ N , 47° 18′ 59″ E) in der Provinz Ardebil und aus der Rhizosphäre von *Cynodon dactylon* in der Hashtroud Region (37 ° 28' 40" N, 47 ° 03' 03 "E) der Ost-Azarbaijan Provinz. ein Isolat von *P. abappressorium* und ein Isolat von *P. spinosum* gewonnen. Basierend auf der Kombination von Wachstumsraten, Kulturmerkmalen, ITS-rDNA Sequenzdaten und Pathogenitäts-Assays wurden die Isolate als *P. abappressorium* und *P. spinosum* identifiziert. Phylogenetische Analysen der ITS-rDNA-Sequenzen gruppierten unsere Isolate mit repräsentativen Sequenzen für diese Arten in der GenBank. Die Arten sind neue Nachweise für die Mykobiota des Iran. Mit dieser Arbeit bieten wir eine vollständige Illustration für diese Arten und diskutieren ihre Phylogenie und Morphologie mit eng verwandten Arten und deren Pathogenität auf Gurken (*Cucumis sativus*)-Sämlingen.

Tab. 1. Code, GenBank accession numbers, locality and references of strains used in the phylogenetic analysis.

Species	Code	Locality	GenBank Accession No. ITS	Reference
P. abappressorium	**OPU 1682**	**Abesh–Ahmad, Ardebil province (39°02′33″N 47°18′59″E)**	**KU695265**	**Present study**
P. spinosum	**OPU 1704**	**Hashtroud, E–Azarbaijan province (37°28′40″N 47°03′03″E)**	**KU695266**	**Present study**
P. abappressorium	020162	United States	DQ091294	SCHROEDER & al. 2006
P. abappressorium	CBS110198	United States	HQ643408	ROBIDEAU & al. 2011
P. abappressorium	B 10-2	United States	HQ862935	Direct submission ALCALA & al. 2009
P. abappressorium	B 3-94	United States	HQ862934	Direct submission ALCALA & al. 2009
P. cryptoirregulare	CBS118731	United States	HQ643515	ROBIDEAU & al. 2011
P. irregulare	BR1000	South Africa	HQ643667	ROBIDEAU & al. 2011
P. irregulare	BR1002	South Africa	HQ643665	ROBIDEAU & al. 2011
P. irregulare	BR1004	South Africa	HQ643663	ROBIDEAU & al. 2011
P. irregulare	BR1008	South Africa	HQ643660	ROBIDEAU & al. 2011
P. irregulare	BR1009	South Africa	HQ643659	ROBIDEAU & al. 2011
P. irregulare	BR1015	Australia	HQ643656	ROBIDEAU & al. 2011
P. irregulare	BR1016	Australia	HQ643655	ROBIDEAU & al. 2011
P. irregulare	BR1021	Australia	HQ643651	ROBIDEAU & al. 2011
P. irregulare	BR1022	Australia	HQ643650	ROBIDEAU & al. 2011
P. irregulare	BR629	Canada	HQ643641	ROBIDEAU & al. 2011
P. cylindrosporum	DA-OM232335	Canada	HQ643517	ROBIDEAU & al. 2011
P. cylindrosporum	CBS21894	Germany	HQ643516	ROBIDEAU & al. 2011
P. mamillatum	BR648	Netherlands	HQ643689	ROBIDEAU & al. 2011
P. mamillatum	BR765	Canada	HQ643688	ROBIDEAU & al. 2011
P. mamillatum	CBS25128	Netherlands	HQ643687	ROBIDEAU & al. 2011
P. spiculum	CBS122645	France	HQ643790	ROBIDEAU & al. 2011
P. kunmingense	CBS55088	China	HQ643672	ROBIDEAU & al. 2011
P. spinosum	CBS27667	Netherlands	HQ643792	ROBIDEAU & al. 2011
P. spinosum	Lev1526	United States	HQ643794	ROBIDEAU & al. 2011
P. spinosum	CBS122663	India	HQ643791	ROBIDEAU & al. 2011
P. paroecandrum	BR601	Australia	HQ643735	ROBIDEAU & al. 2011
P. paroecandrum	BR773	South Africa	HQ643734	ROBIDEAU & al. 2011
P. paroecandrum	BR774	South Africa	HQ643733	ROBIDEAU & al. 2011
P. paroecandrum	BR807	South Africa	HQ643732	ROBIDEAU & al. 2011
P. paroecandrum	BR929	Spain	HQ643730	ROBIDEAU & al. 2011
P. paroecandrum	CBS15764	Australia	HQ643731	ROBIDEAU & al. 2011
P. macrosporum	BR1029	Canada	HQ643685	ROBIDEAU & al. 2011
P. macrosporum	ADC0029	Norway	HQ643686	ROBIDEAU & al. 2011
P. macrosporum	CBS57480	Netherlands	HQ643684	ROBIDEAU & al. 2011
P. emineosum	BR836	Canada	GQ244428	BALA & al. 2010
P. emineosum	BR479	Canada	GQ244427	BALA & al. 2010
P. sylvaticum	BR1045	Canada	HQ643852	ROBIDEAU & al. 2011
P. sylvaticum	BR1069	Canada	HQ643851	ROBIDEAU & al. 2011
P. sylvaticum	BR171	Canada	HQ643850	ROBIDEAU & al. 2011
P. sylvaticum	BR179	Canada	HQ643849	ROBIDEAU & al. 2011
P. sylvaticum	BR599	Canada	HQ643848	ROBIDEAU & al. 2011
P. sylvaticum	BR647	Netherlands	HQ643847	ROBIDEAU & al. 2011
P. sylvaticum	Lev1544	United States	HQ643846	ROBIDEAU & al. 2011
P. sylvaticum	P15580	Canada	HQ261741	ROBIDEAU & al. 2011
P. sylvaticum	CBS45367	United States	HQ643845	ROBIDEAU & al. 2011
P. terrestris	ADC9906	Netherlands	HQ643858	ROBIDEAU & al. 2011

Tab. 1. Continued.

Species	Code	Locality	GenBank Accession No. ITS	Reference
P. terrestris	BR922	United States	HQ643856	ROBIDEAU & al. 2011
P. terrestris	CBS112352	France	HQ643857	ROBIDEAU & al. 2011
P. lucens	CBS113342	Canada	HQ643681	ROBIDEAU & al. 2011
P. viniferum	F-1201	France	AY455694	Direct submission PAUL 2003
P. viniferum	CBS119168	France	HQ643956	ROBIDEAU & al. 2011
P. intermedium	BR1042	Canada	HQ643579	ROBIDEAU & al. 2011
P. intermedium	BR128	Canada	HQ643578	ROBIDEAU & al. 2011
P. intermedium	BR339	Canada	HQ643577	ROBIDEAU & al. 2011
P. intermedium	BR485	Netherlands	HQ643576	ROBIDEAU & al. 2011
P. intermedium	BR707	Canada	HQ643575	ROBIDEAU & al. 2011
P. intermedium	BR734	Poland	HQ643574	ROBIDEAU & al. 2011
P. intermedium	BR869	Canada	HQ643573	ROBIDEAU & al. 2011
P. intermedium	BR924	Spain	HQ643571	ROBIDEAU & al. 2011
P. intermedium	CBS26638	Netherlands	HQ643572	ROBIDEAU & al. 2011
P. attrantheridium	Lev3004	United States	HQ643473	ROBIDEAU & al. 2011
P. attrantheridium	DA-OM230387	United States	HQ643475	ROBIDEAU & al. 2011
P. attrantheridium	DA-OM230383	Canada	HQ643477	ROBIDEAU & al. 2011
P. spinosum	CBS27667	Netherlands	HQ643792	ROBIDEAU & al. 2011
P. spinosum	Lev1526	United States	HQ643794	ROBIDEAU & al. 2011
P. spinosum	CBS122663	India	HQ643791	ROBIDEAU & al. 2011
P. spinosum	CBS27567	Netherlands	HQ643793	ROBIDEAU & al. 2011
P. paroecandrum	ADC9909	Netherlands	HQ643737	ROBIDEAU & al. 2011
P. paroecandrum	ADC9910	Netherlands	HQ643736	ROBIDEAU & al. 2011
P. aphanidermatum	Lev1800	Canada	HQ643442	ROBIDEAU & al. 2011

The genus *Pythium* comprises more than 140 species. For a general short introduction to the genus see BOUKET & al. (2016). Molecular revision was done by LEVESQUE & DE COCK (2004). Clade F contains some of important plant pathogens with a world-wide distribution. Most species do not or rarely produce zoospores. Members of this clade produce either globose, non–proliferating sporangia or globose hyphal swellings (*P. irregulare* develops both of them). They are fast growing and have moderate cardinal temperatures.

Pythium abappressorium is characterized by forming sexual and asexual structures from the appressoria and remaining appressoria attached at the base of sporangia. The species was originally isolated from wheat and apple roots in eastern Washington, United States. *Pythium abappressorium* is pathogenic to *Triticum aestivum*, causing damping–off and stunting, but is not pathogenic to *Malus pumila* (PAULITZ & al. 2003). *Pythium spinosum* is characterized by forming ornamented oogonia. The species was originally isolated from *Anthirrhinum majus* in Taiwan. *Pythium spinosum* has been shown to cause damping-off and root rot of *Antirrhinum majus*, *Lactuca sativa* and *Allium cepa*, in addition to many other plants (VAN DER PLAATS-NITERINK 1981).

Here, based on combining morphological, ecological and phylogenetic species concepts referred to as the Consolidated Species Concept (CHENARI BOUKET & al. 2015a) we describe a new record of *P. abappressorium* from rhizosphere of *Urtica dioica* and *P. spinosum* from rhizosphere of *Cynodon dactylon* in mycobiota of Iran.

We also evaluate their pathogenicity on cucumber (*Cucumis sativus*) seedlings under laboratory conditions.

Tab. 2. Morphological comparison between *Pythium abappressorium* (OPU 1682) and its morphologically and phylogenetically related species.

Characteristics	OPU 1682	***Pythium lucens*** (ALI–SHTAYEH & DICK 1985)	***Pythium viniferum*** (PAUL & al. 2008)
Main hyphae	> 5 µm	3.5–6.5 µm	> 7 µm
Sporangia	More or less globose, 22–27 µm in diameter, terminal or intercalary, formed from appressoria that often attached to the base of sporangium	Globose or subglobose, terminal and occasionally catenulate 2–3 in a chain, intercalary (21–25 µm); discharge tube up to 30 µm long, 1–2 per sporangium; Encysted zoospores 8–10 µm in diam.	Terminal and intercalary, spherical (7–25 µm), ovoid to elongated; Zoospores were never observed in spite of repeated reculturing, at different temperatures, in sterile distilled, pond, and soil extract water
Hyphal swellings	Globose, lemon-shaped or cylindrical, 22–38 × 12–17 µm	Not formed	1–6 germ tubes
Appressoria	Curved to sickle-shaped, often branched	Not formed	Sickle-shaped
Oogonia	Smooth, globose, terminal or intercalary, 13–25 µm in diam.	Smooth walled, globose, 22–35 µm in diam., rarely pyriform, usually terminal, occasionally intercalary, rarely 2–3 in chain	Mostly intercalary, occasionally terminal, sometimes catenulate, 17–29 µm in diam., antheridia and oogonia borne on an appressorium
Antheridia	Sac- to crook-necked-shaped, 1–2 per oogonium, monoclinous and diclinous	1–2(5) per oogonium, monoclinous, usually stalked, originating usually more than 20 µm distance from the oogonium base, occasionally diclinous, antheridial cells clavate, occasionally 2–3 borne on one antheridial branch	Hypogynous, monoclinous sessile or monoclinous on short branches, at times diclinous, 1–5 per oogonium, antheridial cells conspicuous and at times bi-lobed, monoclinous stalked antheridia making a broad apical contact zone with the oogonia
Oospore	Smooth, plerotic, globose 17–31 µm in diam. and rarely oblong 24–26 × 14–16 µm, 1 or occasionally 2 produced in one oogonium	Aplerotic, usually single, occasionally 2 oospores per oogonium, globose, 17–23 µm in diam.	
Daily growth rate at 25 °C	15 mm	9 mm	25 mm

Materials and methods

Isolation: *Pythium abappressorium* and *P. spinosum* isolates were recovered from rhizosphere of *Urtica dioica* Abesh-Ahmad region in Ardebil province and from rhizosphere of *Cynodon dactylon* from Hashtroud region in East-Azarbaijan province, Iran. Pieces of roots of plant were washed in sterile water and then dried on a paper towel, transferred onto *Pythium*-selective medium namely NARF (MORITA & TOJO 2007, CHENARI BOUKET & al. 2015b) and incubated at 25 °C for 1–2 days. Pure cultures were established using a hyphal tip technique. Cultures were preserved on V8A (MILLER 1955) slant vials at 10 °C in the dark until use. Detailed information of the isolates is given in Tab. 1.

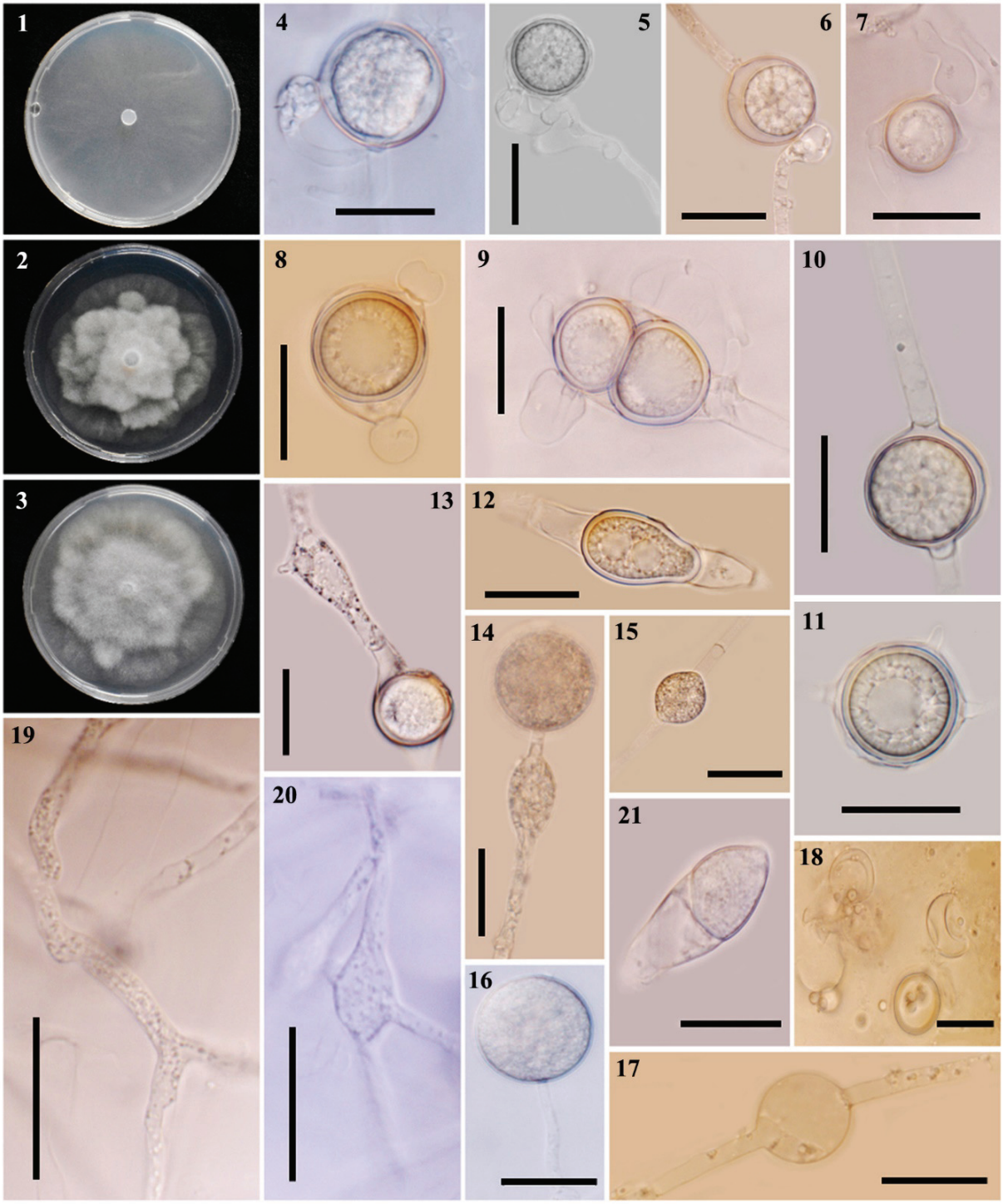

Fig. 1. *Pythium abappressorium*. *1–3* Colony morphology of the isolate OPU 1682 on CMA, PDA, V8A, *4–9* oogonium with 1–2 monoclinous and diclinous antheridia, *10–13* globose and oblong oospore produced within appressorium, *14–17* sporangium, *18* empty sporangia after release of zoospores, *19–21* branched and unbranched appressoria. – Bars: 20 µm.

Morphology and growth temperature relationships: Mycelial patterns of the isolate were recorded three days after inoculation at 22 °C on CMA (Becton Dickinson and Company, Franklin Lakes, NJ, USA), PDA (Sigma Aldrich, St. Louis, MO, USA) and V8A (MILLER 1955). Morphological observations were made on structures produced in sterile grass blades floated in sterile pond water (MARTIN 1992). Thirty measurements were made for each microscopic structure including hyphae, sporangia, antheridia, oogonia and oospores. Photographs were captured using an Olympus-BX43 microscope with the digital camera system (DP2-ASL) (Olympus, Tokyo, Japan). The cultures were deposited in the Culture Collection of Osaka Prefecture University (OPU), Osaka, Japan. The cardinal

temperatures were determined three days after inoculation on PCA at temperature ranging from 4 to 43°C.

Tab. 3. Morphological comparison between *Pythium spinosum* (OPU 1704) and its morphologically and phylogenetically related species.

Characteristics	**OPU 1704**	***Pythium kunmingense*** (YU 1973)
Main hyphae	> 7 µm	> 8.6 µm
Sporangia	Sporangia and zoospores were not formed	(sub)globose, ovoid or limoniform, terminal or intercalary, 13–23 (av. 19) µm in diam., with a smooth, rarely spiny wall. Zoospores not formed
Hyphal swellings	Globose, lemon-shaped, thin-walled, 18–30 µm in diam.	Not formed
Oogonia	Terminal or intercalary, globose or fusiform, 15–26 µm in diam., with a varying number of blunt, finger-like ornamentation, 4–6 µm long and 1–2 µm in diam. at the base	(sub)globose or limoniform, terminal or intercalary, 15–26 (av. 21) µm in diam., ornamented, rarely smooth. Ornamentations papilliform, 2.5–14 µm long and 1.7–2.5 µm diam. at the base
Antheridia	1–2 per oogonium, monoclinous and diclinous, antheridial cells inflated	Antheridia 1–3 per oogonium, mostly monoclinous, occasionally diclinous; antheridial cells clavate, curved, vermiform or sickle–shaped, 13–19 × 5.2–6.8 µm
Oospore	Plerotic, occasionally aplerotic, 14–22 µm in diam., thin-walled. Two oospores in one oogonium	Oospores plerotic, 10–24 (av. 19) µm in diam., wall 0.8–2.0 µm thick
Daily growth rate at 25 °C	32 mm	9 mm

Tab. 4. Pathogenicity of *Pythium abappressorium* and *P. spinosum* isolates to seedlings of cucumber.

Pythium species inoculated	Mortality (%)	Infection (%)
P. abappressorium	80.8 ± 19.2 **A**	100
P. spinosum	76 ± 14.9 **A**	100
Control	0 **B**	0

Data given as mean ± standard errors (N = 3). Values followed by the same letters in a column do not differ significantly according to a Tukey–Kramer honestly significant different (HSD) test ($P < 0.01$).

DNA extraction and amplification, DNA sequencing and phylogenetic analysis: Methodology followed CHENARI BOUKET & al. (2016, this issue). Newly generated sequences were submitted to GenBank (http://www. ncbi.nlm.nih.gov; Tab. 1).

Pathogenicity assays: These were implemented using cucumber (*Cucumis sativus* L., cv. 'Aonagakei-Jibai', Takii & Co. Ltd., Kyoto, Japan) plants. One-week-old seedlings were used that had been grown on moistened filter paper in a growth chamber at 25 °C with continuous light (80 µmol m^{-2} s^{-1} measured) in 9-cm-diameter petri dishes. 5 mm diameter agar (CMA) plugs with growing mycelia of *P. abappressorium* (OPU 1682) and *P. spinosum* (OPU 1704) were taken from the culture, placed on the plants, and incubated in the growth chamber at 25 °C. CMA was used as a control. Mortality of plants was recorded 10 days after incubation. The presence of asexual structures including sporangia and hyphal swellings in the plant tissues was the criterion of *P. abappressorium* and *P. spinosum* infection, respectively. Each experiment included 25 plants (5 plants in each petri dish) and was repeated 3 times. Analysis of variance was conducted on the rates of mortality and infection using IBM SPSS Statistics software (version 22; IBM Corporation, USA). Means of the data were compared by

the least significant difference based on a Tukey–Kramer honestly significant different (HSD) test ($P < 0.01$).

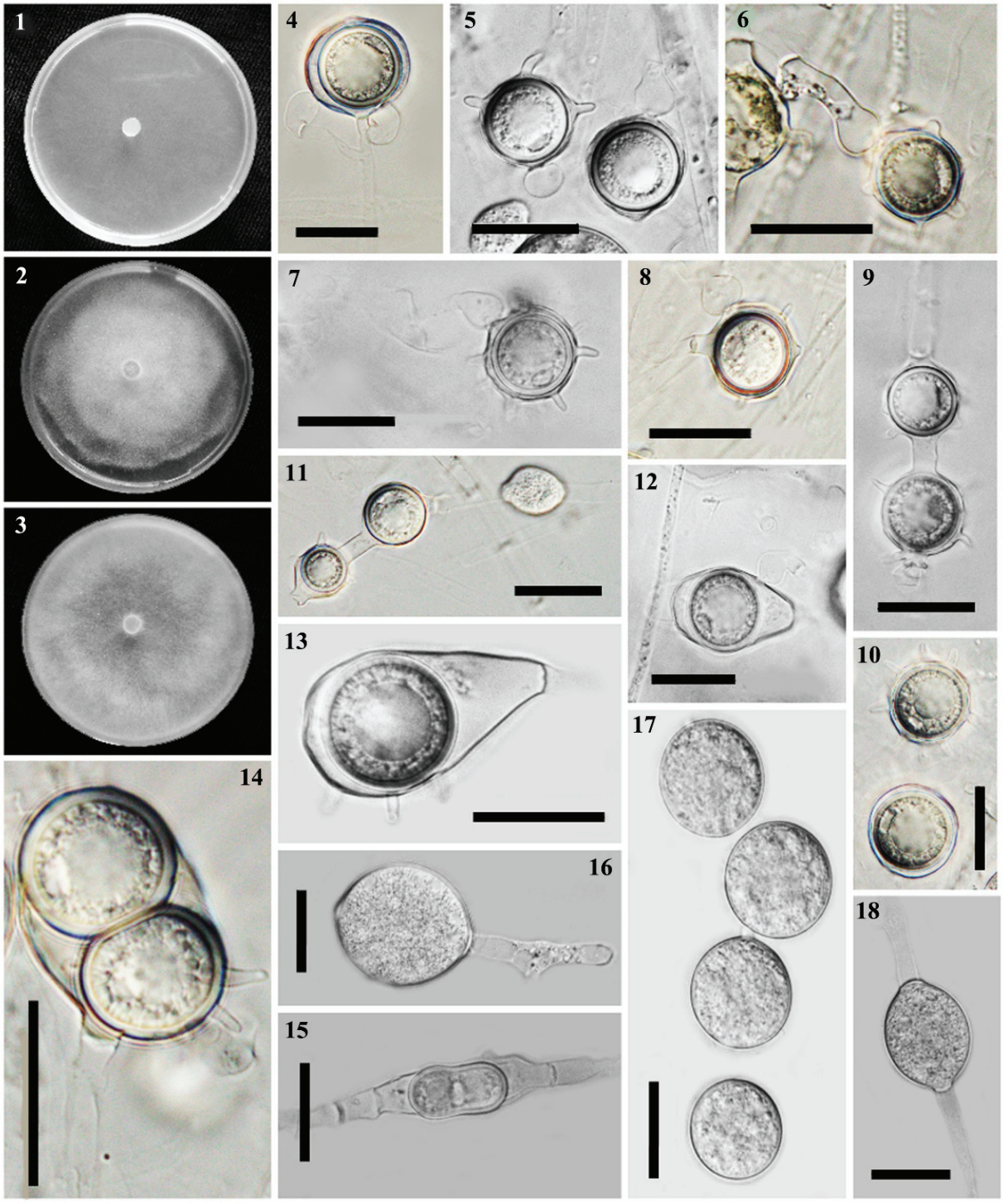

Fig. 2. *Pythium spinosum*. *1–3* Colony morphology of the isolate OPU 1704 on CMA, PDA, V8A, *4–8* oogonium with 1–2 monoclinous and diclinous antheridia, *9–11* plerotic oospores, *12–13* aplerotic oospores, *14* two oospores within one oogonium, *15* fusiform oogonia, *16–18* terminal and intercalary hyphal swellings. – Bars: 20 µm.

Results

Morphological description

Pythium abappressorium isolate OPU 1682. – Fig. 1

M y c e l i a : submerged with vague radiate pattern on CMA, composed of aerial growth with rose pattern-like on V8A and PDA. Optimum growth at 25 °C on PCA, with the average daily growth rate of 15 mm. Minimum, optimum and maximum growth temperature on PCA were 5, 20 and 30 °C, respectively.

M a i n h y p h a e : hyaline and up to 5 µm wide.

A p p r e s s o r i a : curved to sickle–shaped often branched.

S p o r a n g i a : more or less globose, 22–27 µm in diam., terminal or intercalary, formed from appressoria often attached to the base of sporangium.

Z o o s p o r e s : formed at 22 °C.

H y p h a l s w e l l i n g s : globose, lemon–shaped or cylindrical 22–38 × 12–17µm.

O o g o n i a : smooth, globose, terminal or intercalary, 13–25 µm in diam.

A n t h e r i d i a : sac- to crook-necked-shaped, 1–2 per oogonium, monoclinous and diclinous.

O o s p o r e s : smooth, plerotic, globose 17–31 µm in diam. and rarely oblong 24–26 × 14–16 µm, 1 or occasionally 2 in one oogonium.

Pythium spinosum isolate OPU 1704. – Fig. 2

C o l o n y : submerged with slight radiate pattern on CMA, arachnoid-cottony pattern on PDA and with aerial mycelium growth on V8A. Optimum growth at 25 °C on PCA, with the average daily growth rate of 32 mm. Minimum, optimum and maximum growth temperature on PCA 4, 22 and 30 °C.

M a i n h y p h a e : hyaline and up to 7 µm wide.

S p o r a n g i a : and zoospores not formed.

H y p h a l s w e l l i n g s : globose, lemon-shaped and thin-walled, 18–30 µm in diam.

O o g o n i a : terminal or intercalary, globose or fusiform, 15–26 µm in diam., with a varying number of blunt, finger-like ornamentation, 4–6 µm long and 1–2 µm in diam. at the base.

A n t h e r i d i a : 1–2 per oogonium, monoclinous and diclinous, antheridial cells inflated.

O o s p o r e s : plerotic, occasionally aplerotic, 14–22 µm in diam., thin-walled. Two oospores formed within one oogonium.

DNA sequencing and phylogenetic analysis

One isolate of *P. abappressorium* and one isolate of *P. spinosum* were subjected to DNA sequence analyses. For *P. abappressorium* and *P. spinosum*, the final aligned ITS dataset contained 64 ingroup taxa with a total of 329 characters, containing 62

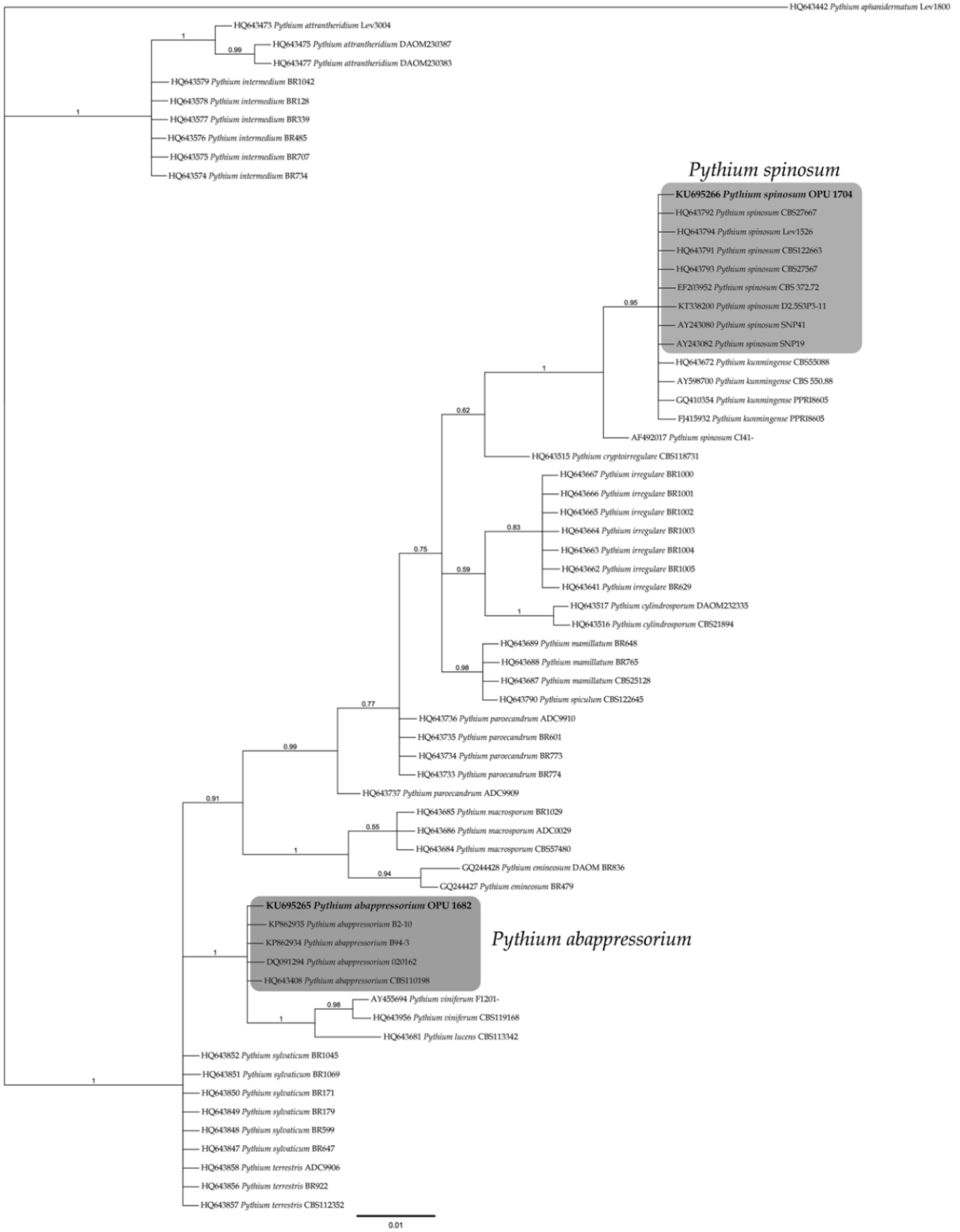

Fig. 3. Consensus phylogram (50% majority rule) of 864 trees resulting from a Bayesian analysis of ITS sequence alignment using MrBayes v.3.2.1. The scale bar indicates 0.01 expected changes per site. Numbers above the branches represent posterior probabilities. The tree was rooted with *Pythium aphanidermatum*.

unique site patterns. *Pythium aphanidermatum* (GenBank accession HQ643442) served as the outgroup taxon. The heating parameter was set to 0.15. The results of MrModeltest recommended HKY+I model with a gamma distributed rate variation

and dirichlet base frequencies. During the generation of the ITS tree, a total of 864 trees were saved, and consensus trees and posterior probabilities were calculated from

Fig. 4. Pathogenicity assay of *P. abappressorium* (2) on cucumber seedlings. Arrows indicate the rotting symptoms after inoculation. Control plants (1). Sporangium of *P. abappressorium* (3) formed in leaf cells of *Cucumis sativus*. – Bars: 20 µm.

Fig. 5. Pathogenicity assay of *P. spinosum* (1) on cucumber seedlings. Arrows indicate the rotting symptoms after inoculation. Control plants (2). hyphal swellings of *P. spinosum* (3) formed in leaf cells of *Cucumis sativus*. – Bars: 20 µm.

the remaining 1152 (75 %) trees. Based on the result of the ITS sequence data, isolates of *P. abappressorium* and *P. spinosum* obtained in this study resided in the well-supported clade together with the representative sequences for *P. abappressorium* and *P. spinosum* isolates from GenBank in the clade F. (Fig. 3).

Pathogenicity assays

Both *P. abappressorium* and *P. spinosum* isolates were pathogenic on cucumber seedlings. Disease symptoms initially appeared as small water-soaked lesions on stem near to the root system. The lesions then coalesced and gave the stems a weak and rotten appearance. Infection rates for both isolates in inoculated plants were 100 %. No significant differences were observed in the mortality induced by different isolates according to a Tukey-Kramer honestly significant difference (HSD) test ($P > 0.01$) (Tab. 4). Sporangia of *P. abappressorium* and hyphal swellings of *P. spinosum* were frequently found within tissues of inoculated plants (Figs. 4, 5). These fungal isolates were consistently reisolated from inoculated plants (100 %) but not from the control plants (0 %).

Discussion

Based on the results of the ITS-rDNA sequence data, *P. abappressorium* and *P. spinosum* isolate sequences obtained in this study nested within *Pythium* clade F species. *Pythium abappressorium* has some unique morphological traits not shared by other *Pythium* species. Appressoria are formed in contact with petri-dish surfaces or grass leaves. In *P. viniferum* antheridia and oogonia arised from appressoria, but they did not form within the appressorium, unlike *P. abappressorium.* In *P. abappressorium*, at maturity, some parts of the appressoria often remain attached at the base of oogonia and sporangia. Another unusual trait of the appressorium of *P. abappressorium* is the branching of the appressoria. In most species of the genus *Pythium*, appressoria are single or in chains. *Pythium abappressorium* differs clearly from *P. lucens* and *P. viniferum* in phylogeny and morphology (Tab. 2).

Pythium spinosum differs from the other *Pythium* species by its digitate ornamentations. Rarely a few spines can be conical, the majority is cylindrical with a blunt tip and 4–6 µm long. *Pythium irregulare* can produce a number of cylindrical protuberances, but these are irregularly arranged and usually fewer in number (VAN DER PLAATS-NITERINK 1981). YAMAMOTO & MAEDA (1961) considered *P. artotrogus* var. *macracanthum* to be synonymous with *P. spinosum*. According to the description, the oogonia, oospores and spines are larger than in *P. spinosum*. Phylogenetically and morphologically, *P. spinosum* differs clearly from *P. kunmingense* (Tab. 3).

Both recovered species, *P. abappressorium* and *P. spinosum* are new records for the mycobiota of Iran. We found that Iranian isolates of the species were pathogenic on cucumber seedlings and formed sporangia and hyphal swellings within plant tissues. Regarding previous studies, *Urtica dioica* and *Cynodon dactylon* are new substrates associated with *P. abappressorium and P. spinosum*, respectively.

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