


## Short Article

***Phytophythium montanum*, a new species for Iran**Negin Ramezanzadeh , Khalil-Berdi Fotouhifar ✉*Department of Plant Protection, Faculty of Agriculture, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*<https://doi.org/10.22092/mi.2025.368635.1307>**ABSTRACT**

To investigate Oomycete species in the aquatic habitats of Tehran Province, Iran, water samples were randomly collected from various aquatic ecosystems based on their geographical distribution from April to November 2021. Oomycete isolates were recovered by the baiting method with citrus (*Citrus limetta*) leaves and cultured on CMA+PARP semi-selective culture medium. Finally, two isolates (KAN0230 and KAN0218) obtained from the Kan River were identified as *Phytophythium montanum* based on the morphological features of the vegetative and reproductive organs as well as molecular data from the internal transcribed spacer (ITS-rDNA) sequences in a maximum likelihood phylogenetic analysis. Based on the available literature, *Phytophythium montanum* is reported for the first time as a new taxon for the Oomycete biota of Iran.

**KEYWORDS**Morphology, Phylogeny, ITS-rDNA, *Oomycota*, aquatic ecosystem.**INTRODUCTION**

*Oomycota*, a monophyletic group of filamentous fungal-like organisms (*Stramenopila*), is ubiquitous in the oceans, freshwater, and terrestrial environments throughout the world (Thines and Kamoun 2010, Jankowiak et al. 2015). The species belonging to the class *Oomycetes* are fungus-like organisms that are much closely related to diatoms and brown algae than to the kingdom of fungi (Beakes et al. 2012). Because they are related to aquatic organisms, their dependence on water is strong as all or some stages of their lifecycle are reliant on water (Walker and van West 2007). Some of these species are saprophytes, however, others can colonize plant tissues and thus be pathogenic and capable of causing severe diseases (Thines 2014). Two genera of *Oomycetes*, *Pythium* and *Phytophythium*, have a variety of nutritional modes and ecological niches. They are well established as soil-borne plant pathogens or saprophytes but are also distributed widely in freshwater ecosystems (Nechwatal et al. 2008). Species of *Pythium* are categorized into 11 clades (A-K), according to their phylogenetic relationships,

morphological characteristics, and host preferences. Members of the genus *Phytophythium* were originally classified in clade K of the genus *Pythium* according to the molecular phylogenetic subdivision of *Pythium sensu lato* by Levesque and de Cock (2004). They are similar to the *Pythium* species based on the zoospore differentiation and release mechanism but produce globose to ovoid, papillate sporangia, often with internal proliferation similar to species of *Phytophthora* (Bala et al. 2010a). As a result, a new genus, *Phytophythium*, an intermediate between *Phytophthora* and *Pythium* was introduced to separate it from other groups in *Pythium* (de Cock et al. 2015).

Oomycete species are pathogens of many plants and they represent one of the biggest threats to global food security and natural ecosystems. Until 2015, only three *Phytophythium* species with previous generic name of *Pythium*, including *P. helicoides*, *P. ostracodes*, and *P. vexans*, have been reported from Iran (Babai-Ahary et al. 2004, Mostowfizadeh-Ghalamfarsa 2015), but subsequent studies using morphological and sequence

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data documented the presence of *P. carbonicum*, *P. litorale*, *P. boreale*, *P. mercuriale*, *P. oedochilum*, *P. babaiaharii*, *P. longitubum*, *P. palingenes* and *P. sterile* in different provinces of Iran, indicating that the most isolated *Phytophthium* species from Iran belong to clade 1 that have been recovered from agricultural soils (Chenari Bouket et al. 2016, Salmaninezhad and Mostowfizadeh-Ghalamfarsa 2017, Hosseini Badrbani et al. 2018, Rezaei et al. 2021, Salmaninezhad and Mostowfizadeh-Ghalamfarsa 2023).

Considering the importance of aquatic ecosystems in the transmission of plant pathogens, identifying these pathogens and determining their characteristics are very important. Before our study, information on *Phytophthium* was scarce and no species of this genus had been reported from Tehran Province, Iran. Therefore, the present study was conducted to identify the Oomycetous species in some aquatic ecosystems of the Tehran Province.

## MATERIALS AND METHODS

Water samples were collected from some aquatic ecosystems (running waters, reservoirs, fountains, and lakes) of Tehran Province, Iran, between April to November of 2021. Isolates were obtained from water samples by baiting technique (van der Plaats-Niterink 1981), using sterile citrus (*Citrus limetta*) leaves. Baits embedded in water samples were incubated at room temperature for 24 h and then, the colonized baits were washed under running tap water to remove the excess contaminants, blotted dry on sterile filter paper and then plated on CMA+PARP agar culture medium (extract of 60 g ground maize, pimaricin 0.01 g, ampicillin 0.25 g, rifampin 0.01 g, PCNB 0.1 g, agar 15 g, distilled water 1l) (Mostowfizadeh-Ghalamfarsa and Banihashemi 2005). The grown mycelia on the culture medium were sub-cultured on 2% water agar (WA) culture medium (agar 20 g, distilled water 1 liter) and the isolates were purified by transferring the hyphal tips onto Petri dishes containing handmade potato-dextrose-agar (PDA; extract of 200 g cooked peeled potato, agar 20 g, dextrose 15 g, distilled water 1l).

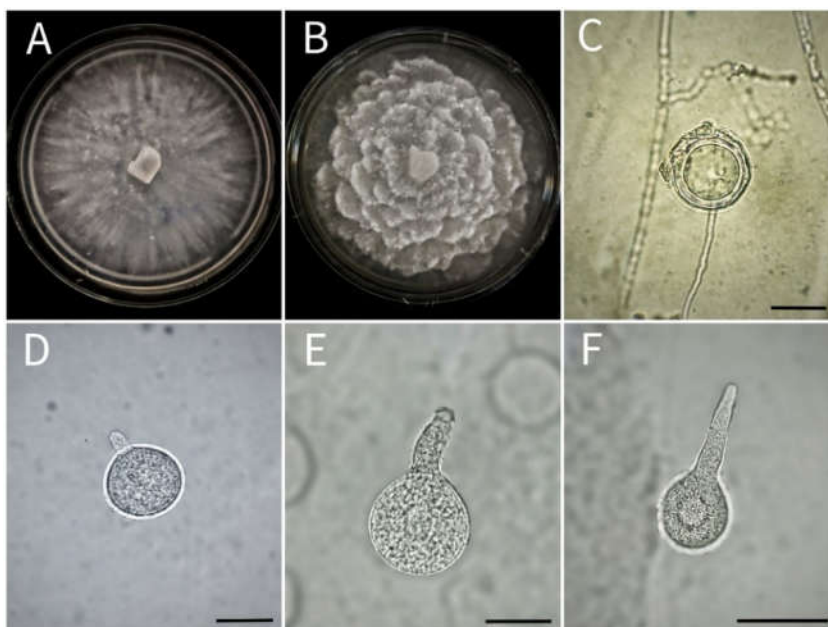
**Table 1.** ITS-rDNA sequences used for phylogeny. The newly generated sequence is in boldface.

Taxon	Origin	Isolate ID	GenBank no.	Reference
<b><i>Phytophthium montanum</i></b>	<b>Iran</b>	<b>IRAN 4844C</b>	<b>OQ825978</b>	<b>Present study</b>
	Japan	CBS111349	AB725883	Baten et al. (2014)
	Germany	ADC9762	HQ643391	Robideau et al. (2011)
	Germany	CBS111349	HQ643389	Robideau et al. (2011)
	Germany	ADC9766	HQ643390	Robideau et al. (2011)
<i>Phytophthium aichiense</i>	Japan	MAB-2014	AB948197	Baten et al. (2014)
<i>Phytophthium babaiaharii</i>	Iran	Iran-Ppy1	PP892678	Rezaei et al. (2021)
<i>Phytophthium boreale</i>	China	CBS55188	HQ643372	Robideau et al. (2011)
<i>Phytophthium carbonicum</i>	France	CBS112544	HQ643373	Robideau et al. (2011)
<i>Phytophthium citrinum</i>	Netherlands	ADC9442	HQ643380	Robideau et al. (2011)
<i>Phytophthium chamaeophyon</i>	Japan	CBS259.30	AB690609	Baten et al. (2014)
<i>Phytophthium cucurbitacearum</i>	Canada	CBS 748.96	AY598667	Levesque and de Cock (2004)
<i>Phytophthium delawarensis</i>	Japan	382B	AB725875	Baten et al. (2014)
<i>Phytophthium dogmae</i>	Philippines	USTCMS 4101	MF353170	Unpublished
<i>Phytophthium helicoides</i>	Japan	CBS286.31	AB725878	Baten et al. (2014)
<i>Phytophthium iriomotense</i>	Japan	CBS292.37	AB690624	Baten et al. (2014)
<i>Phytophthium fagopyri</i>	Japan	FP1	AB690625	Baten et al. (2014)
<i>Phytophthium kandeliae</i>	Taiwan	CBS11191	HQ643134	Robideau et al. (2011)
<i>Phytophthium litorale</i>	France	CBS122662	HQ643385	Robideau et al. (2011)
<i>Phytophthium megacarpum</i>	South Korea	Pet-Stol-SW7	AB725588	Unpublished
<i>Phytophthium mercuriale</i>	Japan	CBS122443	AB690614	Baten et al. (2014)
<i>Phytophthium mirpurensis</i>	Pakistan	CBS124524	KJ831614	Unpublished
<i>Phytophthium nanjingensis</i>	China	Chen 218	MF459636	Unpublished
<i>Phytophthium oedochilum</i>	Japan	CBS292.37	AB108020	Baten et al. (2014)
<i>Phytophthium ostracodes</i>	Spain	CBS76873	HQ643395	Robideau et al. (2011)
<i>Phytophthium palingenes</i>	Vietnam	VN429	MN872742	Jung et al. (2020)
<i>Phytophthium paucipapillatum</i>	South Africa	STE-U7843	KX372749	Unpublished
<i>Phytophthium sindhum</i>	Pakistan	CBS:124522	HM244828	Bala et al. (2010b)
<i>Phytophthium sterile</i>	Iran	KH1-2	KX228096	Salmaninezhad and Mostowfizadeh-Ghalamfarsa (2017)
<i>Phytophthium sterilum</i>	Spain	PE101	DQ217603	Belbahri et al. (2006)
<i>Phytophthium vexans</i>	South Africa	CBS119.80	GU133572	Spies et al. (2011)
<i>Achlya sparrowii</i>	Canada	CBS10249	HQ643108	Robideau et al. (2011)

To induce the formation of sporangia, mycelial plugs from the actively growing colony margins of the isolates grown on PDA culture medium were transferred into Petri dishes containing sterile distilled water or soil extract (10 g/L), and boiled hemp seed (Banihashemi et al. 1992). Petri dishes were incubated at 25°C under the cool white fluorescent light and were examined daily and the shape and size of the asexual structures were determined for each isolate by preparing microscopic slide mounts. To induce the formation of oospores, isolates were grown on hemp seed agar (HSA; ground hemp seed extract 60 g/L; agar 15 g/L) (Salmaninezhad and Mostowfizadeh-Ghalamfarsa 2019) culture medium and the inoculated Petri dishes were incubated at 25°C in continuous darkness. Colony pattern and average daily growth rate at 25°C were measured in three replicates growing on PDA and corn meal agar (CMA) culture media. The living culture of the investigated taxon has been deposited in the Fungal Collection of the Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN).

For molecular study, isolates were grown in handmade Potato Dextrose Broth (PDB; extract of 200 g cooked peeled potato, dextrose 15 g, distilled water 1l) at 25°C for seven days on a rotary shaker and total genomic DNA was extracted from obtained mycelium using the method described by Zhong and Steffenson (2001). The primer pair ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990) and ITS6 (GAAGGTGAAGTCGTAACAAGG) (Cooke et al. 2000) were used to amplify the internal transcript spacer (ITS) region from nuclear rDNA of

the isolates. The PCR cycling parameters were: initial denaturation at 94°C for 2 min followed by 30 cycles of denaturation at 94°C for 2 s, annealing at 62°C for 25 s, extension at 72°C for 1 min and final extension at 72°C for 10 min. The PCR product was analyzed using 1% agarose gel electrophoresis, stained with ethidium bromide (0.5 µg/ml), and visualized under UV light, and then sent to the Codon Genetic Research Center (IRAN) for sequencing. The generated sequence was manually edited with Chromas 2.6.6 software (Technelysium Pty Ltd, Australia) and the edited sequence was saved in FASTA format. The resulting sequence was subjected to BLAST search (Altschul et al. 1997) in the National Center of Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov) to find the most similar and relevant sequences. Sequences of the reference isolates were obtained from GeneBank and *Achlya sparrowii* (HQ643108.1) was used as an outgroup taxon. All obtained sequences (Table 1) were aligned together with the sequence of our isolate (IRAN 4844C) using the Clustal W (Thompson et al. 1994) algorithm implemented in MEGA 11.0 (Tamura et al. 2021). Phylogenetic analysis using the maximum likelihood method (Felsenstein 1973) with default options (nucleotide substitution model: Tamura-Nei model; rates among sites: uniform rates; gaps/missing data treatment: use all sites; tree inference options: ML heuristic method, with nearest-neighbor-interchange, NNI) was conducted in MEGA 11.0. The generated tree was tested using the bootstrap method with 1000 replicates. The newly generated ITS-rDNA sequence of the present study (IRAN 4844C) was submitted to GenBank with accession number of OQ825978.

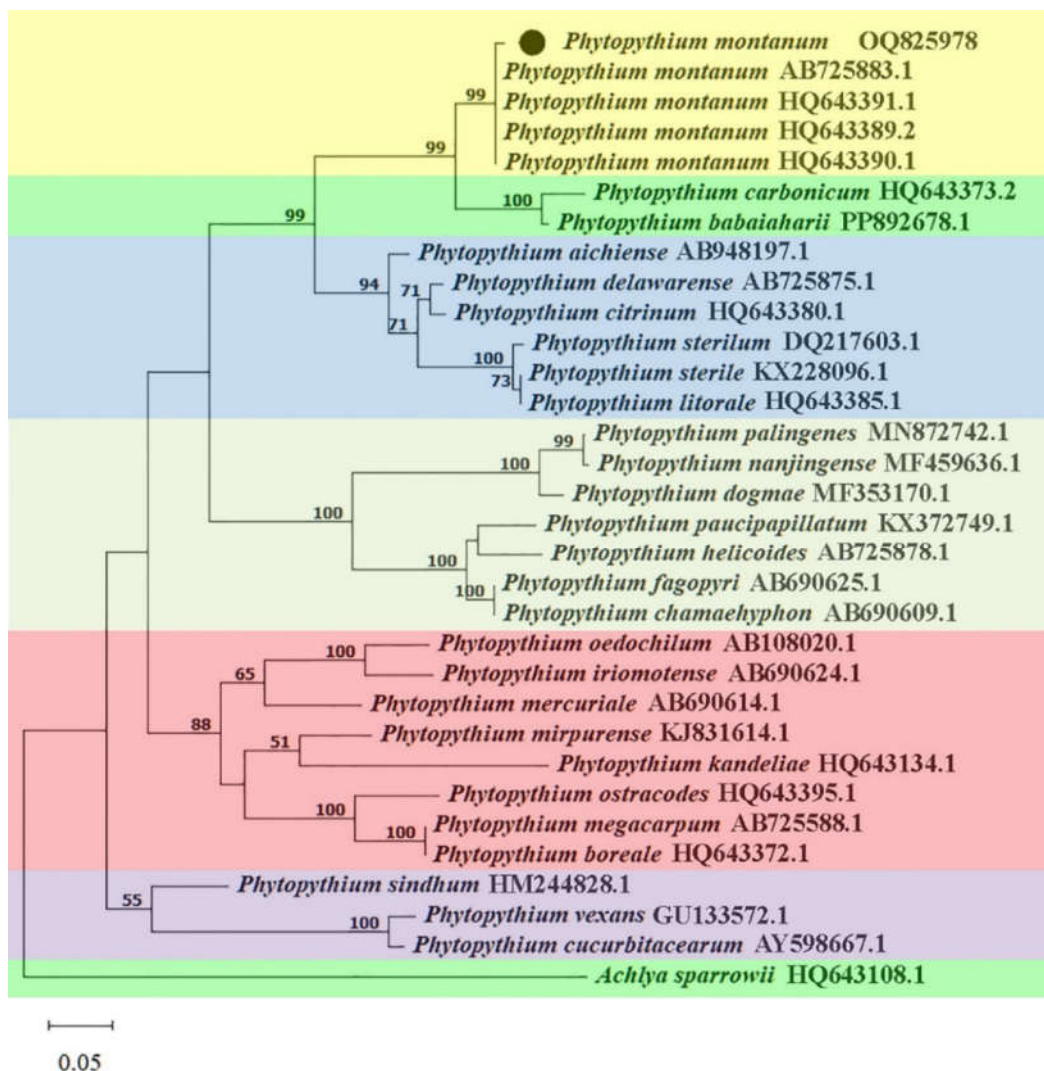


**Fig. 1.** *Phytophythium montanum*, isolate IRAN 4844C: (A) Colony pattern on CMA, (B) Colony pattern on PDA after 10 days in continuous darkness at 25°C, (C) Oospore, (D-F) Sporangia showing various degrees of apical elongation (Bars = 20µm).

## RESULTS AND DISCUSSION

Two Oomycete isolates [KAN0230 (IRAN 4844C) and KAN0218] with similar cultural and morphological features were obtained from samples collected from the Kan River and were identified as *Phytophthium montanum* (Nechw.) Abad, de Cock, Bala, Robideau, Lodhi & Levesque, based on the morphological and molecular data of the ITS-rDNA sequence. Colonies on agar culture media (PDA, CMA and HSA) were submerged, showing a petaloid pattern on PDA and stellate to chrysanthemum pattern on CMA. The average daily growth rate at 25°C ranged from 5.5 mm/day on PDA to 6 mm/day on CMA. The width of the main hyphae was up to 5 µm. Hyphal swelling or

chlamydozoospores were not observed. Sporangia formed in water were terminal, globose, ovoid or ob-pyriform. Mature sporangia with apical or lateral papilla and papilla either giving rise to a short discharge tube with 2-8 µm width, subsequent release of sporangium contents for zoospore development, or developing into a large rostrum up to 20 µm. After zoospore release, new sporangia developed by internal proliferation, either within or outside of the empty sporangium. Oogonia were spherical, smooth-walled and mostly terminal, measuring 22-27 µm (av. 23) in diameter. Antheridia were monoclinal and extremely variable in shape. Oospore was aplerotic, 16-21 µm in diameter (av. 18.5) and oospore walls 1.6-2 µm thick (Fig. 1).



**Fig. 2.** Phylogenetic tree inferred using the maximum likelihood (ML) method based on the nucleotide sequences of ITS-rDNA region in 31 isolates of *Phytophthium* species. The solid circle indicates the isolate obtained in the present study. Numbers on the branches represent the percentage of bootstrap values from 1000 replicates. *Achlya sparrowii* (HQ643108.1) was used as an out-group taxon.

Morphological features of the investigated isolates were similar to those of *Pythium montanum* as described by Nechwatal and Osswald (2003). *Pythium montanum* was first introduced by Nechwatal and Osswald (2003) having a unique combination of sporangial and gametangial characters as well as ITS-rDNA sequence data that could not be assigned to any other known species of the genus *Pythium*. The species has relatively low growth rate, internally proliferating sporangia and extremely variable, monoclinal, sessile or shortly stalked antheridia with frequently direct germination of sporangia in water culture. de Cock et al. (2015) described the new genus *Phytophythium* to accommodate the members of *Pythium* clade K, as described by Lévesque and de Cock (2004), and transferred *Pythium montanum* to the genus *Phytophythium*, establishing the new combination *Phytophythium montanum*.

*Phytophythium montanum* is phylogenetically and morphologically related to but distinct from *P. babaiaharii*. *Phytophythium babaiaharii* has recently been reported from the soil of sugar beet fields in West Azarbaijan as a new species. Morphologically, *P. babaiaharii* is distinguished from *P. montanum* by the production of lateral oogonia and having both plerotic and aplerotic oospores (Rezaei et al. 2021). *Phytophythium montanum* as *Pythium montanum* has been isolated from the rhizosphere soil of Norway spruce [*Picea abies* (L.) H. Karst] in Bavarian Alps at an altitude of 1000 m above sea level. In experimental conditions, the weak aggressiveness of this species on lupine (*Lupinus angustifolius* L.) and Norway spruce has been demonstrated (Nechwatal and Oßwald, 2003). In the present study, the ITS-rDNA sequence of the *Phytophythium montanum* (IRAN 4844C) showed more than 98% similarity with valid sequences such as HQ643389, HQ643390, HQ643391, (Robideau et al. 2011), and AB725883 (Baten et al. 2014) of previously identified and deposited taxa in the GenBank. The maximum likelihood phylogenetic tree constructed based on ITS region sequences indicated that the Iranian specimen is placed in the same clade with *P. montanum* isolates reported by other authors with 99% bootstrap support (Fig. 2). Based on the results of morphological and phylogenetic investigations, *P. montanum* is reported for the first time as a new taxon for the Oomycete biota of Iran. The living culture of the isolate Kan0230 has been deposited in the Fungal Collection of the Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN), with accession number IRAN 4844C.

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#### AUTHOR CONTRIBUTION

The individual contributions of authors to the manuscript are as follows. Negin Ramezanzadeh performed the research and wrote the primary manuscript, Khalil-Berdi Fotouhifar designed and supervised the research and reviewed the primary manuscript. In addition, Kh. -B. Fotouhifar is corresponding author.

#### DATA AVAILABILITY

All generated datasets during the current study are available in the manuscript. The newly generated ITS sequence was deposited in the NCBI with accession number of OQ825978.

#### DECLARATION

The authors declare that there is no conflict of interest.

#### FUNDING

There is no fund for this research to be declared.

#### ETHICS APPROVAL

Not applicable.

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## *Phytophythium montanum*, گونه جدیدی برای ایران

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گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشکدگان کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران

### چکیده

در طی اردیبهشت تا آذر سال ۱۴۰۰، برای بررسی تنوع گونه‌های آُمیستی در برخی از زیست‌بوم‌های آبی استان تهران، نمونه‌های آب از برخی آب‌های جاری، راکد، ذخیره‌ای، چشمه و چاه به صورت تصادفی و با رعایت پراکنش جغرافیایی جمع‌آوری شدند. به منظور جداسازی گونه‌های آُمیستی، قطعات برگ لیمو شیرین (*Citrus limetta*) به ظروف حاوی هر نمونه آب قرار داده شدند و به مدت ۲۴ ساعت در ۲۵ درجه سلسیوس نگهداری گردیدند. سپس برگ‌ها روی محیط کشت نیمه اختصاصی CMA-PARP قرار داده شدند و از میسلیوم‌های رشد کرده در اطراف برگ لیمو شیرین، پرگنه‌های خالص تهیه شدند. از بین جدایه‌های به دست آمده، بر اساس ویژگی‌های ریخت‌شناختی اندام‌های رویشی و زایشی و همچنین داده‌های مولکولی مبتنی بر توالی نوکلئوتیدی نواحی ITS-rDNA، دو جدایه *Phytophythium montanum* (Nechw.) Abad, de Cock, Bala, Robideau) حاصل از رودخانه کن به گونه *Phytophythium montanum* (Nechw.) Abad, de Cock, Bala, Robideau) Lodhi & Levesque تعلق داشتند. بر اساس منابع موجود، این نخستین گزارش از گونه *Phytophythium montanum* در ایران می‌باشد و آرایه جدیدی برای زیواگان آُمیستی ایران است.

### کلمات کلیدی

ریخت‌شناسی، تبارزایی، ITS-rDNA، آُمیکوتا، زیست بوم آبی.