



## The Current Status of *Phytopythium* Species in Iran: Challenges in Identification of an Intermediate Taxon

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**Abstract:** *Phytopythium* is a cosmopolitan genus found in different regions of the world from various substrates. This genus is a newly described taxon that was once a member of the genus *Pythium sensu lato*. *Phytopythium* is an intermediate genus between *Pythium* and *Phytophthora* with 34 formally described species. Recently, some studies focused on the phylogeny of this genus in Iran. Although not many studies concentrated on isolating species assigned to this genus from Iran, some comprehensive studies showed that *Phytopythium* is an important genus with vast distribution in this part of the world. Accurate identification and classification of *Phytopythium* species are quite challenging. Morphological identification of *Phytopythium* is troublesome due to the lack of identification keys, overlapping of some morphological features, the presence of species complexes, pleomorphism, and the absence of certain structures in some species. Besides, most species cannot be separated using only one or two loci for the phylogenetic analyses. In addition, some studies in Iran do not include molecular investigations to support their morphological identification or make it possible to reidentify the reported species. Having no accurate and current checklist of the country's species also adds to the problem. This review focuses on the current systematics of *Phytopythium* species in Iran, discussing the challenges in the morphological and molecular identification of the species in the country. It also proposes some approaches to address the problem of characterizing the species in the genus *Phytopythium*.

**Key words:** Molecular barcoding, Morphology, *Oomycota*, Phylogeny, *Pythiaceae*

### INTRODUCTION

The genus *Pythium* (Pringsh.) is one of the most well-known genera in *Oomycota*. Various species of this genus have been either reported several times from Iran, e.g., *Pythium aphanidermatum* (Edson) Fitzp., *Py. catenulatum* Matthews, *Py. hydnosporum* (Mont.) J. Schröt., and *Py. oligandrum* (Drechsler) (Teymoori *et al.* 2012; Abad *et al.* 2013; Bolboli & Mostowfizadeh-Ghalamfarsa 2016; Badali *et al.* 2016; Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2017a; Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a; Salmaninezhad *et al.* 2021) or even originally described from this country, e.g., *Py. heteroogonium* Mostowf. & Salmaninezhad, *Py. longipapillum* Mostowf. & Salmaninezhad, *Py. oryzicollum* Salmaninezhad & Mostowf. (Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019b). Species of this genus are known to produce a diverse type of asexual structures called sporangia, in which the asexual spores, called zoospores, are formed. Zoospores are then differentiated into a sack-shaped structure called a vesicle (Van der Pläats-Niterink 1981; Zitnick-Anderson 2013). For a long time, it was believed that if a certain isolate produces vesicles, it would be categorized into the genus *Pythium* (De Cock *et al.* 2015). However, from the beginning, it was revealed that this genus might be paraphyletic. The idea of the paraphyletic nature of the genus *Pythium* comes from the formation of different types of sporangia by its species, including globose, subglobose, lobate, strictly filamentous, filamentous inflated, ovoid, pyriform, or even ellipsoid and elongated types of sporangia (Uzuhashi *et al.* 2010). Production of different types of sporangia led to the conclusion that *Pythium* cannot be considered as a single genus and further molecular studies supported this claim which resulted in the description of new genera, such as *Globisporangium*, *Elangisporangium*, *Pilasporangium* and even an intermediate genus, such as *Phytopythium* (Uzuhashi *et al.* 2010; Baten *et al.* 2014; De Cock *et al.* 2015; Nguyen *et al.* 2022). In this review, we aimed to illustrate a general picture of *Phytopythium* species

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reported from Iran and the challenges facing their precise identification.

#### **From *Pythium sensu lato* to *Phytopythium*: A story of separation**

With advances in molecular studies, researchers concluded that the genus *Pythium* “is” paraphyletic and should be divided into other genera. Using only the ITS region, researchers first divided the species associated with the genus *Pythium* into 11 clades, *i.e.*, A to K (Lévesque & De Cock 2004). However, even after this division, researchers proposed that clade K is completely different from other clades of the genus *Pythium* (Uzuhashi *et al.* 2010). Using the multiple gene genealogies approach, scientists revealed that the genus *Pythium* contains at least five different genera, *i.e.*, *Elangisporangium* (Clade H), *Globisporangium* (Clades E, F, G, I), *Pilasporangium*, *Phytopythium* (Clade K), and *Pythium sensu stricto* (Clades A, B, C, D) (Uzuhashi *et al.* 2010). There are several reports of *Pythium sensu stricto*, *Globisporangium*, and *Phytopythium* species from Iran, including *Py. aphanidermatum*, *Py. catenulatum*, *Py. oligandrum*, *Py. rhizo-oryzae* Paul, *Py. plurisporium*, *Py. porphyrae* Takah & Sasaki, *Py. pyrlobum* Vaartaja, *Globisporangium carolinianum* (Matthews) Uzuhashi, Tojo & Kakish, *G. glomeratum* (Paul) Uzuhashi, Tojo & Kakish, *G. heterothallicum* (Campb. & Hendrix) Uzuhashi, Tojo & Kakish, *G. irregulare* (Buisman) Uzuhashi, Tojo & Kakish, *G. nodosum* (Paul, D. Galland, T. Bhatn. & Dulieu) Uzuhashi, Tojo & Kakish, *G. ultimum* var. *ultimum* (Trow) Uzuhashi, Tojo & Kakish, *Phytopythium ostracodes* (Drechsler) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *P. sterile* Belbahri & Lefort, and *P. vexans* (de Bary) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque (Ershad 2022; Badali *et al.* 2016; Bolboli & Mostowfizadeh-Ghalamfarsa 2016; Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2017a; Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019; Salmaninezhad *et al.* 2021).

The genus *Phytopythium* is an intermediate genus between *Pythium* and *Phytophthora*. It is morphologically more similar to *Pythium*, yet genetically more related to *Phytophthora* (Lodhi *et al.* 2020). This genus is known to produce subglobose, ovoid, to ellipsoid sporangia, with or without papillae, as well as with or without internal or external proliferation, very similar to *Phytophthora*. However, in contrast to *Phytophthora*, *Phytopythium* species produce vesicles, and zoospores differentiate in the vesicle, like the genus *Pythium* (Zitnick-Anderson 2013). However, recently some reports showed that the differentiation of zoospores could also occur within the sporangia of some *Phytopythium* species, such as *P. leanoi* Bennett & Thines, *P. dogmae* Bennett & Thines, and *P. kandeliae* (H.H. Ho, H.S. Chang & S.Y. Hsieh) Thines (Marano *et al.* 2014; Bennett *et al.* 2017). *Phytopythium* also produces cylindrical or lobate antheridia. Antheridial attachment to oogonium in the genus *Phytopythium* is

also an intermediate feature. It is known that, in *Pythiaceae*, antheridium can be attached to oogonium as paragynous, amphigynous, or hypogynous. All three types of antheridia have been reported for the *Phytopythium* species, whereas the amphigynous antheridium is only reported in *Phytophthora*, and the hypogynous type has only been reported in the *Pythium* species (Baten *et al.* 2014, 2015; De Cock *et al.* 2015; Lodhi *et al.* 2020) (Fig. 1).

The name *Phytopythium* was first coined by Bala *et al.* (2010) and *P. sindhum* was introduced as the type species (Bala *et al.* 2010). Even though Uzuhashi *et al.* (2010) proposed the name *Ovatisporangium* for this genus, due to the priority, *Phytopythium* was accepted as the valid name. Using ITS and cytochrome c oxidase subunit I (*cox1*), De Cock *et al.* (2015) formally described *Phytopythium* as a new genus belonging to the family *Pythiaceae*, order *Peronosporales*, and *P. sindhum* Lodhi, Shahzad & Lévesque was considered as the type species (De Cock *et al.* 2015). Recently, using the whole genome sequencing of all *Pythium sensu lato* species, researchers also have confirmed *Phytopythium* as an intermediate but distinct genus (Nguyen *et al.* 2022). There are 34 species of *Phytopythium* described so far. It is also revealed that *Phytopythium* species are categorized into three main clades, *i.e.*, 1, 2, and 3 (Baten *et al.* 2014; De Cock *et al.* 2015).

#### **Distribution of *Phytopythium* species in Iran**

Since the beginning of the investigation of oomycetes in Iran, several *Phytopythium* species have been reported from various parts of the country from different hosts (Table 1). However, not until recently were they all identified and reported as *Pythium*, not *Phytopythium*. Using previous names would confuse researchers and should be avoided. Only a few studies reported the correct and new scientific names of the species assigned to *Phytopythium*. Fortunately, recent descriptions of new *Phytopythium* species from Iran tried to address this problem and used the correct name. For example, *P. babaiaharii* Rezaei, Abrinbana & Ghosta and *P. longitubum* Rezaei, Abrinbana & Ghosta have both been reported from Northwest of Iran and were both named correctly (Rezaei *et al.* 2021). Most of the reported *Phytopythium* species from Iran are from the Northwest part of the country as well as Fars Province (Table 1). However, a comprehensive study is required to clarify the actual distribution of the isolates assigned to *Phytopythium*. The species reported from Iran are listed in Table 1.

#### ***Phytopythium* species importance in Iran as plant pathogens**

*Phytopythium* species are agriculturally important (Baten *et al.* 2014; Rezaei *et al.* 2021). Most *Phytopythium* species cause devastating effects on seeds, roots, and crowns of various plants, belonging to different genera (Van der Pläats-Niterink 1981; Spies *et al.* 2011; Benfradj *et al.* 2017). The effects have also been observed on branches and shoots of trees (Rezaei *et al.* 2021). Even though several *Phytopythium* species have been reported from Iran,

most isolates have been collected from the soil. However, it has been indicated that *Phytophthium* species can cause severe damage to crops and ornamental plants (Spies *et al.* 2011). For instance, *P. vexans* complex has been reported from various plants worldwide and in Iran. This species causes rot symptoms on seeds, roots, and crowns on different hosts. While *P. vexans* complex is mainly reported as a plant pathogenic oomycete, some reports stated that it is also a saprophytic species. In addition, *P. helicoides* (Drechsler) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque is also known as a cosmopolitan oomycete causing root and seed rot on a wide range of plant hosts (Spies *et al.* 2011; Lodhi *et al.* 2020; Rezaei *et al.* 2021). In Iran, the pathogenicity of *P. vexans* complex, *P. litorale* (Nechw.) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *P. ostracodes*, and *P. babaiaharii* have been verified (Bolboli & Mostowfizadeh-Ghalamfarsa 2014; Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2017b; D. Delshad & R. Mostowfizadeh-Ghalamfarsa Unpublished data). There are only a few reports of the pathogenicity of *P. ostracodes* worldwide, and Iran is one of them (Rezaei *et al.* 2021). Furthermore, most of the *Phytophthium* species records from the country are reported from infected plants, such as cucurbits and rice (Hosseini Badrbani *et al.* 2018; Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019). Despite several records of *Phytophthium* from Iran, only a few have conducted Koch's postulates for the species assigned to this genus to confirm their pathogenicity. Hence, a comprehensive study is required to fulfill this aim.

#### **Morphological challenges in the identification of the genus *Phytophthium***

One of the most crucial steps in identifying a particular species is morphological identification. Hence, the precise identification of morphological features is of great importance. Regardless of how much molecular markers have facilitated the procedure of species identification, morphology is still an inevitable part of the taxonomy. However, morphological identification is the most challenging step in taxonomical studies, and *Phytophthium* species are no exception. Challenges in isolation of a particular species, the existence of heterothallic or sterile isolates, lack of specific structures, morphological plasticity of a specific structure, and lack of identification data for species would result in difficulties in species characterization (Mostowfizadeh-Ghalamfarsa & Salmaninezhad 2020). Therefore, morphological classification of *Phytophthium* species could be quite challenging.

One of the major problems of most researchers is the existence of both pathogenic and saprobe species in a single sampling. This might not seem a problem at first glance; however, plant pathologists prefer to obtain pathogens rather than saprobes (Mostowfizadeh-Ghalamfarsa & Salmaninezhad 2020). Hence, the current reported number of *Phytophthium* species does not reflect the actual

number of the species. Furthermore, most species could not be easily isolated from soil or plant materials, and if they do, they might not produce the required structures for morphological identification (Kageyama 2014; Mostowfizadeh-Ghalamfarsa & Salmaninezhad 2020). Another problem is the influence of different environments on the production of specific structures. For instance, *P. babaiaharii* was first described as a species readily producing plenty of sporangia and zoospores (Rezaei *et al.* 2021). Nonetheless, the second isolation from Fars Province showed different results. None of the isolates assigned to *P. babaiaharii* from Fars Province were able to produce zoospores with the same method described in the original description (Salmaninezhad *et al.* 2021).

The zoospore development location is another major problem in the morphological identification of the genus *Phytophthium*. All *Phytophthium* species produce vesicles, and zoospores develop within the vesicles. However, recent reports of some *Phytophthium* species, such as *P. leanoi*, *P. dogmae*, and *P. kandeliae*, showed that zoospore development would occur within the sporangium, just like *Phytophthora* species (Marano *et al.* 2014; Bennett *et al.* 2017).

Growth rate and pattern on various media is of great significance in *Pythium sensu lato* species, in particular *Phytophthium* species, identification (Van der Pläats-Niterink 1981; De Cock *et al.* 2015). Yet, strains of a single species show variations in their growth habit. Therefore, specific identification of a particular *Phytophthium* species should not rely only on its growth rate and pattern (Zitnick-Anderson 2013; Mostowfizadeh-Ghalamfarsa & Salmaninezhad 2020).

The inability to produce particular structures by some species is probably the biggest challenge for taxonomists. Some *Phytophthium* species, such as *P. litorale* are heterothallic and do not produce sexual structures. Morphology of the sporangium of *P. litorale* cannot solely separate this species from the others. Hence, this species can only be identified based on molecular approaches. Zoospore production would also not be feasible in some species.

Although producing specific structures for morphological identification is crucial, forming similar structures could also be a hassle for taxonomists. For instance, two known clades of *Phytophthium*, *i.e.*, clades 1 and 2, cannot be separated based on their sporangial shapes. Both clades 1 and 2 of *Phytophthium* produce subglobose sporangia with papillae and internal or external proliferation. Production of similar structures by two different species may lead to the misidentification of a species even when it comes to professionals. Variation of a specific morphological feature can be seen in most oomycetes, in particular *Phytophthium* species, called pleomorphism. Pleomorphism is another important obstacle in the morphological identification of *Phytophthium* species. For instance,

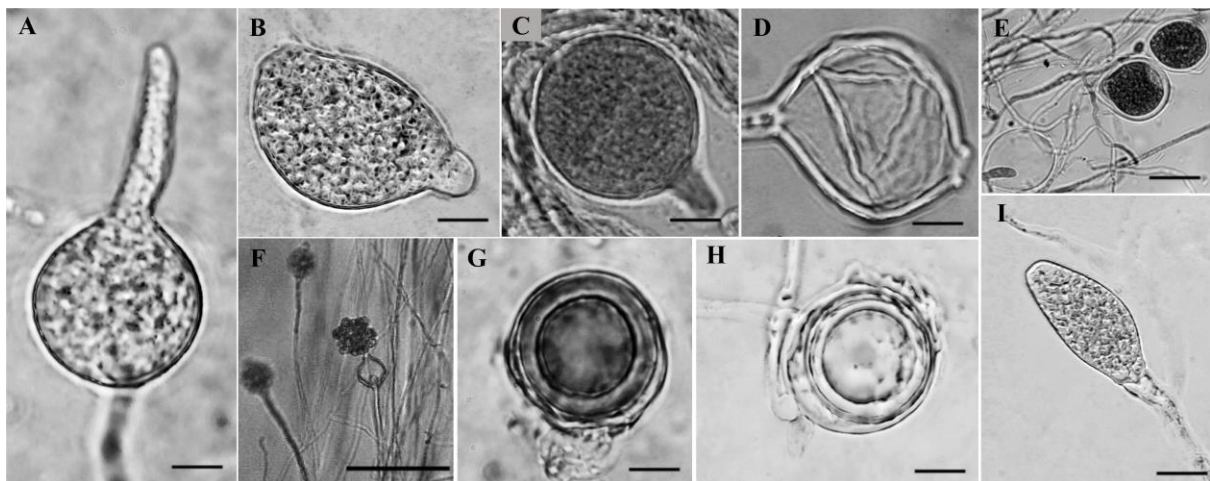
*P. mirpurens* Lodhi, De Cock, Lévesque & Shahzad produces different types of sporangia, globose, subglobose, ovoid, obovoid, and limoniform (De Cock *et al.* 2015). It also has both aplerotic and plerotic oospores, as well as both hypogynous and paragynous antheridia (De Cock *et al.* 2015). These variations have also been reported for other *Phytophthium* species, making the identification quite difficult.

Another major problem in the morphological classification of *Phytophthium* species is species complexes. The term “species complex” is usually used in taxonomy regarding three main situations: I. It is believed that a group of organisms may represent more than one species; II. No species boundaries could be discerned with certainty, *e.g.*, because of morphological similarity or insufficient data; and III. It is hypothesized that these species are related in some way. Among all known *Phytophthium* species, *P. vexans* is reported to be complex (Spies *et al.* 2011; Hyde *et al.* 2014; De Cock *et al.* 2015). This species has been reported several times from different parts of Iran (Table 1).

The morphological identification keys for *Phytophthium* species are still lacking. Therefore, it seems that an interactive key for the identification of *Phytophthium* is required which should be updated regularly. Since there are a limited number of

*Phytophthium* species reported from Iran, providing an interactive key for morphological identification of local species would be beneficial. This interactive key should contain precise morphological description, illustrations, high-quality microscopic pictures, and morphometric data sets as well as the coordinates of the recovered species from Iran.

In addition to obstacles mentioned earlier in the morphological identification of *Phytophthium* species, researchers in Iran encounter other problems. For example, some species are only reported once or, in rare cases, twice from Iran (Table 1), and in some cases, the metadata recordings of the recovered *Phytophthium* species are unavailable for several isolates. Only in recent comprehensive studies metadata recordings, such as matrices, host information, location coordinates, and date of isolation, have appeared in their corresponding literature (Bolboli & Mostowfizadeh-Ghalamfarsa 2014; Mostowfizadeh-Ghalamfarsa 2016; Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2017b; Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019; Rezaei *et al.* 2021). Moreover, host information is quite important in the recordings and generalization of the host names, such as turf grass, cucurbits, etc., could be problematic in future studies.



**Fig. 1.** Morphology of some *Phytophthium* species reported from Iran. A: *Phytophthium palingenes* sporangium with long discharged tube; B: Subglobose to ovoid sporangium in *Phytophthium vexans* complex with short papilla; C: Globose sporangium in *Phytophthium ostracodes*; D: Empty sporangium in *Phytophthium litorale*; E: Sporangia in *Phytophthium sterile*; F: Subglobose to ovoid sporangia in *Phytophthium litorale* as well as the formation of vesicle and differentiation of zoospores; G: Oospore in *Phytophthium palingenes*; H: oospore of *Phytophthium vexans* complex; I: Non-papillate sporangium in *Phytophthium babaiaharii*. Bar = 10  $\mu$ m, except for F in which Bar = 40  $\mu$ m.

**Table 1.** List of *Phytophthium* species reported from Iran.

Species <sup>1</sup>	Matrix	Location <sup>2</sup>	Reference
<b><i>P. babaiaharii</i></b> Rezaei, Abrinbana & Ghosta [1]			
	<i>Acer saccharinum</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Beta vulgaris</i> (rhizosphere soil)	West Azerbaijan (Piranshahr)	Rezaei <i>et al.</i> 2021
	<i>Cupressus sempervirens</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Morus alba</i> (root tissue)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Pinus eldarica</i> * (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Platanus orientalis</i> (rhizosphere, root and crown tissue)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Salix</i> spp. (crown tissue)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
<b><i>P. boreale</i></b> (R.L. Duan) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. boreale</i> , 1]			
	<i>Mentha spicata</i> (rhizosphere soil)	West Azerbaijan (Urmia)	Rezaei <i>et al.</i> 2021
	<i>Malus domestica</i> (rhizosphere soil)	West Azerbaijan (Salmas)	Rezaei <i>et al.</i> 2021
	<i>Prunus persica</i> (rhizosphere soil)	West Azerbaijan (Piranshahr)	Rezaei <i>et al.</i> 2021
	<i>Prunus persica</i> (rhizosphere soil)	West Azerbaijan (Khoy)	Rezaei <i>et al.</i> 2021
<b><i>P. carbonicum</i></b> (B. Paul) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. carbonicum</i> , 1]			
	<i>Helianthus annuus</i> (rhizosphere soil)	West Azerbaijan (Salmas)	Rezaei <i>et al.</i> 2021
<b><i>P. helicoides</i></b> (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. helicoides</i> , 2]			
	<i>Prunus dulcis</i> † (rhizosphere)	Kermanshah (?)	Azizi <i>et al.</i> 2013
	<i>Prunus persica</i> † (rhizosphere)	Kermanshah (?)	Azizi <i>et al.</i> 2012
<b><i>P. longitubum</i></b> Rezaei, Abrinbana & Ghosta [1]			
	<i>Solanum lycopersicum</i> (rhizosphere soil)	West Azerbaijan (Urmia)	Ershad 1977
	<i>Helianthus annuus</i> (rhizosphere soil)	West Azerbaijan (Salmas)	Rezaei <i>et al.</i> 2021
<b><i>P. littorale</i></b> (Nechw.) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. littorale</i> , 1]			
	<i>Citrullus lanatus</i> (?)	Kermanshah (?)	Hosseini Badrbani <i>et al.</i> 2018
	<i>Juncus</i> sp.† (?)	East Azerbaijan (Myaneh)	Bouket <i>et al.</i> 2016
	<i>Oryza sativa</i> * (rhizosphere soil)	Fars (Kamfiruz)	Salmaninezhad & Mostowfizadeh- Ghalamfarsa 2017b
	<i>Platanus orientalis</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad & Mostowfizadeh- Ghalamfarsa 2019a
	<i>Prunus amygdalus</i> * (rhizosphere, root and crown tissue)	Isfahan (Najaf Abad)	Sharifnabi <i>et al.</i> 2019

Table 1. Continued.

Species <sup>1</sup>	Matrix	Location <sup>2</sup>	Reference
<b><i>P. mercuriale</i></b> (Belbahri, B. Paul & Lefort) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. mercurial</i> , 1]			
	<i>Cucumis sativus</i> (?)	Kermanshah (?)	Hosseini Badrbani <i>et al.</i> 2018
	<i>Cucurbita maxima</i> (?)	Kermanshah (?)	Hosseini Badrbani <i>et al.</i> 2018
	<i>Solanum lycopersicum</i> (rhizosphere soil)	West Azerbaijan (Urmia)	Rezaei <i>et al.</i> 2021
<b><i>P. oedocheilum</i></b> (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. oedocheilum</i> , 1]			
	<i>Circium</i> sp. † (rhizosphere)	Ardabil (Meshginshahr)	Bouket <i>et al.</i> 2016
	<i>Cupressus sempervirens</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a
	<i>Pinus brutia</i> var. <i>eldarica</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a
	<i>Ulmus boissieri</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a
<b><i>P. ostracodes</i></b> (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. ostracodes</i> , 1]			
	<i>Begonia sepefloreans</i> † (rhizosphere)	Tehran (?)	Ershad 1977
	<i>Beta vulgaris</i> † (rhizosphere)	West Azerbaijan (Miandoab)	Babai-Ahari <i>et al.</i> 2004
	<i>Cupressus arizonica</i> (root tissue)	Fars (Shiraz County)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a
	<i>Oryza sativa</i> * (rhizosphere soil)	Fars (Firuzabad)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2017b
	<i>Pinus eldarica</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Platanus orientalis</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a
	Soil	Fars (Fasa, Shiraz)	Mostowfizadeh-Ghalamfarsa & Banihashemi 2005
	<i>Triticum aestivum</i> † (rhizosphere)	Fars (Fasa, Shiraz)	Mostowfizadeh-Ghalamfarsa & Banihashemi 2005
	Water *	Fars (Firuzabad)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2017b
<b><i>P. palingenes</i></b> (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. palingenes</i> , 1]			
	<i>Ailanthus altissima</i> (crown)	Fars (Shiraz)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a

Table 1. Continued.

Species <sup>1</sup>	Matrix	Location <sup>2</sup>	Reference
<b><i>P. palingenes</i></b> (Drechsler) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. palingenes</i> , 1]			
	<i>Cupressus sempervirens</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a
	<i>Eucalyptus obliqua</i> (root)	Fars (Shiraz)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a
<b><i>P. sterile</i></b> Belbahri & Lefort [ <i>Py. sterilum</i> , 1]			
	<i>Oryzae sativa</i> <sup>§</sup> (rhizosphere)	Fars (Kamfiruz, Ramjard)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2017b
<b><i>P. vexans</i></b> species complex (de Bary) Abad, De Cock, Bala, Robideau, Lodhi & Lévesque [ <i>Py. vexans</i> , 3]			
	<i>Acer saccharinum</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Citrus aurantifolia</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Cupressus sempervirens</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Eucalyptus obliqua</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Hedera helix</i>	Fars (Shiraz)	Bolboli & Mostowfizadeh-Ghalamfarsa 2014
	<i>Juglans regia</i>	Fars (Abbarik, Lapui, Roodbal, Zarqan)	Ghaderi & Banihashemi 2008
	<i>Cupressus sempervirens</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2019a
	<i>Mespilus</i> sp. (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Morus alba</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Populus</i> sp. (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Pinus elderica</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Pinus nigra</i>	Mazandaran (Amol)	Ershad 1977
	<i>Platanus orientalis</i> (rhizosphere)	Fars (Shiraz)	Salmaninezhad <i>et al.</i> 2021
	<i>Salix</i> sp. (rhizosphere)		Salmaninezhad <i>et al.</i> 2021
	Soil	Fars (Abadeh)	Mostowfizadeh-Ghalamfarsa & Banihashemi 2005
	Turfgrass (?)	Fars (Shiraz)	Barzegar Marvdasti & Banihashemi 2011

<sup>1</sup> *Phytophthium* species [*Pythium sensu lato* name, Clade *sensu* Lévesque & de Cock 2004]<sup>2</sup> Province (place)

\* Pathogenic ability is reported after Koch's postulates were confirmed

† Originally was reported as a *Pythium* species

§ It colonizes root and crown issues of the associated plant without causing any symptoms



## Phylogeny of the genus *Phytophthium* and evaluation of current species in Iran

### *Phytophthium* Clade 1

Clade 1 of *Phytophthium* is known to produce papillate sporangia with internal or external proliferation. This clade is the largest clade of *Phytophthium* and consists of 20 species, *i.e.*, *P. aichiense* Baten & Kageyama, *P. babaiaharii*, *P. boreale* (R.L. Duan) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *P. carbonicum* (B. Paul) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *P. citrinum* (B. Paul) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *P. delawarensis* (Broders, P.E. Lipps, M.L. Ellis & Dorrance) Abad, de Cock, Bala, Robideau, Lohdi & Lévesque, *P. dogmae*, *P. iriomotense* Baten & Kageyama, *P. kandeliae*, *P. leanoi*, *P. litorale*, *P. longitubum*, *P. mercuriale* (Belbahri, B. Paul & Lefort) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *P. megacarpum* Kirk, *P. montanum* (Nechw.) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *P. oedochilum* (Drechsler) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *P. ostracodes*, *P. sindhum*, and *P. sterile* (Baten *et al.* 2014, 2015; De Cock *et al.* 2015). Among all these species, *P. megacarpum* and *P. sterile* were claimed to be invalid (De Cock *et al.* 2015). *Phytophthium megacarpum* is invalid because no type was indicated; hence, it is believed to be *P. boreale* (De Cock *et al.* 2015). However, recent studies revealed that this species can still be considered a valid species since it was recently isolated from various parts of the world (Lodhi *et al.* 2020). *Phytophthium sterile* is also morphologically very similar to *P. litorale* and has the same ITS sequence; therefore, it is also considered an invalid species. However, just like in the case of *P. megacarpum*, it is currently considered a valid taxon because it has been reported several times from different parts of the world, and its mitochondrial loci analyses showed that it is indeed a valid taxon (Lodhi *et al.* 2020; Rezaei *et al.* 2021). Besides, *P. kandeliae* has been split into different lineages, and further studies are required to solve its problem (Marano *et al.* 2014; Lodhi *et al.* 2020). *Phytophthium kandeliae* was first described as *Halophytophthora kandaliae* (Ho *et al.* 1991). Since *Halophytophthora* is also another intermediate genus between *Pythium* and *Phytophthora*, most of the isolates assigned to *Halophytophthora* are now considered to be related to other genera, one of which is *Phytophthium*. *Phytophthium kandeliae* produces ovoid to obovoid, semipapillate sporangia with internal proliferation. However, zoospore differentiation takes place in two different ways: A. within a vesicle, like *Pythium* species, and B. part of plasma moves out in a vesicle through the exit pore, and zoospore development occurs inside the sporangium and in the extruded vesicle (Marano *et al.* 2014). These findings as well as multiple gene genealogy data from analyzing ITS region and *cox1*

loci led to the description of *P. kandeliae* (Marano *et al.* 2014).

Most of the *Phytophthium* species reported from Iran are also included in clade 1 *sensu* Baten *et al.* (2014). *Phytophthium babaiaharii*, *P. boreale*, *P. carbonicum*, *P. litorale*, *P. mercuriale*, *P. montanum*, *P. oedochilum*, *P. ostracodes*, *P. palingenes* and even *P. sterile* (Mostowfizadeh-Ghalamfarsa & Banihashemi 2005; Bolboli & Mostowfizadeh-Ghalamfarsa 2014; Bouket *et al.* 2016; Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2017b; Sharifnabi *et al.* 2019 Rezaei *et al.* 2021; Salmaninezhad *et al.* 2021) (Table 1).

### *Phytophthium* Clade 2

Clade 2 of *Phytophthium* is the second largest clade and consists of 12 species, including *P. chamaehyphon* (Sideris) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, *P. fagopyri* (S. Takim. ex S. Ito & Tokun.) Kageyama & Baten, *P. helicoides*, *P. indigoferae* (E.J. Butler) P.M. Kirk, *P. kandeliae* (H.H. Ho, H.S. Chang & S.Y. Hsieh) Thines, *P. mirpurensis* A.M. Lodhi, De Cock, Lévesque & Shahzad, *P. nanjingense* Jia J. Chen & X.B. Zheng, *P. palingenes*, *P. paucipapillatum* S.D. Langenhoven, W.J. Botha & L. Mostert, and *P. polytylum* (Drechsler) Abad, de Cock, Bala, Robideau, Lohdi & Lévesque (Baten *et al.* 2014, 2015; Marano *et al.* 2014; De Cock *et al.* 2015; Bennett *et al.* 2018; Chen *et al.* 2019). This clade cannot be morphologically distinguished from clade 1 based on sporangial shape. Among all species from Clade 2 of *Phytophthium*, only *P. helicoides* have been reported from Iran (Table 1). No other reports of the remained species within clade 2 of *Phytophthium* are available in Iran, which could be due to the preferences of ecological niches of these species since most species have been reported from mangrove and aquatic ecosystems of tropical regions (Bennett *et al.* 2018; Chen *et al.* 2019).

### *Phytophthium* Clade 3

Clade 3 is the smallest clade of *Phytophthium*, encompassing only two species, *i.e.*, *P. cucurbitacearum* P.M. Kirk and *P. vexans* complex (de Bary) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque. However, *P. cucurbitacearum* is now considered an invalid species due to the lack of formal description and unviable nature of the holotype (De Cock *et al.* 2015). *Phytophthium vexans* complex is known to produce various types of sporangia, such as globose, subglobose, ovoid, and ellipsoid without papillae (Van der Pläats-Niterink 1981; Baten *et al.* 2014). Several reports of *P. vexans* complex are available from Iran from different hosts (Table 1). This species could be considered the most important species assigned to *Phytophthium* because it has been reported several times from important crops and trees causing severe damage worldwide and also in Iran (Van der Pläats-Niterink 1981; Spies *et al.* 2011; De Cock *et al.* 2015; Mostowfizadeh-Ghalamfarsa 2016; Lodhi *et al.* 2020).



Recent studies suggested the existence of at least 3 phylogenetic groups within the *P. vexans* complex (Spies *et al.* 2011; De Cock *et al.* 2015). However, these studies concluded that *P. indigoferae* would also be in the same clade as *P. vexans* complex. Nevertheless, *P. indigoferae* is now grouped with clade 2 of *Phytopythium* (Lodhi *et al.* 2020). Furthermore, previous studies only investigated a limited number of *P. vexans* isolates from a restricted region, *i.e.*, South Africa, and on a limited number of hosts (Spies *et al.* 2011). No more studies have been conducted to resolve the problem of *P. vexans* complex from various parts of the world and on different hosts. Recently, several morphological groups of *P. vexans* complex have been identified from ornamental trees in Iran (Salmaninezhad *et al.* 2021). These groups produce different types of sporangia (from amorphous to ovoid and ellipsoid) with different dimensions of sexual structures (F. Salmaninezhad & R. Mostowfizadeh-Ghalamfarsa, Unpublished data). Besides, basic molecular studies have shown that these isolates are located in different lineages, which results in a conclusion that the Iranian isolates assigned to *P. vexans* complex require further investigation concerning their morphological features as well as their phylogenetic relationship with other isolates worldwide to create a verified phylogeny of this group as well as their pathogenicity on different hosts.

#### Challenges in the phylogeny of the genus *Phytopythium*

Generally, using the ITS region of the rDNA is the most common region to identify oomycetes (Robideau *et al.* 2011). Although multiple advantages, such as the availability of many sequences in public databases, ease of amplification, and interspecific variation level, will be acquired using this region, using this region alone cannot solely address the problem of species identification in most oomycetes, including *Phytopythium* species (Robideau *et al.* 2011; Spies *et al.* 2011; Hyde *et al.* 2014; Mostowfizadeh-Ghalamfarsa & Salmaninezhad 2020). For example, *P. litorale* and *P. sterile* have the same ITS sequences, none of them produce sexual structures, and their asexual structures are similar. Hence, it was concluded that *P. sterile* could be an invalid taxon. Yet, recent studies using multiple gene genealogy approaches revealed that *P. sterile* differs from *P. litorale*, especially regarding its mitochondrial genes (Lodhi *et al.* 2020). The ITS is especially ineffective when the researchers encounter *P. vexans* complex. The ITS region cannot separate different groups of *P. vexans* complex. In addition, it seems that the problem of *P. vexans* complex can neither resolve using the ITS region nor other regions, such as cytochrome c oxidase subunit I and II (*cox1* and *cox2*) (Spies *et al.* 2011). Using the multiple gene genealogy approach, researchers could only reveal at least 3 groups within *P. vexans* complex (Spies *et al.* 2011). However, no other attempts have been conducted ever since to address this problem. Hence,

further studies are required to resolve the problem of *P. vexans* complex and to reveal whether there are any new combinations or cryptic species within this complex. Furthermore, no universal DNA barcode for *Phytopythium* species has been introduced.

#### CONCLUSION

Since the first description of the genus *Pythium sensu lato*, *Phytopythium* species have been reported several times from different regions worldwide and in Iran. There are 34 *Phytopythium* species described worldwide, and 12 species have been reported from Iran (Table 1). The pathogenicity of some species has also been confirmed. Using the current name of *Phytopythium* instead of *Pythium* or *Ovatisporangium* is of great significance to avoid further confusion among researchers.

Major *Phytopythium* species isolates from Iran belong to clade 1 recovered from agricultural soils (Table 1). However, recently some species, including *P. babaiaharii*, *P. ostracodes*, and *P. palingenens* have been isolated from ornamental trees, especially conifers (Table 1). *Phytopythium litorale* and *P. ostracodes* are pathogenic on rice and their pathogenicity have been confirmed (Salmaninezhad & Mostowfizadeh-Ghalamfarsa 2017b). *Phytopythium babaiaharii* was originally reported from sugar beet rhizosphere; nevertheless, it was later isolated from infected root and crown tissues of ornamental trees, and its pathogenicity was also confirmed (Salmaninezhad *et al.* 2021; D. Delshad & R. Mostowfizadeh-Ghalamfarsa Unpublished data).

From clade 2 of *Phytopythium*, only *P. helicoides* have been recovered from *Prunus* sp. (Table 1). Since most species from clade 2 of *Phytopythium* have been isolated from mangrove and aquatic ecosystems of tropical regions, it would be an advantage to investigate the *Phytopythium* flora of Bushehr mangrove forests to evaluate whether we have previously species from clade 2 or new ones.

The precise identification of *Phytopythium* species should be conducted based on a consolidated approach using both morphological and molecular data sets. Identification of *Phytopythium* species traditionally has been conducted based on morphological features such as sporangia, oogonia, and antheridia, the type and size of oospores, homothallism vs. heterothallism, growth habit, and growth rate in culture media. Such features might vary under different cultural conditions and many species show similar morphological characteristics. Some of these characteristics can also change or be acquired or lost easily. The ITS region of the nuclear rDNA has been established to be variable at the family, genus, and species level for *Phytopythium* (De Cock *et al.* 2015). Even though the application of the ITS region seems to be the most popular choice of many researchers working with this genus (Villa *et al.* 2006; Hyde *et al.* 2014), it is recommended to use more than one gene to describe a new species (Villa *et al.* 2006; Robideau *et al.* 2011; Hyde *et al.* 2014).

While using even more than one locus would be problematic to identify a certain species (De Cock *et al.* 2015; Mostowfizadeh-Ghalamfarsa & Salmaninezhad 2020), whole genome sequencing of the species as well as using new molecular barcodes are recommended. It would be beneficial to include and test other loci, such as NADH1,  $\beta$ -tubulin, and other commonly used barcodes for oomycetes to resolve the problem of identifying *Phytophthium* species. One should point out the importance of the *cox2* locus in identifying *Pythium sensu lato* species, including *Phytophthium* spp., because *cox1* mostly does not work for their precise identification (Hyde *et al.* 2014).

Since only a limited number of *Phytophthium* species are reported from Iran, one can design an interactive identification key for accurate and prompt identification of species within the country. However, it should be regularly upgraded based on new reports and descriptions.

Almost all Iranian *Phytophthium* species are reported from either the Northwest of the country or Fars Province; it is of great importance to conduct a comprehensive study regarding the *Phytophthium* flora of different regions of Iran from different hosts to evaluate the main distribution of this genus throughout the country and identify the new species and the pathogenic ones.

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## وضعیت کنونی گونه‌های *Phytophythium* در ایران: چالش‌هایی در شناسایی آرایه‌ای حدواسط

فاطمه سلمانی‌نژاد و رضا مستوفی‌زاده قلمفرسا

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**چکیده:** جنس *Phytophythium* پراکنش جهانی دارد و در مناطق مختلف از بستره‌های متفاوتی جداسازی شده است. این جنس، آرایه‌ای تازه توصیف شده محسوب می‌شود و قبلاً از اعضای جنس *Pythium sensu lato* به شمار می‌رفته است. جنس *Phytophythium*، حدواسطی بین *Pythium* و *Phytophthora* با 34 گونه‌ی توصیف شده‌ی رسمی است. به تازگی برخی مطالعات در ایران بر روی فیلوژنی این جنس متمرکز شده‌اند. اگرچه بررسی‌های زیادی در ایران روی جداسازی گونه‌های منتسب به این جنس صورت نگرفته، برخی مطالعات جامع نشان داده‌اند که *Phytophythium*، جنسی مهم با پراکنش وسیع در این بخش از جهان است. شناسایی دقیق و رده‌بندی گونه‌های *Phytophythium* بسیار چالش‌برانگیز است. شناسایی ریخت‌شناختی *Phytophythium* به دلیل فقدان کلیدهای شناسایی، همپوشانی برخی خصوصیات ریخت‌شناختی، وجود گونه‌های مرکب، چندشکلی ساختاری و عدم وجود ساختارهای مشخص جنسی یا غیرجنسی در برخی گونه‌ها، دشوار است. از طرفی، اکثر گونه‌ها را نمی‌توان تنها با استفاده از یک یا دو ژن‌گاه برای واکاوی‌های فیلوژنتیکی تفکیک کرد. به علاوه در برخی از مطالعاتی که در ایران انجام شده، برای تأیید تشخیص ریخت‌شناختی، یا شناسایی مجدد گونه‌های گزارش شده، مطالعات مولکولی انجام نگرفته‌اند. فقدان فهرست دقیق و به روز گونه‌های موجود در کشور نیز به این مشکل افزوده است. تمرکز این نوشتار روی سیستماتیک کنونی گونه‌های *Phytophythium* در ایران است و چالش‌های موجود در شناسایی ریخت‌شناختی و مولکولی این گونه‌ها را در کشور به بحث می‌گذارد. همچنین راه‌بردهایی را برای حل مسئله‌ی شناسایی گونه‌های موجود در جنس *Phytophythium* پیشنهاد می‌کند.

**کلمات کلیدی:** ریخت‌شناسی، فیلوژنی، نشان‌گذاری مولکولی، *Pythiaceae*, *Oomycota*