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The current status of *Pythium* species in Iran: challenges in taxonomy

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Abstract: *Pythium* species are cosmopolitan, fungus-like oomycotes which may cause diseases in plants, animals and even human beings. Some of the species, however, are saprophytes and also well-proven biological control agents of plant pathogenic fungi. Since 1968, when the first *Pythium* species was reported from Iran, *ca* 44 species, two varieties, and five groups of this genus have been reported from different regions of Iran. Nevertheless, morphological or morphometric characters of only 35 species are more or less described. On the other hand more than 50% of these species have only been reported once. In the course of identification and classification of *Pythium* taxa in Iran, in addition to general obstacles, there exist some other problems. The lack of identification keys for the species; the absence of comprehensive checklists of the species, their dispersal and matrices; the lack of or the inadequacy of the species descriptions; unillustrated descriptions; generalization about the host names; unspecific geographical locations; unknown isolation matrices; the absence of type specimens in type culture collections; and the overlooking of molecular analyses, especially in the case of phylogenetic species are some of the challenges ahead. In this review, the prospects of future studies on the taxonomy of the *Pythium* species in Iran and the proposed solutions for the taxonomic challenges are discussed.

Key words: *Pythium* spp., *Straminopila*, *Oomycota*, classification, identification

INTRODUCTION

The genus *Pythium* Pringsheim 1858 includes various species of fungus-like *Oomycota* which are living as saprophytes or parasites in soil or fresh water niches. Some of the species, however, are among the well-proven biological control agents of plant pathogenic fungi. *Pythium* species are pathogenic to many plants and some aquatic and terrestrial animals and they also can parasitize some algae and fungi.

Species of *Pythium* are ecologically scattered all over the world and considered as cosmopolitan taxa. They can be found in tropical, temperate and even in cold regions. Tropical species can also be recovered from greenhouses in temperate regions (van der Plaats-Niterink 1981). Many of the plant pathogenic *Pythium* species are economically significant and cause devastating diseases on crops and ornamental plants. Therefore, in order to have a better understanding of biology, ecology and evolutionary relationships among species, identification and characterization of the species are of importance.

The earliest recorded report of a *Pythium* sp. inducing a plant disease in Iran returns to around 1947, when Esfandiari (1947) reported *Pythium debaryanum* R. Hesse as the causal agent of tobacco root rot based on his observations. Nevertheless, the first report based on laboratory tests has not been published until 21 years later about *Pythium ultimum* Trow on lentil (Kaiser et al. 1968) and a few years after, Fatemi (1971) published the first formal description of a *Pythium* sp. for *Pythium aphanidermatum* (Edson) Fitzp. from Iran. There has been ongoing attention to the species of this genus ever since. The objectives of this paper are to describe the current taxonomic status of *Pythium* species in Iran and to illustrate the obstacles existing in the way of identification and description of these species. The paper also reviews the prospects of future studies on taxonomy of the *Pythium* species in Iran and discusses the solutions for the taxonomic challenges ahead.

Pythium Genus, Species and Groups

There are almost 307 described *Pythium* species (www.mycobank.org) which are classified in the Kingdom *Straminopila* (Webster & Weber 2007) and the Phylum *Oomycota*. The genus concept of *Pythium* has been an ongoing controversy since the time of the description and there is still no consensus on the alternative genera (*e.g.* Fischer 1892; Schröter 1897; Sparrow 1931; Waterhouse 1967; van der Plaats-Niterink 1981; Dick 1990; Bala et al. 2010; Ko et al. 2010; Uzuhashi et al. 2010). Most of the authors tried to attend *Pythium* spp. into other genera based on the sporangial shapes, which is reviewed by Ho (2013). This state of flux forced me to adopt the classical definition of *Pythium* (Waterhouse 1974), *i.e.* all zoospore forming oomycetes which produce variable shapes of non-deciduous sporangia in water and in which zoospores form in a membranous vesicle

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connected to a discharge tube at the tip of the sporangium.

Pythium species are sexually homothallic (self-fertile) or need mating types in heterothallic species to produce oospores. There are some isolates which never produce some of the sexual organs. Theses isolates are categorized into some "Groups" (van der Plaats-Niterink 1981). Some isolates only form sporangia or hyphal swellings, but never produce oogonia or any other sexual organs in single or dual cultures. If these isolates only produce filamentous sporangia, they are grouped as Group "F". Isolates with swollen, toruloid sporangia are categorized as Group "T". If these isolates produce globose, non-proliferating sporangia, they are grouped as Group "G". Globose, proliferating sporangia producing isolates are Group "P", and isolates which only have hyphal swellings are categorized as Group "HS".

Taxonomic Criteria for Species Identification

The available identification keys of *Pythium* species are generally based on morphological, biological and morphometric characteristics (*e.g.* Matthews 1931; Middleton 1943; Waterhouse 1967; van der Plaats-Niterink 1981; Dick 1990). Therefore, the recognition of these criteria and the factors influencing them will be helpful for accurate identification of the species. The main characteristics for conventional identification of *Pythium* species are: possessing or lacking the sexual organs (the shapes, sizes and positions of the oogonia the oogonial ornamentations and their sizes; the shapes, sizes, numbers, origins and positions of the antheridia; the sizes, numbers and configurations of the oospores (*i.e.* if they are plerotic or aplerotic); and the thickness of the oospore wall), as well as asexual organs (the shapes and diameters of the hyphae; the presence and absence of hyphal swellings and their configurations; the presence and absence of chlamydospores, their sizes and colors; the presence and absence of appressoria, their shapes and configurations; the shapes and prolife- rations of zoosporangia), the colony patterns on various media and the temperature relationships. Due to the simple morphology of the species, the accurate identification of the species requires careful and precise examination of the isolates.

The Taxonomic Challenges of *Pythium*

Working with *Pythium* spp. isolates, one might encounter some obstacles during the course of species identification. Some of these complications were related to the biology of these microorganisms and some of which had something to do with the scientific tools and software availability. Most of the *Pythium* spp. isolates need sterols to produce sexual or asexual reproduction organs, such as zoosporangia and oospores in artificial media. It is possible to add some phytosterols in the form of sterol-rich plant materials, such as hemp seed extract or pure sterols, for instance β-Sitosterol, into media. However, for some isolates it takes a long time to produce any sexual or asexual organs and some of them never produce anything but a mycelial mat of coenocytic hyphae. Additionally, some sexual organs, such as antheridia may decline quickly soon after they appear. This makes the culture observation a time-consuming and laborious task. Some *Pythium* species produce more than one type of sporangia or antheridia. To avoid any confusion related to this multimorphism, the cultures must be absolutely pure. On the other hand, there is a high level of morphological overlapping among convergent species, especially phylogenetically related ones, which makes them an identification challenge. It is not that easy to find a compatible mating type for a heterothallic *Pythium* isolate to stimulate oospore production. Therefore, the identification must be solely based on asexual morphology, which is prone to error.

The biology of *Pythium* species is not the only issue in the course of identification and there are several software-based concerns which have something to do with the accessible tools for the identification. The most comprehensive *Pythium* species identification key available (van der Plaats-Niternk 1981) only covers 120 out of 307 reported species. Additionally, the latest identification key (Dick 1990) is almost 25 years old. On the other hand, there are also no descriptive sheets, no web-based database and no molecular barcode metadatabase for *Pythium* species. If a molecular identification is the approach of choice, species-specific primers are designed for only around 20 species (http://sppadbase.ipp.cnr.it/), most of which are developed for plant pathogens. There were some attempts for generating web-based interactive keys based on Lucid Builder platform (Moorman et al. 2014). Nevertheless, it is a modification of van der Plaats-Niternk (1981) identification key and not all the species in the original key are included.

The Current Status of *Pythium* spp. Identification in Iran

From the time when the first *Pythium* species was formally reported from Iran (Kaiser et al. 1968), 44 species, two varieties, and five groups of this genus have been reported (Table 1) from 76 plant taxa, including 55 plant species, as well as agricultural soil (data not shown). At the moment, there are 371 records of *Pythium* spp. incidence from various provinces of Iran, which are not evenly distributed in each province (Fig. 1). These reports, however, vary enormously in content, quality, detail, and format. Apart from Fars, Razavi Khorasan, Hamadan, Khuzestan and a few other provinces, there are few or no (*e.g.* Bushehr, South Khorasan, and Qom) reports in the most provinces.

Table 1. The list of *Pythium* spp. reported from Iran.

Taxon	Matrices†	Location†	Reference
P. acanthicum Drechsler 1930	*Prunus cerasus* L. [*Rosaceae*]; *Prunus persica* (L.) Batsch [*Rosaceae*]	Kermanshah (?)	Azizi et al. 2012
P. adhaerens Sparrow 1931	Soil [rice nursery]	Fars (Arsenjan)	Bolboli & Mostowfizadeh-Ghalamfarsa 2015
P. amasculinum Y.N. Yu 1973	*Citrullus lanatus* (Thunb.) Mansf. [*Cucurbitaceae*]; *Cucumis melo* L. [*Cucurbitaceae*]; *Cucumis melo* L. [*Cucurbitaceae*]; *Cucumis sativus* L. [*Cucurbitaceae*]; *Lycopersicum esculentum* Mill. [*Solanaceae*]; *Solanum melongena* L. [*Solanaceae*]; Soil	Razavi Khorasan (?)	Askari Farsangi et al. 2011
P. aphanidermatum (Edson) Fitzp. 1923	*Citrullus lanatus* (Thunb.) Mansf. [*Cucurbitaceae*]; *Cucumis melo* L. [*Cucurbitaceae*]; *Cucumis sativus* L. [*Cucurbitaceae*]; Soil	Alborz (Karaj); Fars (Marvdasht, Moharloo); Gilan (Manjil); Golestan (Gorgan)	Banihashemi 1969
P. aquatile Höhnk 1953	Soil	Fars (Sepidan)	Mostowfizadeh-Ghalamfarsa & Banihashemi 2005
P. aristosporum Vanterp. 1938	*Triticum aestivum* L. [*Poaceae*]	Fars (?)	Ravanlou & Banihashemi 2002
P. carolinianum V.D. Matthews 1931	Soil	Fars (Mamasani)	Bolboli & Mostowfizadeh-Ghalamfarsa 2015
P. catenulatum V.D. Matthews 1931	Turfgrass [*Poaceae*]	Tehran (Tehran)	Khodashenas Roudsari et al. 2010
P. coloratum Vaartaja 1965	*Helichrysum bracteatum* Andrews [*Asteraceae*]	Khuzestan (Ramin)	Ershad 1977
P. debaryanum R. Hesse 1874	*Lens esculenta* Moench [*Fabaceae*]	Khuzestan (Dezful)	Ershad 1977
P. deliense Meurs 1934	*Beta vulgaris* L. [*Chenopodiaceae*]	Fars (Marvdasht)	Afzali & Banihashemi 2000
P. diclinum Tokun. 1935	Soil	Fars (Bajgah, Borazjan)	Mostowfizadeh-Ghalamfarsa & Banihashemi 2005
P. dissotocum Drechsler 1930	Soil	Fars (Bajgah, Kazeroon, Zarqan)	Bolboli & Mostowfizadeh-Ghalamfarsa 2015
P. echinulatum V.D. Matthews 1931	Soil	Fars (Darab)	Mostowfizadeh-Ghalamfarsa & Banihashemi 2005
P. grandisporangium Fell & Master 1975	Soil	West Azerbaijan (?)	Badali & Abrinbana 2013
P. helicoides Drechsler 1931	*Prunus persica* (L.) Batsch [*Rosaceae*]	Kermanshah (?)	Azizi et al. 2012
P. heterothallicum W.A. Campb. & F.F. Hendrix 1968	*Triticum aestivum* L. [*Poaceae*]	Fars (?)	Ravanlou & Banihashemi 2002
P. hydnosporum (Mont.) J. Schröt. 1879	*Plumbago europaea* L. [*Plumbaginaceae*]	Hamadan (Hamadan)	Abad et al. 2013
P. inflatum V.D. Matthews 1931	Soil	Fars (Bajgah)	Mostowfizadeh-Ghalamfarsa & Banihashemi 2005

Table 1. Continued

Taxon	Matrices†	Location†	Reference
P. intermedium de Bary 1881	*Begonia semperflorens* Link & Otto [*Begoniaceae*]	Tehran (Tehran)	Ershad 1977
P. irregulare Buisman 1927	*Beta vulgaris* L. [*Chenopodiaceae*]	Khorasan (?)	Afzali & Ershad 2006b
P. kashmirense B. Paul 2008	Soil	Fars (Lar)	Bolboli & Mostowfizadeh-Ghalamfarsa 2015
P. macrosporum Vaartaja & Plaäts-Nit. 1981	*Rosa hybrida* Vill. [*Rosaceae*]	Hamadan (Hamadan)	Abad et al. 2013
P. marsipium Drechsler 1941	Soil	Fars (Arsanjan, Kamfirooz)	Bolboli & Mostowfizadeh-Ghalamfarsa 2015
P. middletonii Sparrow 1960	*Cucumis sativus* L. [*Cucurbitaceae*]	Kerman (Jiroft)	Hatami et al. 2010
P. minus Ali-Shtayeh 1985	Soil	West Azerbaijan (?)	Badali et al. 2014
P. myriotylum Drechsler 1930	Turfgrass [*Poaceae*]	?	Mirabolfathi & Ershad 2002
P. nunn Lifsh., Stangh. & R.E.D. Baker 1984	Soil	Fars (Sormaq)	Bolboli & Mostowfizadeh-Ghalamfarsa 2015
P. okanoganense P.E. Lipps 1981	*Beta vulgaris* L. [*Chenopodiaceae*]	Khuzestan (?)	Zamani Noor et al. 2004
P. oligandrum Drechsler 1930	*Amaryllis* sp. [*Amaryllidaceae*]; *Lantana* sp. [*Verbenaceae*]; *Pinus* sp. [*Pinaceae*]; Turfgrass [*Poaceae*]	Tehran (Tehran); Mazandaran (Amol)	Ershad 1977
P. orthogonon Ahrens 1971	Soil	Fars (Bajgah)	Mostowfizadeh-Ghalamfarsa & Banihashemi 2005
P. ostracodes Drechsler 1943	Soil	West Azerbaijan (Miandoab)	Babai-Ahari et al. 2004
P. paroecandrum Drechsler 1930	*Papaver somniferum* L. [*Papaveraceae*]	Kurdistan (Sanandaj)	Ershad 1977
P. periplocum Drechsler 1930	*Triticum aestivum* L. [*Poaceae*]	Fars (?)	Ravanlou & Banihashemi 2002
P. perplexum H. Kouyeas & Theoh. 1977	*Rosa hybrida* Vill. [*Rosaceae*]; *Petunia* sp. [*Solanaceae*]	Hamadan (Hamadan)	Abad et al. 2013
P. pyrilobum Vaartaja 1965	*Cucumis sativus* L. [*Cucurbitaceae*]	Kerman (Jiroft)	Hatami et al. 2010
P. rostratum E.J. Butler 1907	Soil	Fars (Bajgah)	Mostowfizadeh-Ghalamfarsa & Banihashemi 2005
P. salinum Höhnk 1953	*Beta vulgaris* L. [*Chenopodiaceae*]	Khuzestan (?)	Zamani Noor et al. 2004
P. splendens Hans Braun 1925	*Papaver somniferum* L. [*Papaveraceae*]	Fars (Marvdasht)	Banihashemi 1975
P. torulosum Coker & P. Patt. 1927	Turfgrass [*Poaceae*]	Fars (Shiraz)	Barzegar Marvasti & Banihashemi 2011
P. tracheiphilum Matta 1965	*Beta vulgaris* L. [*Chenopodiaceae*]	Khuzestan (?)	Zamani Noor et al. 2004
P. ultimum Trow 1901	*Lens esculenta* Moench [*Fabaceae*]	Tehran (?)	Kaiser et al. 1968
P. ultimum var. *sporangiiferum* Drechsler 1960	*Actinidia chinensis* Plunch. [*Actinidiaceae*]	Gilan (?); Mazanderan (?)	Taheri et al. 2008

Table 1. Continued

Taxon	Matrices†	Location†	Reference
P. ultimum var. *ultimum* Trow 1901	*Beta vulgaris* L. [*Chenopodiaceae*]	Hamadan (?)	Kashi et al. 2000
P. vanterpooli V. Kouyeas & H. Kouyeas 1963	Turfgrass [*Poaceae*]	Fars (Shiraz)	Barzegar Marvasti & Banihashemi 2011
P. vexans de Bary 1876	*Pinus nigra* Link [*Pinaceae*]	Mazandaran (Amol)	Ershad 1977
Pythium Group "F"	*Triticum aestivum* L. [*Poaceae*]	Fars (?)	Ravanlou & Banihashemi 2002
Pythium Group "G"	*Beta vulgaris* L. (*Chenopodiaceae*]	Khuzestan (?)	Zamani Noor et al. 2004
Pythium Group "HS"	*Beta vulgaris* L. (*Chenopodiaceae*]	Ardabil (Ardabil); West Azerbaijan (Miandoab)	Babai-Ahari et al. 2004
Pythium Group "P"	*Beta vulgaris* L. [*Chenopodiaceae*]	Khorasan (?)	Afzali & Ershad 2006a
Pythium Group "T"	*Beta vulgaris* L. [*Chenopodiaceae*]	Ardabil (Ardabil); West Azerbaijan (Khoy)	Babai-Ahari et al. 2004

? = unknown.
† Matrix(ces) and location from which the taxon was reported for the first time in Iran.

The Taxonomic Challenges of *Pythium* spp. In Iran

In the course of identification and classification of *Pythium* taxa in Iran, in addition to general obstacles, there are some other problems in the way. There are almost no identification keys for the species from Iran. The only available key (Mostowfizadeh-Ghalamfarsa & Banihashemi 2005) just covers the isolates from Fars Province in southern Iran. Furthermore, the shortage of comprehensive checklists of the species, their dispersal and isolation matrices make the confirmation of species identification difficult for the researchers.

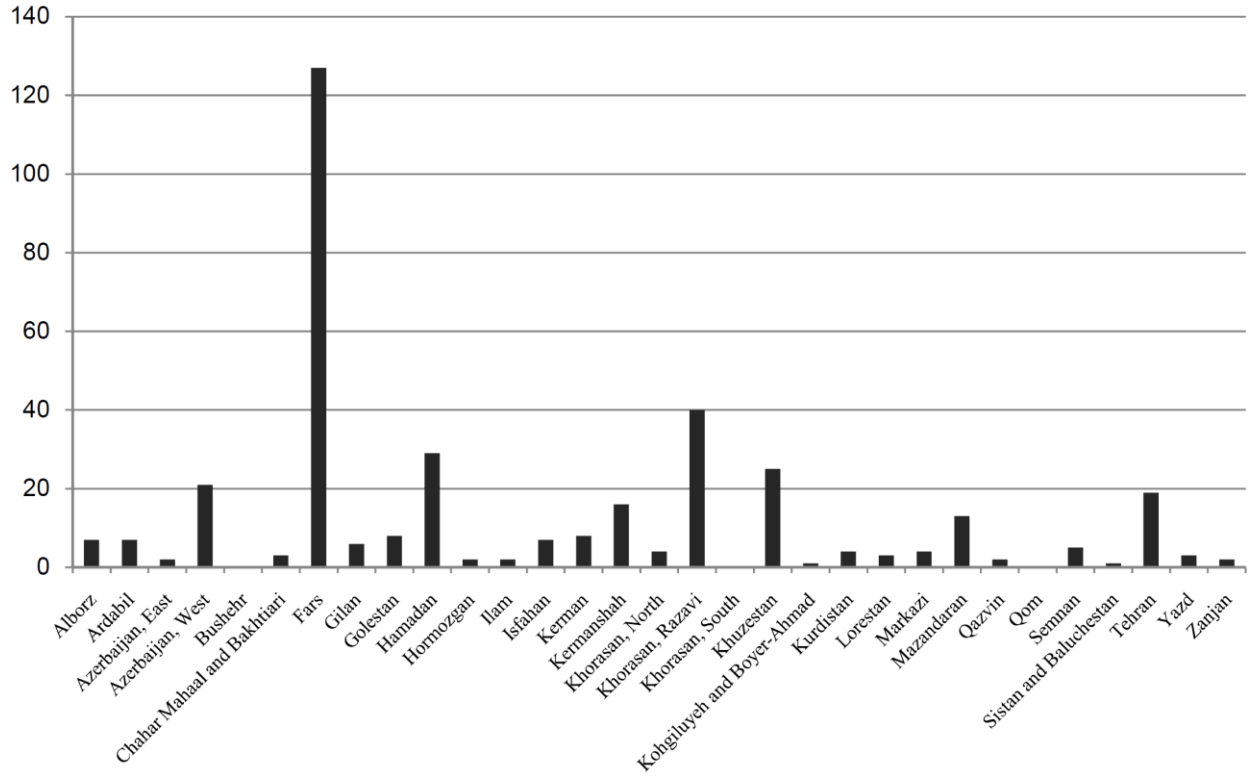

Fig. 1. The number of *Pythium* spp. reports from each province of Iran.

In most of the *Pythium* spp. reports from Iran, there are no or few morphometric data, illustrations or high resolution images. Lack of species descriptions sometimes makes it difficult or even impossible to reevaluate the identification. As a matter of fact, morphological or morphometric characters of only 35 species are more or less described. On the other hand, more than 50% of the recorded species have only been reported once. This could bring the possibility of a flowed identification procedure for these isolates.

Another problem is about the metadata recording during recovering the *Pythium* spp. isolates. Data such as matrices, host names, locations, and the time of isolation could be confirmation sources for systematic identification of the species. Generalization about the host names was frequently happened in these reports. Names such as paper, turf grass, cucurbit, kitchen garden and summer crops are some of the instances which are vague and do not refer to a specific plant species. Additionally, unspecific geographical locations can be observed in some of the reports, for example referring to a province instead of the exact location of the isolation or geographical coordinates. The variable names of some provinces is also another source of confusion in the *Pythium* spp. reports. Some Iranian provinces have been recently named and were previously a part of another province (*e.g.* Alborz, Ardabil, Golestan, North Khorasan, Qazvin, Qom, Razavi Khorasan, and South Khorasan). Therefore, the old name of a province could easily be mistaken for the location it currently refers to. Furthermore, unknown isolation matrices is another problem in some of the records emerging due to the inadequacy of the metadata recording. While the name of a specific plant is mentioned, it is not clear that the isolate(s) was (were) recovered from roots or other plant tissues and materials, or even soil around the plants.

Our observations (Bolboli & Mostowfizadeh-Ghalamfarsa 2015) showed that Iranian agricultural soils are rich in *Pythium* spp. flora. Although there are some findings on the new species in Iran (*e.g.* Bolboli 2014; Chenari Bouket 2015; Salmaninejad & Mostowfizadeh-Ghalamfarsa, Unpub. data), no new *Pythium* species description has been published for Iranian mycoflora so far. On the other hand, the absence of type specimens for most *Pythium* spp. in Iranian type culture collections make the identification and description of new species difficult and the reevaluation of previous reports are even more complicated.

Finally, in the majority of Iranian reports, even in the recent ones, the molecular phylogenetic analyses is neglected and only few papers can be found in which morphological identification was backed up with molecular data. These kinds of molecular analyses are important, especially in the case of phylogenetic species or morphologically convergent ones.

An Outlook

Apart from dry sandy deserts of Iran, *Pythium* species are isolated from a range of eco- and agroecosystems including agricultural fields, nurseries, grasslands, parks, forests, ponds, and surface water in almost all climates. This indicates the rich *Pythium* spp. mycoflora in Iran. However, the above mentioned challenges need to be addressed to identify and describe all existing taxa. A comprehensive *Pythium* spp. monograph for Iran with cross references and checklists seems to be of the priority. This monograph should include a key consisted of all of the species recorded and also their morphological descriptions and morphometric data. A step-by-step protocol would also help the newcomers to plan their identification tasks properly. Additionally, in order to have a better identification process, more experts must be trained through academic programs or retraining workshops. In these training projects, the main focus should be on both morphological and molecular identification.

Any reports of *Pythium* species, practically the same as other fungi and fungus-like microorganisms, must be well reviewed before any publications. This is especially more important in conferences where the time for any evaluations is limited. As a final step, setting up an internet-based database consisting morphological, morphometric and molecular data of *Pythium* spp. isolates from Iran could be a pragmatic approach to overcome some of the taxonomic challenges ahead.

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وضعیت فعلی گونه‌های پیتیوم در ایران: چالش‌های آرایه‌بندی

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چکیده: گونه‌های پیتیوم شبه‌قارچ‌های اُمیستی همه‌جازی هستند و ممکن است در گیاهان، جانوران و حتی انسان بیماری ایجاد کنند. با این حال برخی از گونه‌های این جنس پوده‌رُستاند و تعدادی از آن‌ها نیز از اثبات شده‌ترین مهارگرهای زیستی قارچ-های بیماری‌زای گیاهی به شمار می‌روند. از سال ۱۳۴۷ که نخستین گونه‌ی پیتیوم در ایران گزارش شد تاکنون حدود ۴۴ گونه، دو جوره و پنج گروه از این جنس از مناطق مختلف ایران گزارش شده است. اما در بین آن‌ها فقط ویژگی‌های ریخت‌شناختی یا ریخت-سنجی ۳۵ گونه کم‌وبیش توصیف شده است. همچنین بیش از ۵۰ درصد از این گونه‌ها فقط یک‌بار از مناطق مختلف ایران گزارش شده‌اند. هنگام تشخیص و رده‌بندی آرایه‌های پیتیوم در ایران علاوه بر وجود مشکلات عمومی که گریبان‌گیر گونه‌های این جنس است، مسائل دیگری هم سد راه قرار می‌گیرد. عدم وجود کلیدهای تشخصی در مورد گونه‌ها، نبود فهرست‌های تفصیلی از گونه‌ها، پراکنش و بستره‌ی گونه‌ها، نبود یا نقص در توصیف جامعی از هریک از گونه‌ها، مصور نبودن توصیف‌ها، همچنین کلی‌گویی در مورد نام میزبان، مشخص نبودن مکان دقیق جغرافیایی آرایه و نامشخص بودن بستره‌ی جداسازی در گزارش‌ها؛ نبود نمونه‌های تیپ در مجموعه‌ی کشت‌های تیپ و انجام نشدن واکاوی‌های مولکولی به خصوص در مورد گونه‌های فیلوژنتیکی از چالش‌های موجود است. در این نقد، چشم‌انداز پژوهش‌های آتی در مورد آرایه‌بندی گونه‌های پیتیوم در ایران و راه‌کارهای پیشنهادی بحث شده است.

واژه‌های کلیدی: *Pythium* spp.، *Straminopila*، *Oomycota*، رده‌بندی، شناسایی

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