

FIRST RECORD OF THREE SPECIES OF *PYTHIUM* FROM MOROCCAN WATERS

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ABSTRACT. *First record of three species of Pythium from Moroccan waters.* Species of *Pythium*, including *P.torulorum*, *P.catenulatum* and *P. "Group F"*, isolated from dam near Rabat city (capital of Morocco) in northern Africa, are described. Taxonomic and morphological details of the fungi are discussed. This work is the first report of these species from Moroccan waters.

Keywords. Antheridia, biodiversity, Morocco, oogonia, *Pythium*, sporangia.

RESUMEN. *Primeras citas de tres especies de Pythium para Marruecos.* Se describen especies de *Pythium*, incluyendo *P. torulosum*, *P. catenulatum* y *P. "Group F"*, aislados cerca de la ciudad de Rabat (Marruecos) en el norte de Africa. Se discuten detalles taxonómicos y morfológicos del hongo. Este trabajo es la primera referencia de estas especies en aguas de Marruecos.

Palabras clave. Anteridios, biodiversidad, Marruecos, oogonios, *Pythium*, esporangios.

INTRODUCTION

Morocco, a country characterized by semi-arid conditions, the resort to surface water supply demands is always a challenge, given the scarceness of water and the limit of economical and social development. Currently 62% of potable water production is assured from dam reservoirs (ONEP, 1998). These latter are often affected by the eutrophication phenomenon which lead to

the excessive growth of microorganisms. Aquatic pythiaceous fungi, particularly species within the genus *Pythium*, together with other aquatic microorganisms constitute the fungal flora of fresh water (Plaats-Niterink, 1981).

Pythium species are widely distributed in the world. They are the major pathogens associated with damping-off of seeds and seedlings (Garret, 1970; Hendrix and Campbell, 1973; Raynal, 1980; Walker *et al.*,

1998; Kageyama and Nelson, 2003). Within this context a study to isolate and identify some aquatic pythiaceus fungi from Moroccan waters has been undertaken at the University in Rabat.

A number of water samples taken from dam were examined. This dam situated in the Bassin of Bourgreg has a surface of 28 km² and a total capacity of 493 million m³. It makes it possible to supply drinking and industrial water to the centers and cities located in the bassin of Bourgreg and the cities between Kénitra and Casablanca (ONEP, 1996).

In this report three species of *Pythium* are reported. These species have been described in others countries (Plaats-Niterink, 1981; Paul, 1982, 1994 a) but not from Morocco's waters. This paper is the first record of its occurrence from Moroccan waters.

MATERIALS AND METHODS

Water samples were collected from different places of the dam reservoir. These were baited with boiled hemp-seed halves (Paul, 1986 a-c). The colonised baits were washed thoroughly with sterilised distilled water. With a fine pointed forceps one hypha was removed, placed on the surface of potato carrot agar (PCA) and incubated in the dark at room temperature. A small block of agar with hyphal tips was cut from the edge of the colony and transferred to a fresh plate of PCA.

To study asexual and sexual reproduction, small piece of agar block having bacteria-free mycelium was placed in a petri dish in shallow layer of sterile distilled water, to which several boiled hemp-seed halves and 1-2 cm of boiled grass leaf were added (Plaats-Niterink, 1981). In 3-4 days the baits were colonized by the

fungus. Changing the water additionally favours the production of sporangia and discharge of zoospores (Plaats-Niterink, 1981). Temperature/growth relations were observed on potato carrot agar. Identification of the fungi was done with the help of keys and descriptions of Middleton (1943), Waterhouse (1967), Plaats-Niterink (1981) and Dick (1990).

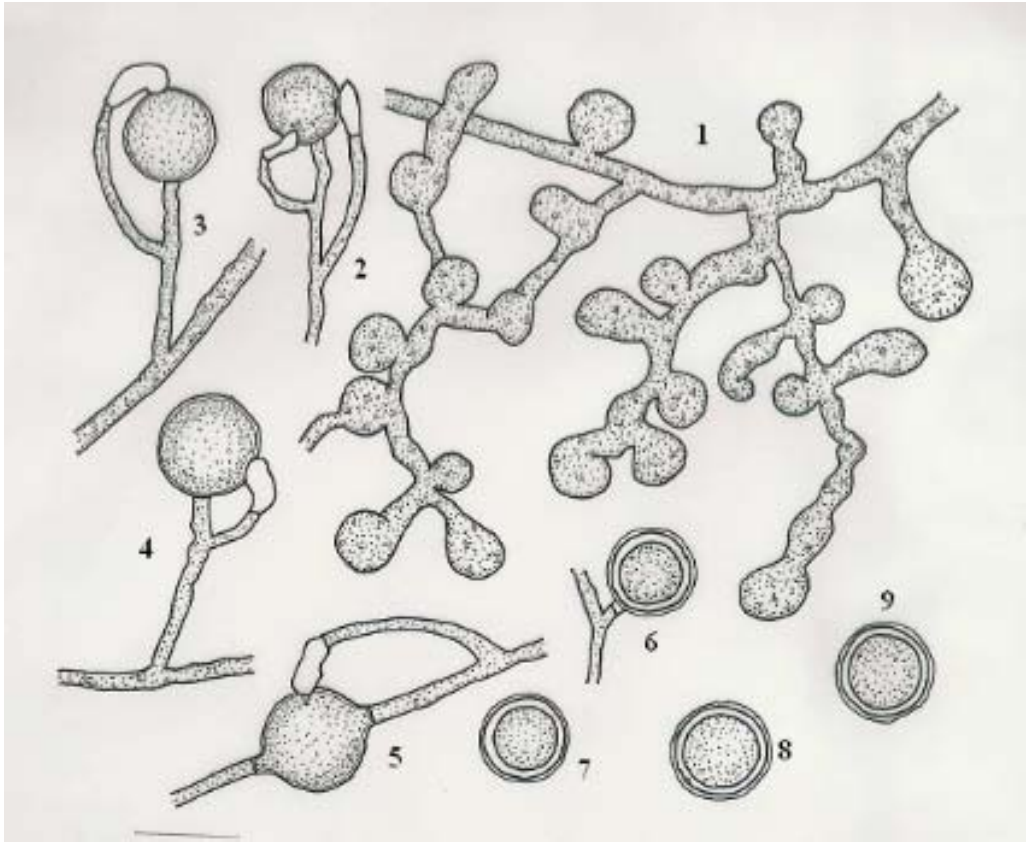
RESULTS

Pythium torulosum Coker & Patterson (Figures 1-9)

Mycelium hyaline, well branched. Main hyphae up to 5µm wide. Colonies on PCA are submerged showing a rosette pattern. On this medium the average daily growth of the fungus at 25°C is 14.5 mm. Sporangia consisting of filamentous inflated, branched outgrowths of the mycelium, forming toruloid elements of various sizes. Vesicles and zoospores are formed plentifully in water cultures at room temperature (18-24°C). Encysted zoospores measure between 6.5-9µm in diameter (av. 7.5µm). Oogonia smooth walled, terminal but at times intercalary, 12-20µm (av. 17µm) in diameter. Antheridia 1-2 per oogonium, monoclinous, antheridial cells clavate making apical contact with the oogonium. Oospores plerotic, single, 10-19µm in diameter (av. 15µm). Wall 1-2 µm in thickness.

Pythium catenulatum Matthews (Figures 10-21)

Mycelium hyaline, well branched. Main hyphae up to 5 µm wide. Colonies on PCA submerged, giving a narrow chrysanthemum pattern. Daily growth rate on this medium at 25°C is 12mm. Sporangia composed of globose, ellipsoidal to irregular swollen elements, connected with branched parts of the mycelium. Vesicles and zoospores formed at



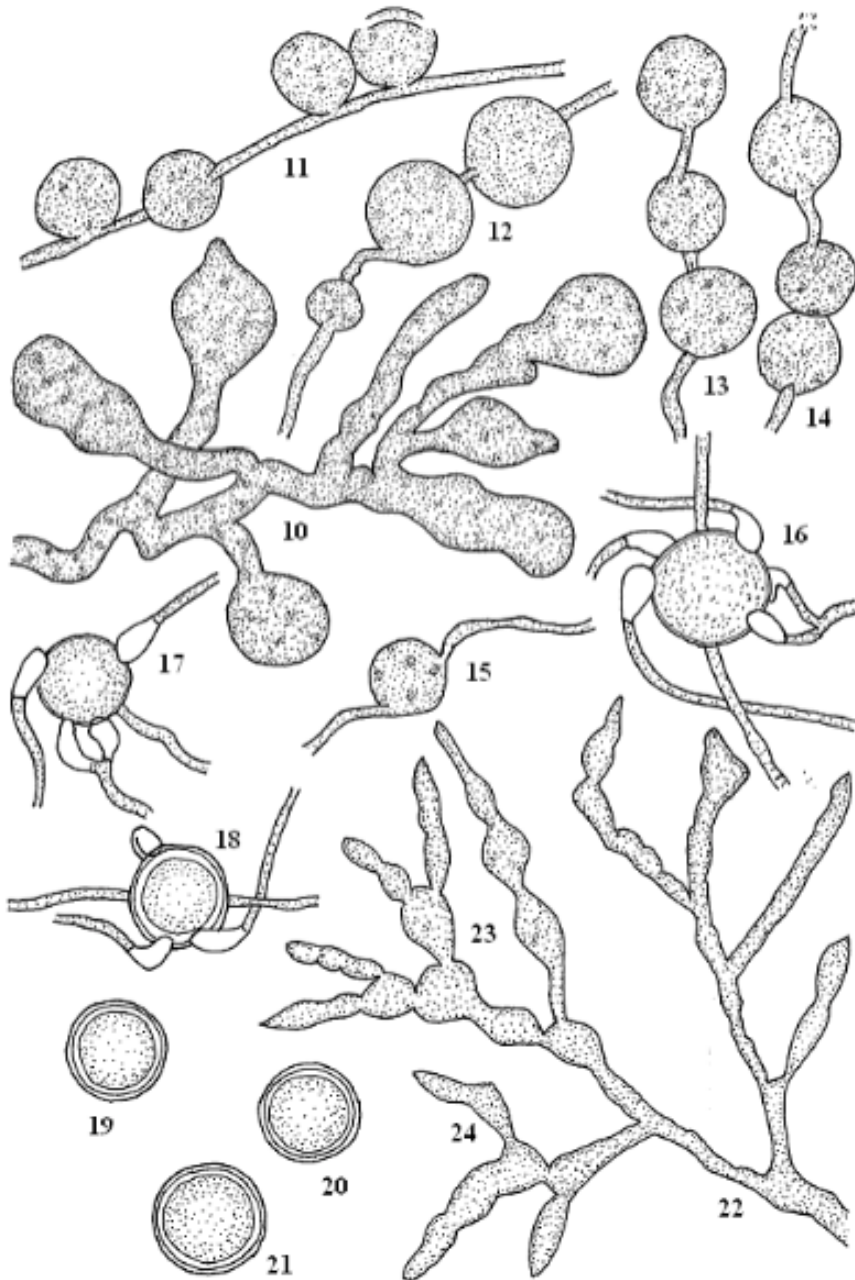
Figures 1-9: *Pythium torulosum*. 1: Filamentous inflated sporangia. 2: Terminal oogonia and monoclinuous antheridia (two per oogonium). 3 and 4: Terminal oogonia and monoclinuous antheridia (one per oogonium). 5: Intercalary oogonia and monoclinuous antheridia. 6-9: Plerotic oospores. (Bar = 20 μ m).

room temperature. Encysted zoospores measures about 8-9 μ m in diameter. Hyphal swellings abundant, of various shapes and sizes, mostly spherical but at times ellipsoidal or oval shaped. The spherical ones measure 10 to 23 μ m in diameter (av. 17 μ m). They can either be terminal or intercalary, mostly in chains of 3-8. Oogonia terminal or intercalary, smooth-walled, 13-29 (av. 22 μ m). Antheridia diclinuous, occasionally monoclinuous, 2-8 per oogonium. Oospores plerotic, single, 12-27 μ m in diameter (av. 20 μ m) with a wall of about 1.5 μ m thick.

***Pythium* "Group F"**

(Figures 22-24)

Mycelium hyaline, well branched. Main hyphae up to 4.5 μ m. Daily growth rate on PCA at 25°C is 8.5mm. On this medium the colonies form little loose aerial mycelium with a rizoidal pattern, and a heap of mycelium like points in some places. Microscopically an aggregate of chlamydospores were observed. These latter are in various shapes, spherical, ellipsoidal or totally irregular. Sporangia were of filamentous non-inflated type, which



Figures 10-21: *Pythium catenulatum*. 10: Irregularly swollen sporangia. 11-14: Catenulate hyphal swellings. 15: Spherical hyphal swelling. 16-18: Oogonia with diclinuous antheridia. 19 and 21: Plerotic oospores. (Bar = 20 μ m). 22-24: *Pythium "Group F"*. 22: Hyphae. 23-24: Chlamydospores. (Bar = 25 μ m).

produce vesicles at the top of long evacuation tubes. Zoospores were formed at room temperature (18-24°C). Sexual reproduction absent.

DISCUSSION

Pythium species are spread all over the world. They have been reported in ponds, lakes, rivers (Paul, 1982, 1986, 1994a), canals (Abdelzaher *et al.*, 1997), dam reservoirs (Paul, 1986b, 1986c) and soils (Paul, 1994a, 1994b, 2002). Many *Pythium* species are well known to affect a wide range of plants, mainly juvenile or succulent tissues and causing considerable economical losses to a wide of agricultural crops (Hendrix and Campbell, 1973; Cline *et al.*, 1988).

Pythium torulosum is a common pythiaceous fungi. It has been isolated from barley, grasses, maize, conifers, soils and waters (Plaats-Niterink, 1981). In Africa this species has been described from Algeria (Paul, 1982) and from the republic of Benin (Paul, 1994a). However, our study is the first report in Morocco. Most of the morphological features of this isolate are in common to those described elsewhere.

Pythium catenulatum was originally isolated in the USA from plant debris in water, and later also from soil and turf grasses (Plaats-Niterink, 1981). The species is quite rare, and in the north of Africa, it has been recorded only from Algeria (Paul, 1982). This is the first report of its presence in Morocco. A part from its slow growth, 12 mm/day instead of 14 mm/day recorded by Plaats-Niterink (1981), most of the morphological characters fit closely the description of the species found in the literature.

Pythium "Group F", a typical aquatic fungus, has been isolated from different

Algerian waters (Paul, 1982) and also from canal water in Egypt (Abdelzaher *et al.*, 1997). This species belongs to isolates which did not produce any oogonia in single or dual cultures. The morphological description of the Moroccan isolate treated in this paper resembles in almost all the characters of *Pythium* "Group F" reported in Algeria (Paul, 1982). This is also the first taxonomic treatment of the species in Morocco.

Only a very limited area was studied during this work and it is expected that the inclusion of other regions in Morocco in future investigations will increase the list of the striminipilan *Pythium* species.

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