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## *Pythium folliculosum*, a New Species from the Bank of Lake Zürich.

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**Summary:** *Pythium folliculosum* sp. nov. has been isolated and identified from soil samples taken at the bank of lake Zürich. This fungus is characterized by its filamentous, inflated type of sporangia, together with both spherical, elongated sac-like oogonia, and strictly monoclinal antheridia.

**Résumé:** *Pythium folliculosum* sp. nov. a été isolé à partir d'échantillons de sol prélevés au bord du lac de Zürich. Ce champignon est caractérisé par ses sporanges filamenteux enflés: ses oogones sphériques et allongées (en forme de sac) et par ses anthéridies exclusivement monoclinales.

### Introduction

The genus *Pythium* comprises about 100 species which are mostly cosmopolitan in distribution. Most of these live in aquatic or other humid habitats. Many species of this genus occur as saprophytes in the soil, while others are parasites that cause a variety of diseases to plants. The most common disease caused by *Pythium* is the "damping-off" of young seedlings in the nurseries, green houses or in the field.

During the course of investigation of these fungi in Switzerland, a number of soil samples, specially forest soils, were collected from different parts of the country. Some humid soil samples were also collected from the banks of "lac Léman" and the lake of Zürich.

A new species of *Pythium* was discovered in one sample taken from the bank of lake Zürich at Zollikon. This fungus belongs to the group of *Pythium* with filamentous inflated sporangia and can easily be distinguished from related species by the presence of sac like oogonia.

### Materials and Methods

In July 1989 soil samples were collected at the bank of lake Zürich in sterile capped, disposable plastic bottles. This was done with a spoon which was disinfected with alcohol after each sampling. The samples were brought to the

was transferred into a sterile capped bottle containing 20 ml of sterile distilled water. This was shaken vigorously to obtain a homogeneous soil suspension. Ten ml of this suspension were then placed in a sterile Petri dish containing 10 ml of sterile distilled water. This was, in turn, baited with boiled hemp-seed halves. After 3-4 days the bait was colonized by fungal mycelium. To suppress the growth of other quick-growing soil fungi 0.5 grams of benomyl was dissolved in 1 litre of distilled water and autoclaved. This Benomyl water was then used to culture the fungus. Repeated subculturing on hemp seeds in sterile distilled water and benomyl water allowed to produce a bacteria-free colony of *Pythium*. Potato carrot agar (PCA) and corn meal agar (CMA) were used for the study of the isolates (Plaats-Niterink, 1981). Small pieces of fungal mycelium were inoculated on PCA and CMA plates, which were in turn incubated at different temperatures.

The isolates were identified with the help of keys provided by Middleton (1943), Waterhouse (1967) and Plaats-Niterink (1981). A comparative study of this isolate with the descriptions of all the recently discovered species of *Pythium* was also done (Ali-Shtayeh & Dick 1985, Paul 1986 a,b,c; 1987 a,b; 1988 a,b )

*Pythium folliculosum* sp. nov. Plates 1-2

Hyphae hyalinae, 4-7  $\mu\text{m}$  diametro. Sporangia filamentosa dilatata. Oogonia terminalia vel intercalaria, globosa, prolata, folliculosa, 10-28  $\mu\text{m}$  diam. et ad 70  $\mu\text{m}$  elongatae. Antheridia monoclina, cellulae antheridiales clavatae. Oogonia continentia unam, interdum duas, raro tres oosporas. Oosporae apleroticae et pleroticae, 6-24  $\mu\text{m}$  in diametro, paries 0.8-3  $\mu\text{m}$  crassa, globulosa. Temperatura optima 25°C. Isolatum ex terra in Zürich (Zollikon), confederatione Helvetica. Holotypus in herbario universitatis Oranensis conservatus (S-24).

**Etymology** :The fungus is being named as *Pythium folliculosum* because of the presence of sac-like oogonia (folliculus = sac).

Mycelium is well branched, measuring 4-7  $\mu\text{m}$  in diameter. Sporangia are produced at 5-25°C. Formation of sporangial vesicles and liberation of zoospores were not observed either in water cultures or in any of the solid media. The sporangia are of the filamentous inflated type. Toruloid inflated elements line up to form an irregularly swollen contiguous structure with densely granular content.

Oogonia are spherical, pear-shaped to elongated, forming sac-like structures, terminal, intercalary, and sometimes catenulate; when terminal they are borne on oogonial stalks measuring 5-60  $\mu\text{m}$  in length. Spherical oogonia measure 10-28  $\mu\text{m}$  in diameter (av. 19.5  $\mu\text{m}$ ), when elongated they can be as long as 70  $\mu\text{m}$  (av. 32.7  $\mu\text{m}$ ).

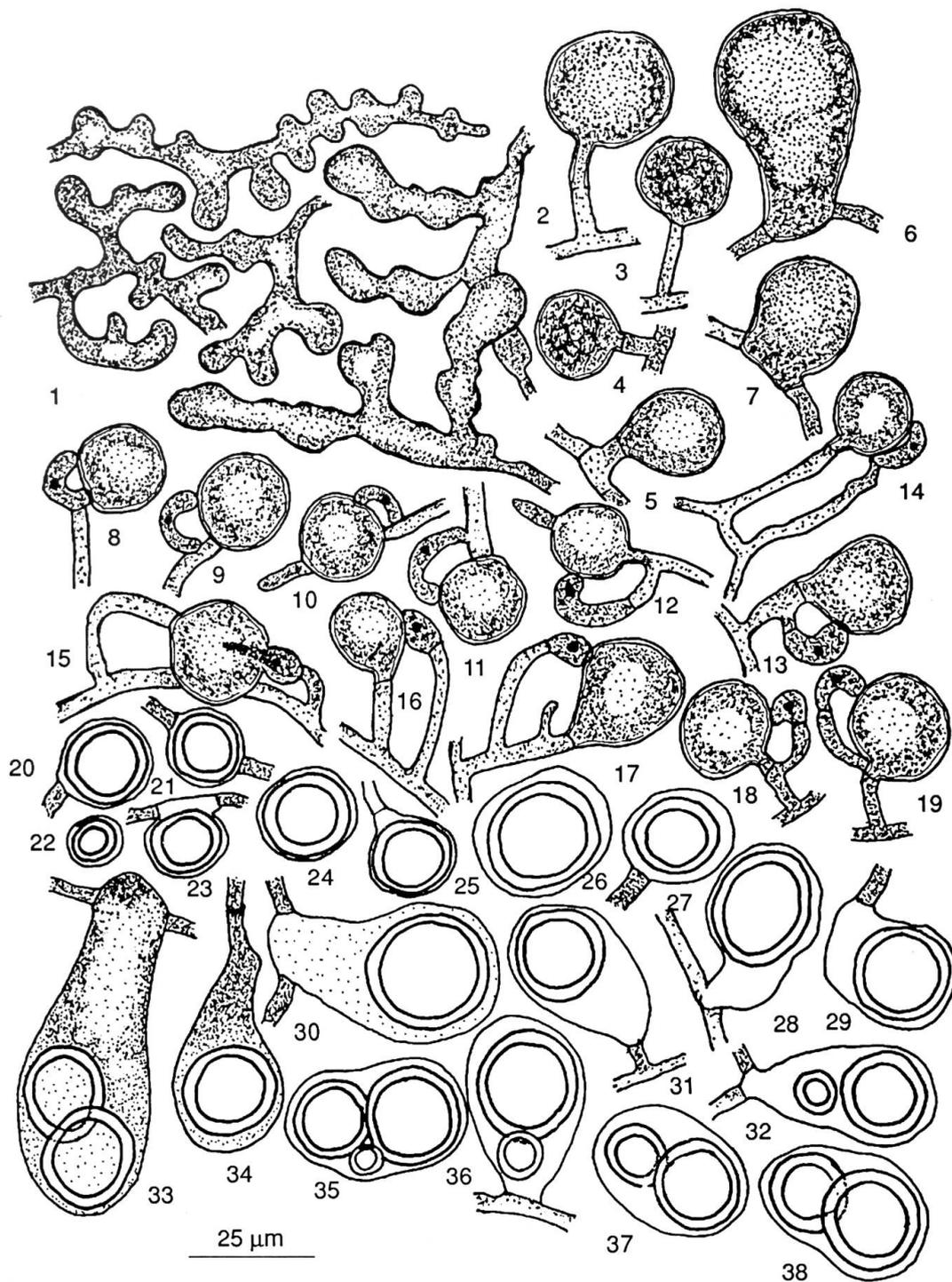


Plate 1: *Pithyum folliculosum*. 1: Filamentous inflated sporangia; 2-5: terminal oogonia; 6-7: intercalary oogonia; 8 -11: oogonia with sessile antheridia; 12-19: oogonia with monoclinal stalked antheridia; 20-25: plerotic oospores; 26-29: aplerotic oospores; 30-38: elongated sac like oogonia containing one to three oospores.

Antheridia are strictly monoclinal, unbranched, stalked or sessile, usually one rarely two per oogonium. Antheridial cells are clavate, making a narrow or wide contact with the oogonia.

Oospores single, often two, rarely three per oogonium, mostly spherical, rarely slightly elongated, measuring 6-24  $\mu\text{m}$  in diameter (av. 16.7  $\mu\text{m}$ ), and are both plerotic and aplerotic. The oospore wall measures 0.8-3  $\mu\text{m}$  in thickness.

*Pythium folliculosum* grows luxuriantly on PCA, CMA and on hemp seeds in water. On both solid media the colony of the fungus is submerged, giving a narrow chrysanthemal pattern on CMA and radiate pattern on PCA. The optimal temperature for its growth was 25°C at which the average daily growth of the fungus was 14.4 mm on CMA and 14 mm on PCA.

### Results and Discussion

*Pythium folliculosum* grows well on hemp seeds in water and on solid media. It belongs to the group of *Pythium* having filamentously inflated type of sporangia. Plaats-Niterink (1981) in her monograph on the genus *Pythium* has described about 15 species belonging to this group. However, none have the sac-like oogonia typical of *P. folliculosum* measuring as long as 70  $\mu\text{m}$ . Most of the species of *Pythium* have globose, spherical oogonia. Recently, Ali-Shtayeh & Dick (1985) described *P. pachycaule* from England. This species comes very close to *P. folliculosum* as it has also sac-like elongated oogonia. A close look to the different characteristics presented by these two fungi makes it clear that they are closely related but not the same. The main differences are underlined in table 1.

TABLE 1 : Differences between *P. folliculosum* and *P. pachycaule*

	<i>P. pachycaule</i>	<i>P. folliculosum</i>
Sporangia	non- inflated or slightly inflated	inflated with contiguous elements.
Oogonia	24-34 $\mu\text{m}$ in diam. (av. 26.6)	smaller oogonia 10-28 $\mu\text{m}$ (av. 19.5 $\mu\text{m}$ ).
Antheridia	mono- and diclinous, 1-3 per oogonium	strictly monoclinal, usually one per oogonium.
Oospores	aplerotic, 18-25 $\mu\text{m}$ in diam. (av. 22 $\mu\text{m}$ ).	both plerotic and aplerotic, 6-24 $\mu\text{m}$ (av. 16.7 $\mu\text{m}$ ).

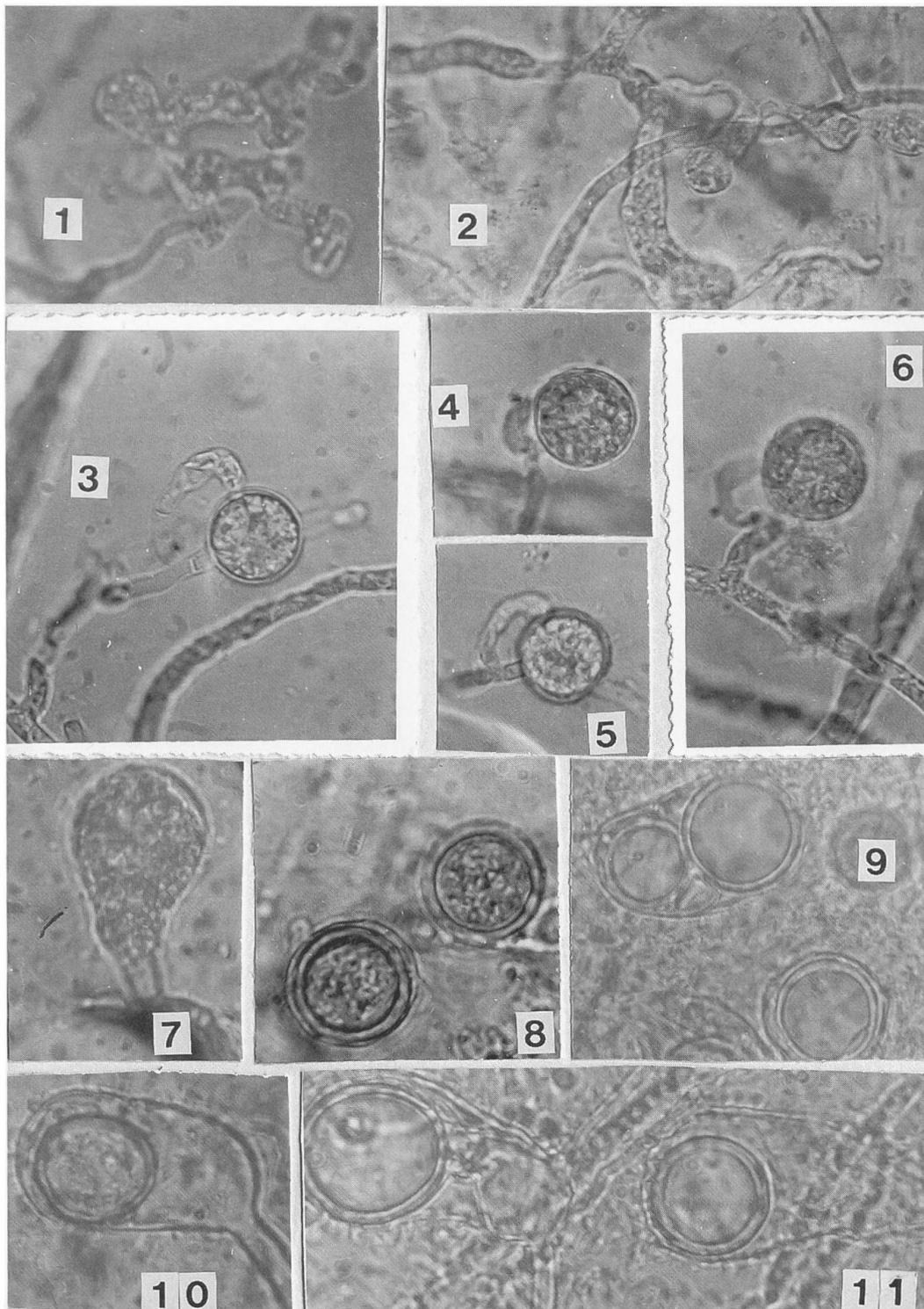
25  $\mu$ m

Plate 2: *Pithyum folliculosum*. 1-2: filamentous inflated sporangia; 3-6: oogonia and monoclinal antheridia; 7: elongated sac like oogonia; 8: plerotic oospores; 9: oogonia containing multiple oospores; 10-11: elongated oogonia containing aplerotic oospores.

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