A new variety of Pythium isolated from cultivated soil in France

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A new variety of *Pythium* isolated from cultivated soil in France

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Summary: *Pythium pachycaule* var. *ramificatum* var. nov. is being described from France. The fungus has the usual sac like or trumpet shaped oogonia, but its dimensions which can attain the length of 300 µm are unique for the genus. Moreover the oogonia in this case are at times branched. Morphological details and a comparison of this variety with other related fungi are being discussed in this article.

Résumé: *Pythium pachycaule* var. *ramificatum* var. nov. est isolée à partir d'un sol prelevé en France. Le champignon est unique par les dimensions de ses oogones qui peuvent atteindre jusqu'à 300 µm en longueur et qui peuvent être ramifiées. Les détails taxonomiques et les aspects biologiques de cette variété ainsi qu'une comparaison avec d'autres espèces voisines font l'objet de cette communication.

Zusammenfassung: *Pythium pachycaule* var. *ramificatum* var. nov. ist von einem in Frankreich entnommenen Boden isoliert worden. Der Pilz ist einzigartig durch die Grösse seiner Oogonien, die bis zu 300 µm lang und verzweigt sein können. Die vorliegende Arbeit bespricht morphologische Charakteristika und die taxonomische Situation, und sie vergleicht diese Varietät mit anderen nah verwandten Arten.

Introduction

Ali-Shatyeh & Dick (1985) reported the presence of a new species, *P. pachycaule* in a dry uncultivated soil in the Reading area. This fungus is redily distinguishable from all other species of *Pythium* by the presence of sac like or trumpet shaped oogonia which generally have a long neck cell. A similar fungus has been isolated from the outskirts of Lille in northern France. The soil sample was taken from a wheat field in December 1990. The fungus was easily identified as *Pythium pachycaule* because of the presence of elongated oogonia. A close study of the isolate however revealed many consistent differences, sufficient enough to create a new variety status. The oogonia of this variety can be as long as 300 µm and moreover it can be branched into 2-6 branches.

Materials & methods

Soil samples were collected in sterilized capped bottles and brought to the laboratory. Fungi were isolated using the usual baiting techniques in water described elsewhere (Paul 1986 a, b; 1987). The baits used were boiled hempseed halves introduced to a watery solution of the soil. Temperature growth

relations were observed on solid media, potato carrot agar (PCA) and corn meal agar (CMA). Benomyl was used to suppress the growth of *Fusarium* like fungi (Paul 1991). ß-sitosterol was used to induce sexual reproduction on solid media. 0,2 mg. of this sterol was dissolved in 10 ml. ethanol. 10µl of this solution was placed aseptically at 10 spots on the surface of the solidified PCA plate. The fungus was then inoculated on the plate. Identification was done with the help of keys provided by Middleton (1943), Waterhouse (1967), an Plaats-Niterink (1981).

Pythium pachycaule var *ramificatum* var. nov. Plates 1-3

Mycelium bene ramificatum, sine loculis, hyphae principales 4-9 µm diam. Sporangia filamentosa, terminalia, parum dilatata. Zoosporae incapsulatae 8-12 µm diam. Oogonia terminalia aut intercalaria, laevia, globosa, saccata vel buccinata, prolata, 13-32 µm diam et ad 300 µm elongatae. Antheridia diclinata, aut circa oogonium. Cellae antheridiales inflatae. Oosparae singulae, 11-28 µm diam., apleroticae et pleroticae, paries 1-3 µm crassa. Incrmentum radiale quotiadianum 20 mm ad 25°C in agaro Solani tuberosi et Dauci carotae (PCA). Secretum ex terra in Loos (Lille) France. Holotypus in herbario universitatis Lille -II conservatus (F-65).

ETYMOLOGY: the varietal name refers to the branched sac like oogonia.
Mycelium hyaline, well branched. Main hyphae 4-9 µm wide. Colonies on PCA and CMA arre submerged showing a radiate pattern of growth. Daily growth rate of the fungus at 25°C on PCA and CMA is 18-20 mm.

Sporangia are not produced in abundance. Thes are occasionally formed in water cultures on boiled hemp-seed halves at room temperature (18-24°C) and are of the filamentous non-inflated or slightly inflated type, up-to 10 µm wide. Encysted zoospores measure between 8-12 µm in diameter.

Oogonia are smooth walled, terminal or interclary, globose, sac-like or trumpet-shaped. Most of the spherical ones are intercalary while the elongated ones are terminal. The sac-like oogonia are occasionally branched into digitate oogonial branches and each branch behaves like an individual oogonium. There can be up to 8 branches. The spherical ones measure 13-31 µm in diameter (av. 22.6), while the elongated ones can grow to the length of up to 300 µm, unknown for any other species *Pythium*.

Antheridia are usually diclinous and intercalary, applied to various ramifications of an oogonium or to different oogonia. The antheridial branches generally wrap around the oogonia and vanish soon after fertilization. Antheridial cells are clavate: 1-4 of these cells are borne on the same antheridial branch making a narrow or sometimes wide contact with the oogonia.

Oospores are both aplerotic and plerotic. The plerotic ones are usually found in the spherical oogonia. In sac-like oogonia these are mostly abortive but at times spherical, spindle, to peanut-shaped. When spherical these measure 11-28 µm in diameter (av. 18 UUm), with a moderately thick wall of 1-3 µm width. More than one oospore per oogonium was not observed in this variety.

Results and Discussion

P. pachycaule which was originally described near Reading, England (Ali - Shtayeh and Dick 1985) is itself unique among the genus *Pythium* because of its sac-like or trumpet-shaped oogonia and the oogonial dimensions which in the case of the Reading isolate were up-to 110 µm long. *Pythium pachycaule* var. *ramificatum* is morphologically similar in some aspects to the one described from England, but there are some consistent differences that merit at least a new varietal status for this fungus:

Character	*P. pachycaule*	*P. pachycaule* var *ramificatum*
Growth	15.4 mm.24 h -1	18-20 mm.24 h -1
Oogonia	24-34 µm diam. (av 26.6) elongated ones up-to 110 µm in length, unbranched.	smaller, 13-32 µm (av.22.6) elongated oogonia upto 300 µm long, ramified in upto 8 branches
Antheridia	mono & diclinous not wrapping around oogonia.	usually diclinous, wrapping around oogonia.
Oospores	aplerotic, 18-25 µm diam. (av.22.2)	both aplerotic and plerotic 11-28 µm diam. (av.18).

Paul (1991) reported a new species of *Pythium, P. folliculosum* from the bank of lake Zürich in Switzerland which has sac like, clongated oogonia. However this species is much different from *P. pachycaule* var. *ramificatum* as it has filamentous inflated type of sporongia and its oogonia and oospore dimensions are smaller than this new variety.

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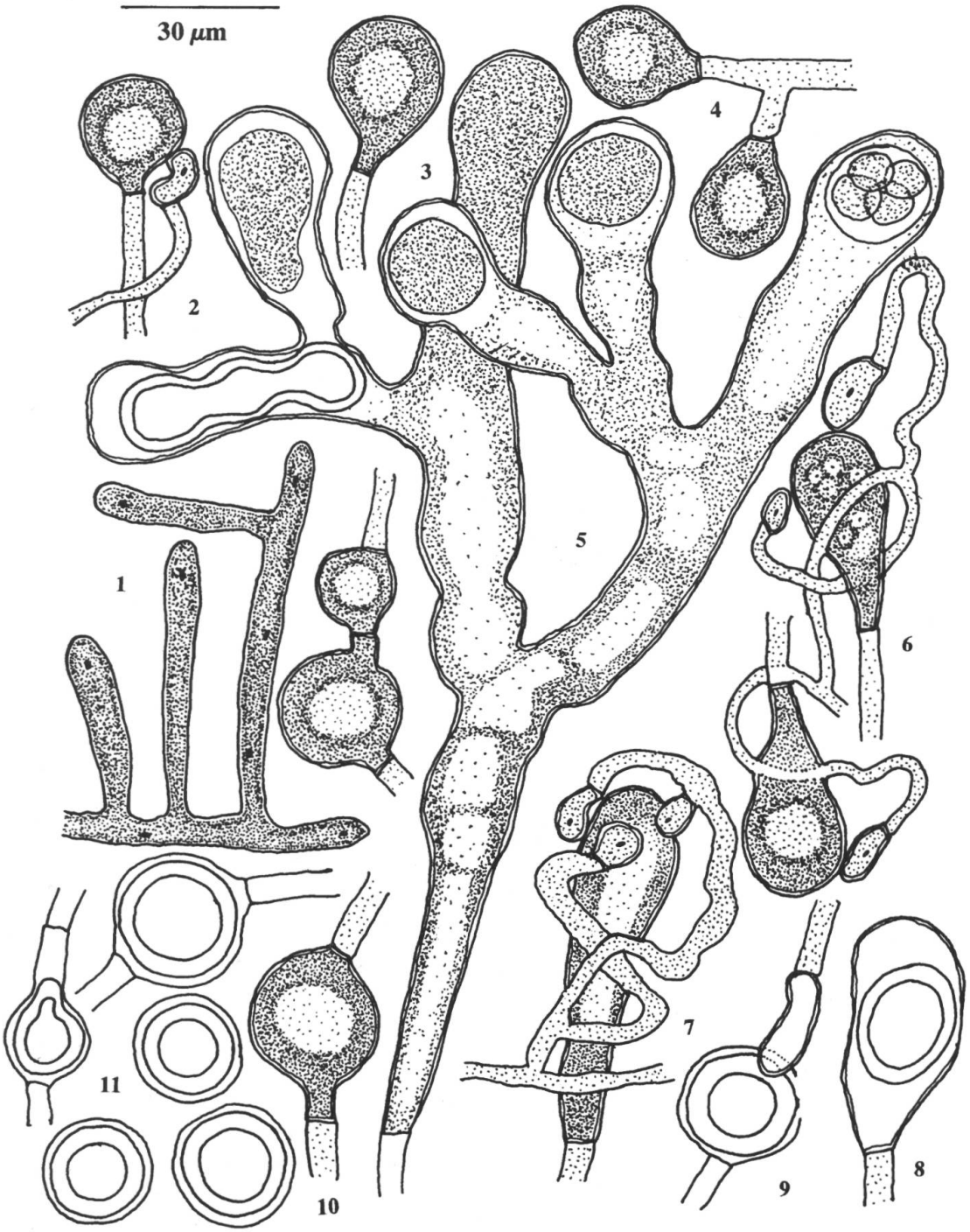

Plate 1: *Pythium pachycaule* var. *ramificatum*: 1: Filamentous slightly inflated sporangia; 2: spherical oogonia with diclinous antheridia; 3-4: terminal oogonia; 5: elongated ramified oogonia; 6-7: elongated oogonia with diclinous antheridia; 89: oogonia containing oospores; 10: intercalary oogonia; 11: plerotic or almost plerotic oospores.

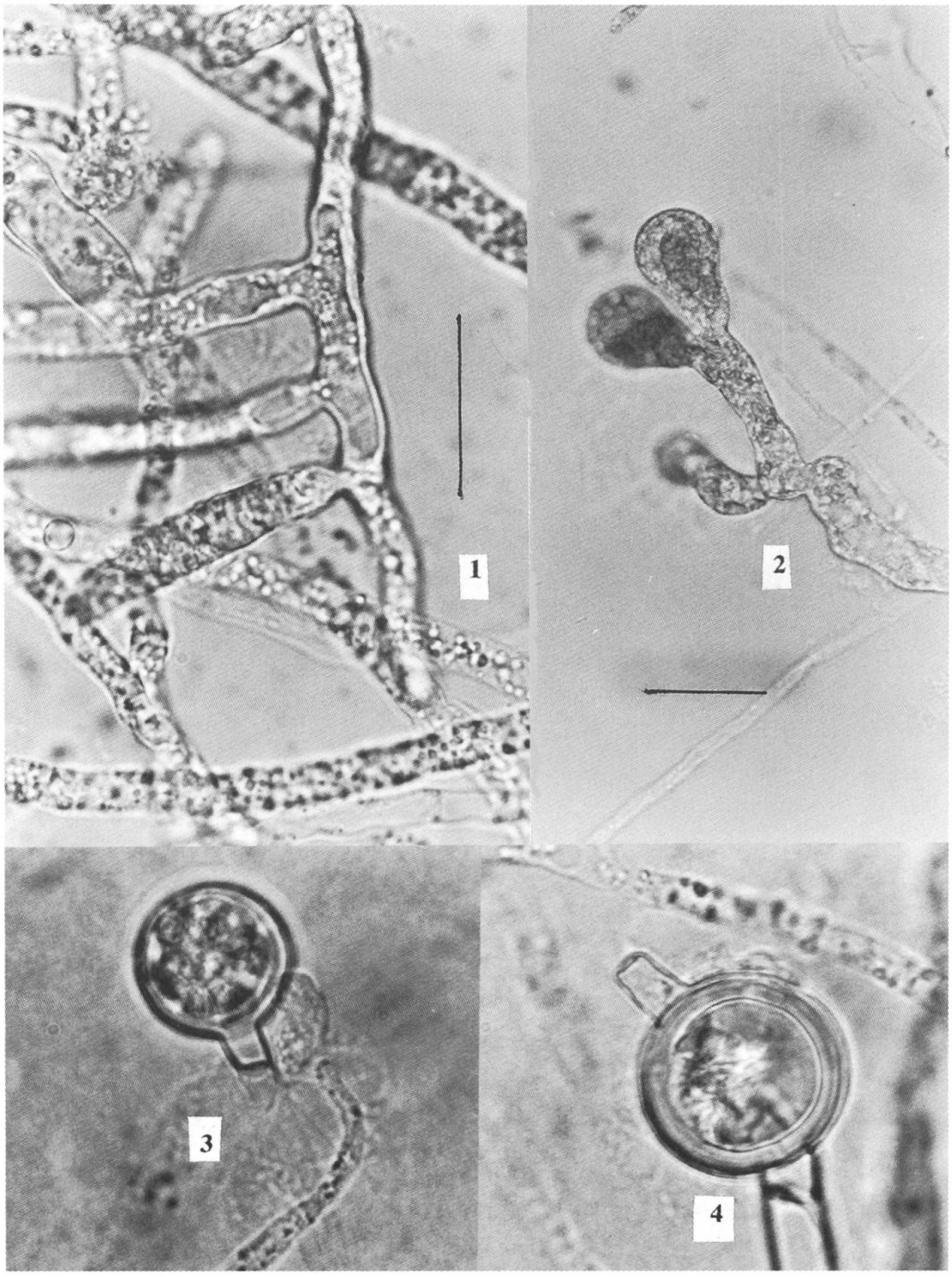

Plate 2: *Pythium pachycaule* var. *ramificatum*: 1: Filamentous slightly inflated sporangia; 2: ramified elongated oogonia; 3: terminal oogonia with diclinous antheridia; 4: intercalary oogonia. (Figs. 1,3 & 4 bar = 20 μm, Fig. 2 bar = 40 μm).

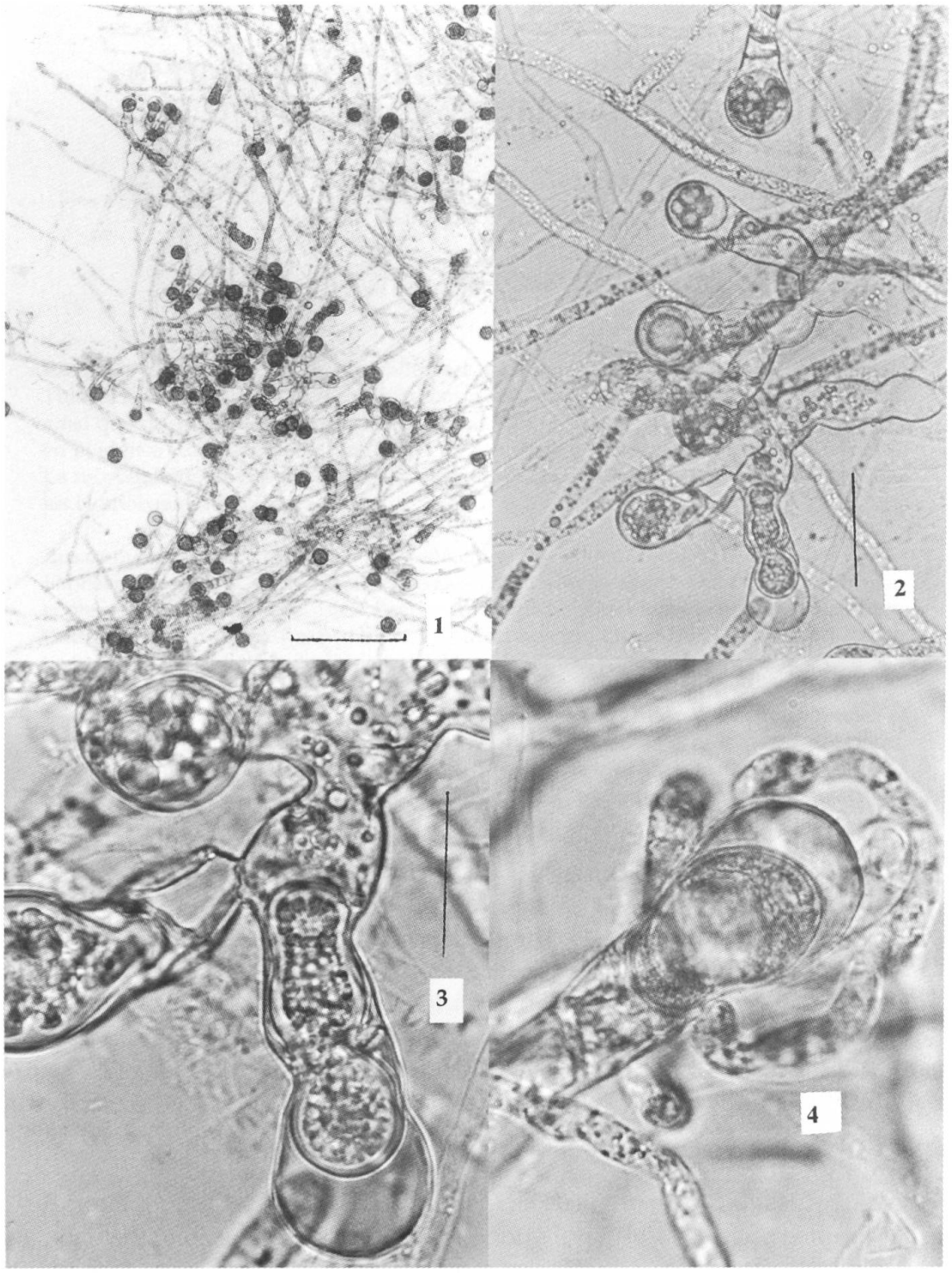

Plate 3: *Pythium pachycaule* var. *ramificatum*: 1: habit in wolér cultures; 2–3: ramified elongated oogonia; 4: elongated oogonia and antheridia. (Fig. 1 bar = 200 μm, Fig. 2 bar = 40 μm, Fig. 3-4 bar = 20 μm).