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Pythium spp. present in irrigation water in the Poniente region of Almería (south-eastern Spain)

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Abstract

Pythium catenulatum, P. diclinum and *P. paroecandrum*, as well as asexual forms with filamentous sporangia were isolated from irrigation water in the Poniente region of Almería (south-eastern Spain). The taxonomic and morphological details of these fungal isolates are described. *P. catenulatum* and *P. diclinum* are new reports to Spain.

Key words: Almería, irrigation water, *Pythium*.

Introduction

The province of Almería is a centre for the production of more than 600 million market crop seedlings in commercial nurseries [1]. Most of the 900 ha of hydroponic crops grown in Spain are concentrated in this province and the neighbouring one of Murcia [2]. Given the scale of the operation, and the importance of irrigation water in the production of these crops, knowledge of the genus *Pythium* in the aquatic environment is of obvious interest.

Various studies have demonstrated the wide distribution of the genus *Pythium* in the small reservoirs and irrigation channels of the Poniente region of Almería, or have estimated the *Pythium* population density in irrigation water [3–5]. However, in these former studies species were not identified. The current paper completes this knowledge, identifying and describing these isolates.

Materials and methods

The study area corresponds roughly to the delta of the river Adra [6], within the Poniente region of Almería. It comprises around 2,000 ha of intensive horticulture, mostly greenhouse crops.

Between October 1994 and August 1997 a total of 120 samples of irrigation water were collected on ten different occasions from the river Adra, irrigation channels and irrigation reservoirs. The water samples were collected in 300 ml sterilized plastic bottles and hermetically sealed. They were taken from the top 40 cm of water and transported to the laboratory in dark at 5 °C [7]. Isolates were derived from 13 of 19 sampling points. Universal Time Mercator (UTM) geographic coordinates of sampling points were annoted (Table 1).

Isolates of *Pythium* spp. were obtained using baits of grass leaves (*Cynodon dactylon*). Ten pieces of grass each 1 cm long, previously boiled for 10 minutes [8], were introduced into the bottles, which were then incubated in darkness at room temperature (20–25 °C). The bottles were periodically shaken [9]. After three days the baits were placed on a Ponchet's P medium [10] except neither the antibiotics nor the fungicides were autoclaved, and 5 ppm Rose Bengal was added to the medium [11] and incubated for two days in darkness at 20 °C.

Pure cultures were made from selected isolates onto potato-carrot agar (PCA) [8] and maintained on PCA in darkness at 20 °C.

To stimulate the formation of the different structures of *Pythium* sp., pieces of the pure culture (on PCA) were placed in Petri dishes with sterilized dis-

Table 1. UTM geographic coordinates of sampling points [11].

No.	(1)	UTM coordinate	No.	(1)	UTM coordinate
1	riv	30S VF 979 754	16	res	30S VF 982 679
2	riv	30S VF 982 739	19	res	30S WF 004 687
4	ch	30S WF 001 693	22	res	30S WF 031 680
8	ch	30S VF 994 666	23	res	30S WF 029 686
9	ch	30S VF 995 665	33	res	30S WF 040 691
10	ch	30S WF 015 692	37		30S WF 051 683
12	ch	30S WF 029 680			

(1) *Source of irrigation water:* riv: River Adra; ch: irrigation channel; res: irrigation reservoir.

Table 2. Frequency of recovery of *Pythium* spp. from the various sources of irrigation water [11].

Pythium species	River Adra	Irrigation channels	Irrigation reservoirs	Total
P. catenulatum	0	1	15	16
P. diclinum	1	1	0	2
P. paroecandrum	2	0	0	2
n.i. isolates	18	6	11	25
Total	21	29	26	55

n.i.: non identified.

tilled water (SDW), baited with hemp cotyledons (*Cannabis sativa*). Various strategies were employed to stimulate production of sporangia (by subjecting the Petri dishes to different temperatures of between 5 and 30 °C and mixtures of SDW/reservoir water), and oogonia (by adding 5 ppm β-sitosterol in SDW, dual cultures on PCA).

Fungal structures on the colonies grown on the hemp cotyledon baits were observed with the light microscope. Observations were made during the first 48 hours, after one week, two weeks, and over the following six months [11].

Colony growths were measured on PCA in darkness at 25 °C. Colony patterns were described from PCA at 25 °C in darkness [8].

Identification was made with the aid of keys and descriptions [8, 12–16].

Results and observations

Fifty-five isolates of *Pythium* spp. were obtained from irrigation water taken from the Poniente region of Almería (Table 2). Of these, a total of three species could be identified. The remaining isolates, which lacked sexual structures, have filamentous sporangia. All these are described below using representative strain for each species. The cited occurrences in Spain are also described.

Pythium catenulatum **Matthews** (Figures 1 and 5A–C)

Isolate described: P-2, obtained from a water reservoir (UTM 30S WF 029 668). Other isolates of the same species: P-1, P-4, P-7, P-10, P-11, P-12, P-13, P-14, P-15, P-17, P-18, P-19, P-20, P-24 and P-25 [11].

Colony on PCA with chrysanthemum pattern, developing some aerial mycelium. Radiate growth rate 15 mm/day on PCA at 25 °C. *Hyphae* 2–7 μm in diameter. *Appressoria* small, single. *Hyphal swellings* globose, 10–15 μm in diameter, in chains or single. *Sporangia* filamentous, inflated, 20 μm wide. *Oogonia* smooth-walled, usually terminal but sometimes intercalary, (20–)25(–27) μm in diameter. *Antheridia* (1–)2(–6) per oogonium, monoclinous and diclinous. *Oospores* aplerotic, (18–)22(–25) μm in diameter, wall thickness (1.5–)2(–3) μm.

Pythium catenulatum is the only species in the genus with inflated filamentous sporangia, as well as catenulate hyphal swellings (globose structures) [15]. In *P. catenulatum* these swellings are found singly or in chains of two or three, or up to six [13]. These characteristics were considered sufficient for the identification of the isolates referred to above.

Although all the isolates exhibited long chains of hyphal swellings, inflated filamentous sporangia were not identified in all cases. Even when typical sporangia were lacking, mycelia with irregularly inflated hyphae were observed.

Of the seventeen isolates identified as *Pythium catenulatum*, only P-2 produced oogonia on PCA medium, when 5 mg/l of β-sitosterol were added. This ability was lost in successive sub-culturing. Oogonia did not develop in dual cultures of the various isolates.

This isolate exhibited a lower than normal number of antheridia per oogonium for this species, although still within the range defined for the species: mostly about 5(–12) per oogonium [8]. It also developed monoclinous antheridia, which occur occasionally in homothalic isolates of this species [8].

The oospore is aplerotic, a circumstance described as occasional in this species [8]. The wall of the oospore in this isolate was somewhat thicker than is

Table 3. Characteristics of development on PCA, at 25 °C, of the non identified isolates of *Pythium* obtained from irrigation water from the Poniente region of Almería.

P	(1)	(2)	(3)	Main hiphae thick (μm)	*Appressoria* Thick (μm)	(4)	*Sporangia* Thick (μm)	(5)	Comments
3	rad	sub	19	8	8–12	cl, nt	4–8	fil	
5	rad	aer	19	8	8–16	cl, ch	4–8	fil	
6	rad	sub	19	8	8–16	cl, ch	4–8	fil	
8	rad	aer	19	8	8–16	cl, nt	4–8	fil	
9	rad	aer	19	8	8–14	cl, s	4–8	fil	
16	ros	sub	19	6	8–16	cl, nt	4–10	fil	
21	ros	aer	15	4	8	cl, ch	4–10	sli	Wavy mycelia
22	ros	aer	11	–	–	–	–	–	
23	chr	aer	18	6	8–12	cl, nt	4–8	fil	
26	ros	aer	15	–	–	–	–	–	
27	ros	aer	12	5	8	cl, s	4–8	fil	
29	rad	sub	19	8	8–14	cl, nt	4–8	fil	
30	rad	sub	10	4	8	cl, s	4–6	den	Scarcely present appressoria
31	rad	sub	16	6	4–6	cl, nt	4–5	fil	Scarcely developed appressoria
32	ros	sub	16	8	4–10	cl, nt	4–8	fil	Idem
34	chr	sub	16	6	4–6	cl, nt	2–6	fil	Idem, rolled mycelia present
37	chr	sub	11	6			4–6	fil	Absent appressoria
40	rad	sub	19	–	–	–	–	–	
41	rad	aer	21	8	8–14	cl, ch	6–8	fil	
42	rad	aer	20	8	8–14	cl, nt	6–8	fil	
43	rad	aer	21	8	8–10	cl, nt	6–8	fil	
44	rad	sub	21	8	8–14	cl, ch	4–8	fil	
45	rad	aer	20	8	8–18	cl, ch	4–8	fil	
47	rad	sub	20	8	8–16	cl, s	4–8	fil	
48	rad	aer	19	6	8–14	cl, s	4–8	fil	
49	rad	sub	19	6	12–14	cl, nt	4–8	fil	
50	rad	aer	18	10	10–16	cl, ch	4–6	fil	
52	rad	aer	18	10	12–21	cl, s	4–6	den	
53	rad	sub	18	–	–	–	–	–	
54	rad	sub	19	8	10–12	cl, ch	4–6	fil	
55	rad	sub	20	8	8–14	cl, ch	4–6	fil	
56	rad	sub	20	8	8–12	cl, nt	4–6	fil	
57	ros	sub	16	6	6	al, ch	3–6	fil	
58	ros	sub	16	6	6–10	al, ch	4–6	fil	
59	ros	sub	16	6	4–6	cl, ch	4–6	fil	Rolled mycelia present

(1) *Colony pattern:* chr: chrysanthemum; rad: radiate; ros: rosette.
(2) *Mycelia: aer:* some aerial; sub: submerged.
(3) *Radial growth:* mm/day.
(4) *Appressoria type:* al: allantoid; cl: club; s: single; ch: chained; n: network.
(5) *Sporangia type:* fil: filamentous; den: dendroid; sli: slightly inflated; inf: inflated.

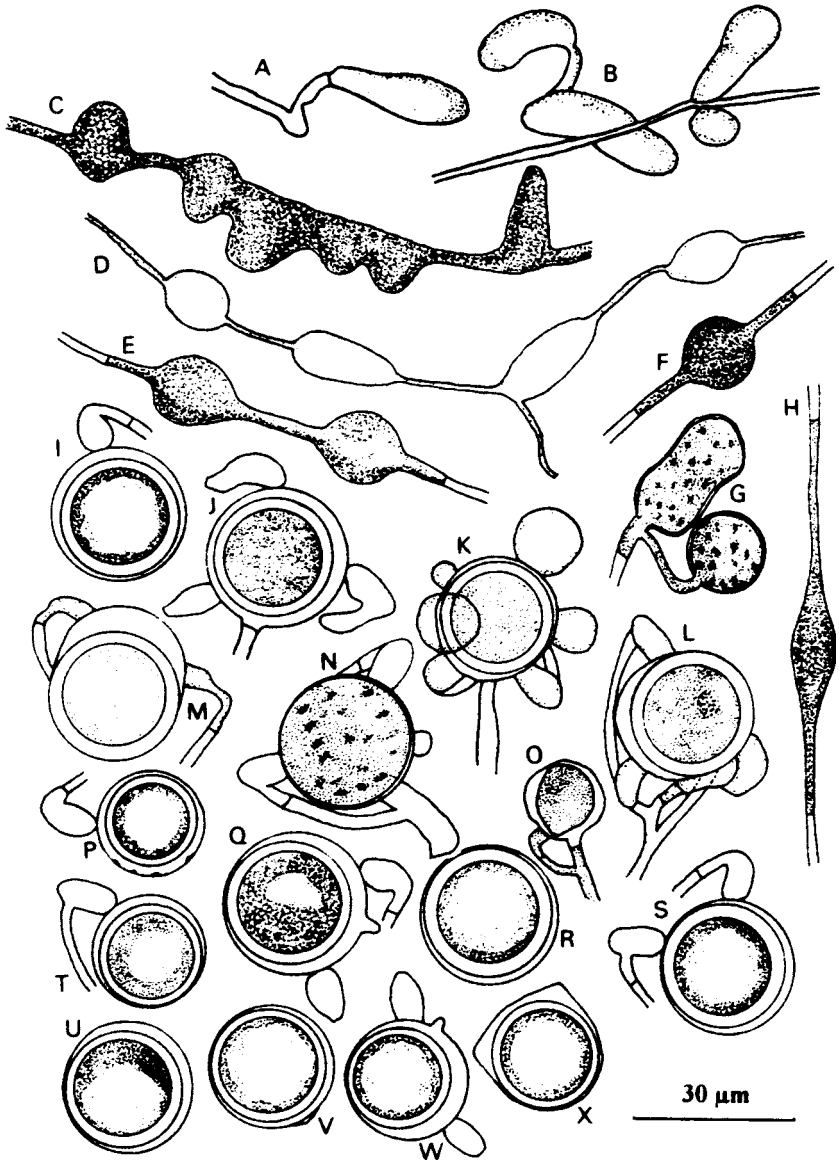

Figure 1. Pythium catenulatum. A–B: appressoria, C: inflated sporangium, D–H: hyphal swellings, I–X: oogonia and antheridia.

normal for the species, though still within the range described: about 1.5 μm thick [8].

Lastly, variability was observed among the appressoria of the different isolates which formed against the base of the Petri dishes (on PCA medium at 25 °C). Appressoria varied in abundance, the degree of grouping and the relative proportion of club-shaped (claviform) to sausage-shaped (allantoid) forms. Grouped, club-shaped appressoria were more frequently observed than other forms.

This is the first time that *P. catenulatum* has been reported in Spain.

***Pythium diclinum* Tokunaga** (Figures 2 and 5D–G)

Isolate described: P-28, obtained from water from an irrigation ditch (UTM 30S WF 001 693). Other isolates of the same species: P-33 [11].

Colony on PCA with radiate pattern and with mycelium submerged in the agar medium. Radiate growth rate 19 mm/day on PCA at 25 °C. *Hyphae* 3–6 μm in diameter. *Appressoria* chained, frequently club-shaped with a twisted basal hypha. *Sporangia* filamentous, occasionally slightly inflated, 50 μm long and 6 μm wide. *Oogonia* smooth-walled, usually in-

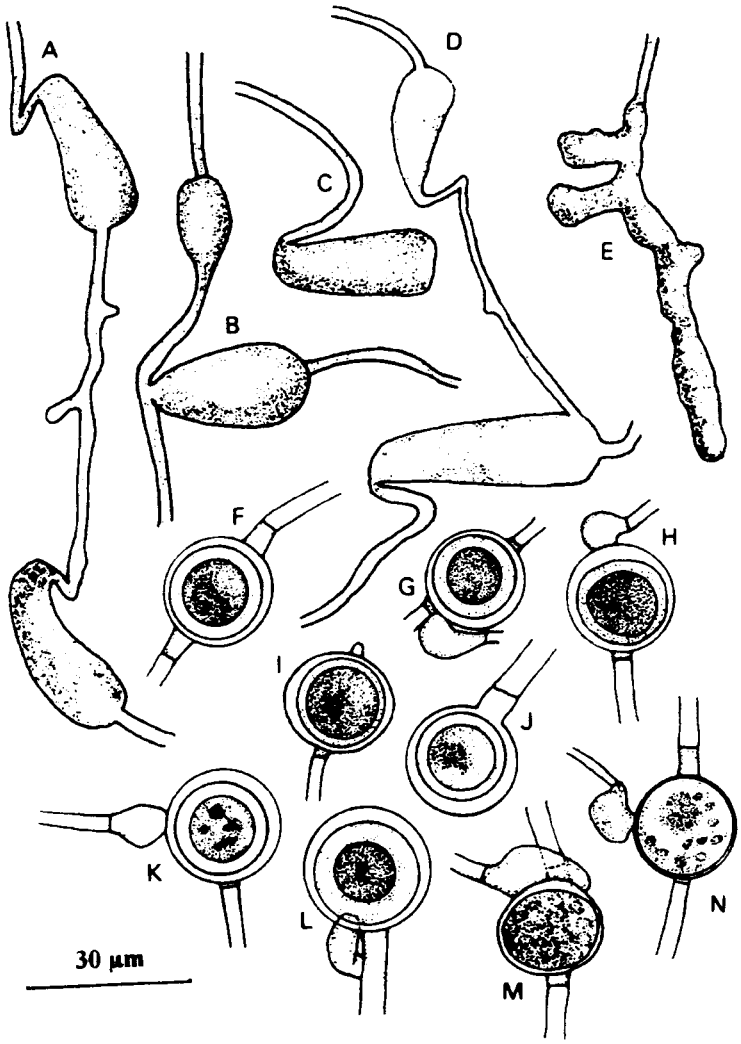

Figure 2. Pythium diclinum. A–D: appressoria, E: slightly inflated sporangia, F–N: oogonia and antheridia.

tercalary but sometimes terminal, (18–)20(–23) μm in diameter. *Antheridia* 1(–2) per oogonium, diclinous or sometimes monoclinous with a short stalk. *Oospores* aplerotic, (15–)17(–19) μm in diameter, wall thickness (1–)2(–3)μm, cell-wall a light violet colour and cell-interior a pale yellow.

Despite the "coloured" oospores, this isolate seems to be closer to *P. diclinum* than *P. coloratum*. The hyhal diameter (main hyphae up to 5.6 μm, but not up to 10 μm [8]), size of oogonia (av. 20.5 μm, but not 22.7 μm [8]) and number of antheridia (1–2 antheridia per oogonium, but not 1–5 [8]) point towards P. *diclinum*. The colour of the oospores may be caused by refraction of the light rather than being caused by a pigment.

This is the first time that *P. diclinum* has been reported in Spain.

***Pythium paroecandrum* Drechsler** (Figures 3 and 6A–C)

Isolate described: P-36, obtained from the river Adra (UTM 30S WF 979 754). Other isolates of the same species: P-35 [11].

Colony with radiate pattern, mycelium with some aerial development on agar medium. Radiate growth rate 23 mm/day on PCA at 25 °C. *Hyphae* (3–)5(–10) μm in diameter. *Appressoria* most frequently grouped and usually allantoid. *Sporangia* spherical or citriform, usually intercalary but sometimes terminal,

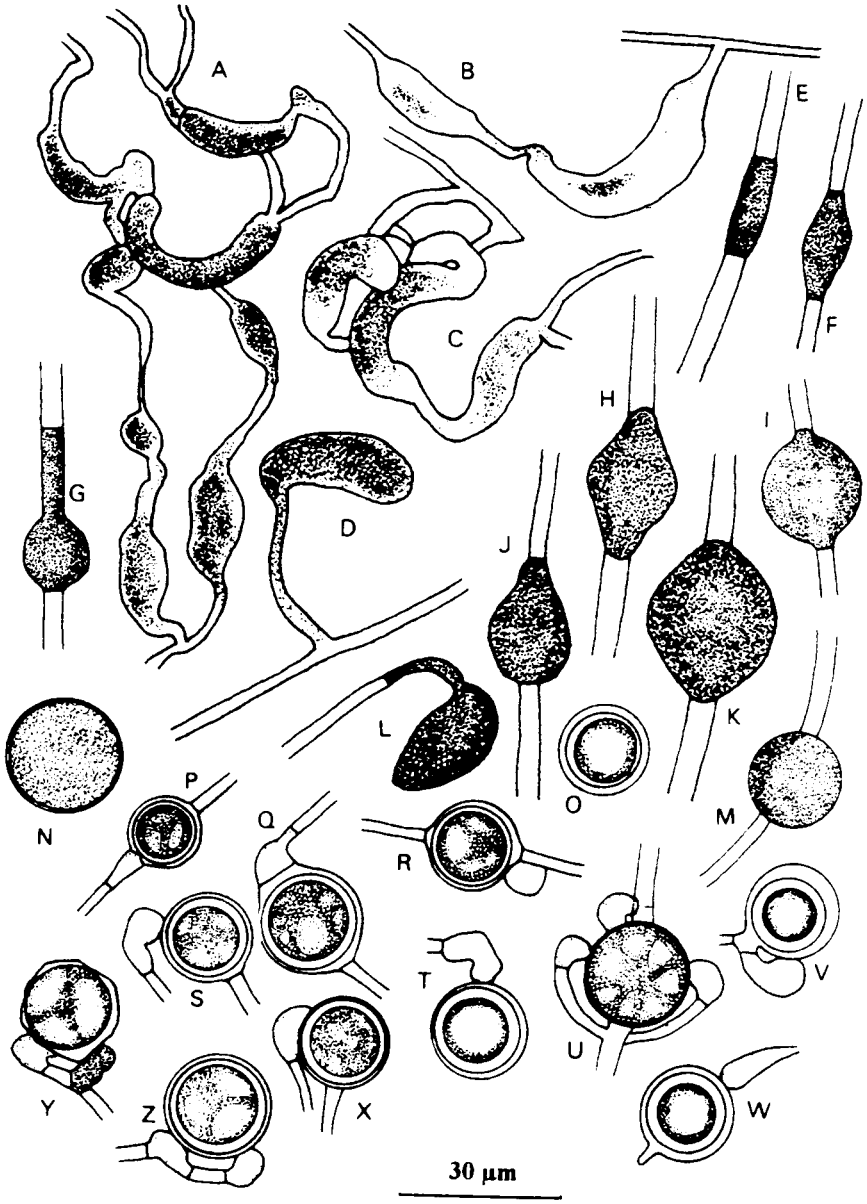

Figure 3. Pythium paroecandrum. A–D: appressoria, E–M: globose sporangia, N–W: oogonia and antheridia.

10–22.5 × 22.5–35 μm in size. *Oogonia* smooth-walled, usually terminal but sometimes intercalary, (15–)19(–20) μm in diameter. *Antheridia* 1(–3) per oogonium, monoclinous (sometimes, sessile), usually diclinous but rarely hypogynous. *Oospores* aplerotic, (14–)17(–19) μm in diameter, wall thickness (1–)1.5(–2.5) μm.

Only *P. paroecandrum* and *P. irregulare* have oogonia with smooth walls, globose sporangia, aplerotic oospores with a diameter of less than 40 μm, and in which the majority of oogonia are intercalary [14]. Although deformations in the oogonium wall were observed in some cases, these were not the typical deformations of *P. irregulare* and monoclinous antheridia were sessile or had a short stalk.

P. paroecandrum forms a complex of species with *P. ultimum* [18]. The close similarity between these two species has been highlighted [12]. In the current study, the species was difficult to distinguish because the isolate exhibited oospore walls which were

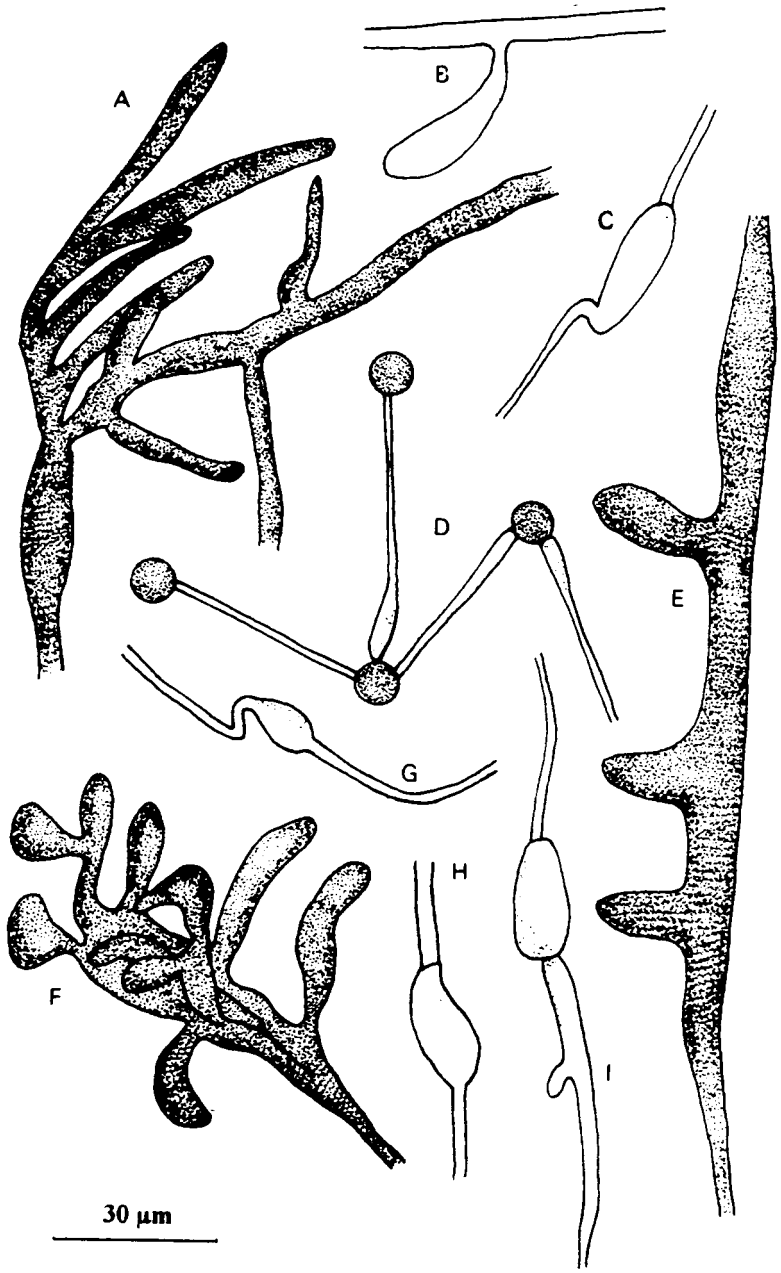

Figure 4. Non identified isolates of *Pythium*. A: dendroid sporangium, B–D: appressoria, E–F: slightly inflated sporangia, G–I: appressoria.

sometimes thicker than have been indicated as being the norm for *P. paroecandrum* [8]. However, *P. paroecandrum* can be differentiated from *P. ultimum* by the presence of each of the different types of antheridia (compared to the more frequent sac-like form in *P. ultimum*), and by a radiate growth rate which was somewhat lower than the 30 mm/day indicated for *P. ultimum* on PCA at 25 °C [8].

The presence of *P. paroecandrum* in soils in ornamental nurseries in Madrid has been reported [19], though a description of the fungus was not provided.

The remaining isolates (Table 3) have filamentous sporangia but lack oogonia [8]. Most of the isolates derived from the samples of irrigation water were found to be from this group (Table 2). The majority of aquatic isolates of *Pythium* appear to have lost

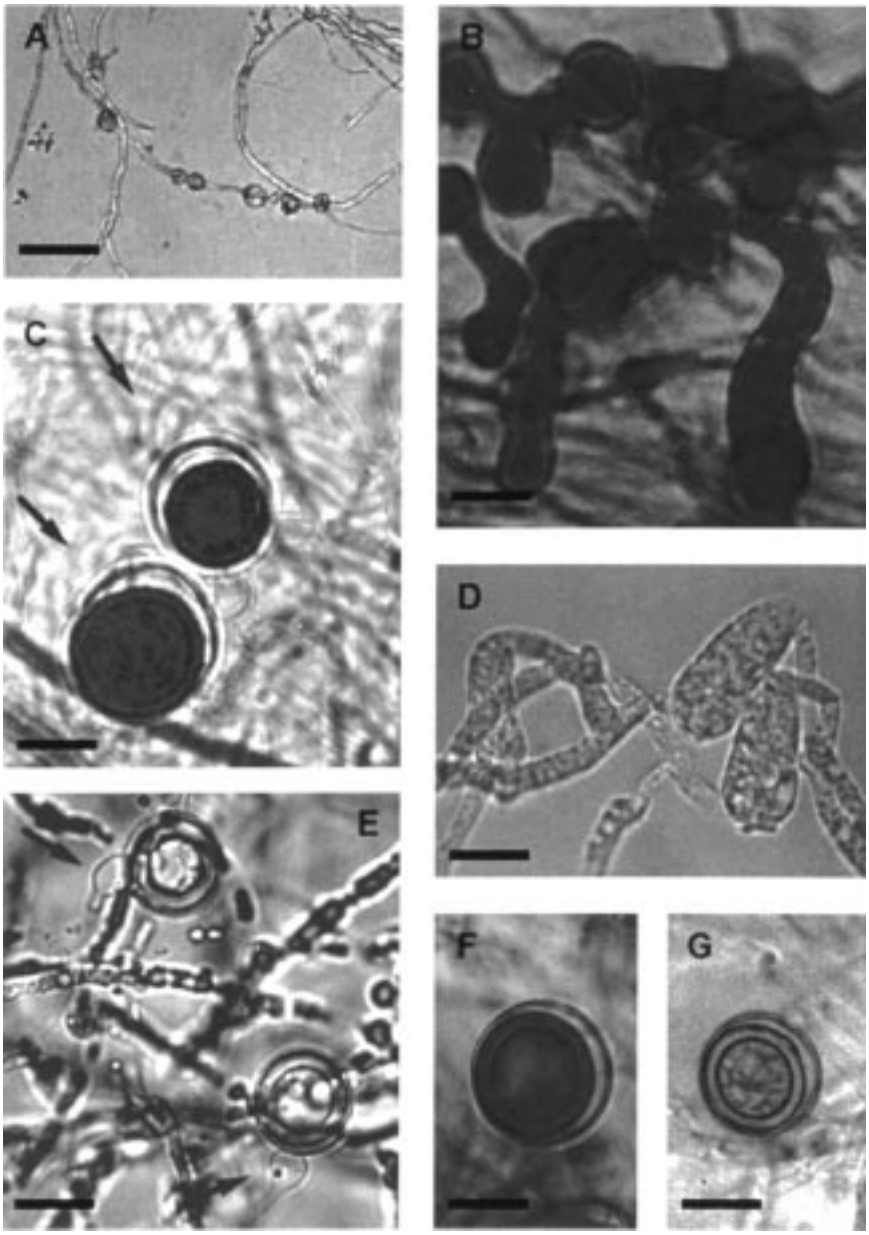

Figure 5. A–C: *Pythium catenulatum*. A: chains of hyphal swellings, B: inflated sporangia, C: oogonia and antheridia, D–G: *Pythium diclinum*, D: appressoria, E: oogonia and antheridia, F: oogonium with oospore, G: oogonium with oospore. (A: bar = 80 μm, other photographs: bar = 20 μm).

their ability for sexual reproduction during the course of their evolution in water [20]. The production of zoospores appears to be the most efficient means of propagation and of maintaining the largest number of individuals in the aquatic medium.

In this isolates, an appreciable diversity of form in both the mycelia and appressoria was noted. The form of the colony was variable (radiate, rosette or chrysanthemum), as well as its growth rate (11 mm/day, 15 mm/day or 20 mm/day on PCA at 25 °C), and the sporangia were filamentous, dendroid or slightly inflated. Accordingly, a study of their relationship at molecular level is required, using PCR techniques [21] or the more recent reverse dot blot hybridization technique [22], in order to determine whether the observed differences have a genetic basis.

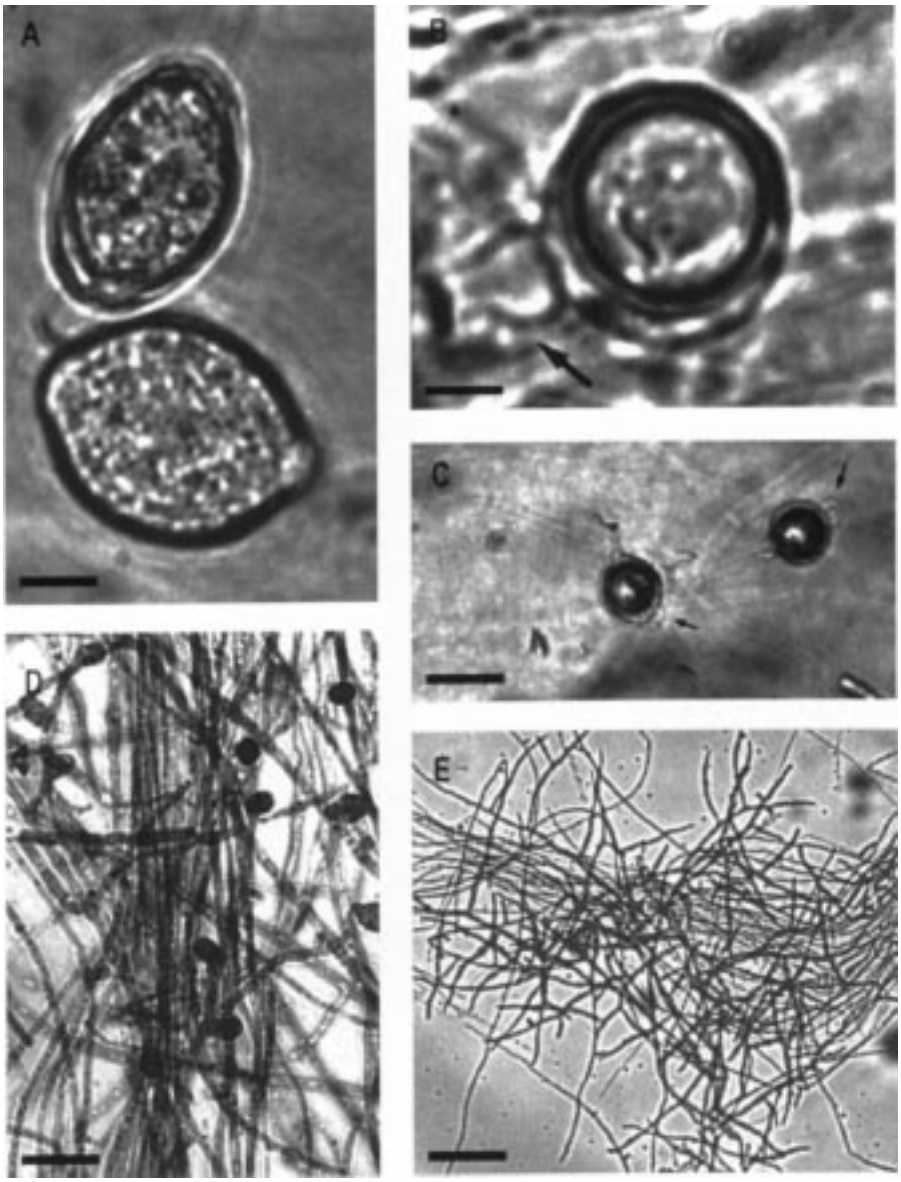

Figure 6. A–C: *Pythium paroecandrum*. A: citriform sporangia, B: oogonium and antheridium, C: oogonia and antheridia, D–E: Non identified isolates of *Pythium*, D: slightly inflated sporangia, E: dendroid sporangia. (A–B: bar = 8 μm, C–D: bar = 40 μm, E: bar = 80 μm).

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