

## A Phaeohyphomycotic Cyst and Peritonitis Caused by *Phialemonium* Species and a Reevaluation of its Taxonomy

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Two cases of human fungal infections caused by members of the genus *Phialemonium*, a genus proposed by Gams and McGinnis (1983) for fungi intermediate between the genera *Acremonium* and *Phialophora*, are presented. The first case was a phaeohyphomycotic cyst on the foot of a renal transplant recipient. The fungus was detected by direct examination and histopathology and was recovered by several procedures over 4 months. It was flat, glabrous, and white becoming yellow with the production of a diffusible yellow pigment; it had conidiophores that were mostly solitary and lateral and terminal phialides and adelophialides with distinct collarettes producing cylindrical to curved conidia. The isolate resembled both *Phialemonium dimorphosporum* and *Phialemonium curvatum*, although its characteristics were more consistent with those of the latter. The second case was peritonitis in a renal transplant recipient. The fungus was white-to-cream colored and yeast like, but later became black with a green diffusible pigment, and produced obovoid conidia; it was easily identified as *Phialemonium obovatum*. Difficulties encountered in the identification and taxonomy of members of this genus highlight the need for standardized conditions, e.g., potato dextrose agar culture incubated at 24 to 25°C for morphologic comparisons, to control significant variations due to culture conditions.

Phaeohyphomycosis is an infection caused by a wide range of genera and species of dematiaceous fungi (8). Infections include cutaneous, subcutaneous, ocular, sinus, pulmonary, bone, and central nervous system infections. A phaeohyphomycotic cyst is a well-defined mycotic infection within the spectrum of phaeohyphomycosis. The entity is characterized by a well-circumscribed, usually single, subcutaneous nodule, typically following trauma, which may occur in both immunocompetent and immunocompromised hosts, although more frequently in the latter. In the early stages, a small (<1 cm in diameter) subcutaneous mass, which usually is not fixed to the skin, forms. The mass enlarges and becomes cystic, with the central portion filled with a liquified, purulent material containing fungal hyphae. Hyphae may also occur in the wall of the cyst. The mass may enlarge to several centimeters in diameter, become firm, and eventually ulcerate. The first case which we present is a phaeohyphomycotic cyst caused by a fungus resembling both *Phialemonium curvatum* W. Gams et McGinnis and *Phialemonium dimorphosporum* W. Gams et McGinnis 1983 (10) and diagnosed in a renal transplant recipient.

A second of type of phaeohyphomycosis, albeit an unusual one, is peritonitis. Fungal peritonitis accounts for approximately 1 to 10% of infections in patients undergoing continuous peritoneal dialysis (11, 29, 34), often with a high mortality and morbidity rate (29). Predisposition to fungal infection has been associated with antibiotic treatment for chronic bacterial infections (6), immunosuppressive therapy after transplantation (5, 17, 30), and infection with the human immunodeficiency virus (9).

Yeasts and yeast-like fungi causing peritonitis include species of *Candida* (24), *Torulopsis*, *Saccharomyces*, and *Cryptococcus* (22), *Hansenula* (23), *Malassezia* (13), *Trichosporon* (1, 31), and the alga *Prototheca* (12). The identification of this group of fungi is relatively easy because their identification is based on a combination of biochemical and microscopic characteristics. In contrast, the identification of filamentous fungi is usually more difficult because they are identified primarily by microscopic criteria. Filamentous fungi reported as etiologic agents of peritonitis include *Rhizopus* spp. (4, 25), *Blastomyces dermatitidis* (26), *Coccidioides immitis* (3), *Histoplasma capsulatum* (19), *Alternaria* spp. (32), *Aspergillus* spp. (27), *Aureobasidium pullulans* (28), *Curvularia* spp. (16), *Fusarium* spp. (7), and *Lecythophora mutabilis* (2). To our knowledge, we report the first case of fungal peritonitis caused by *Phialemonium obovatum* W. Gams et McGinnis 1983. This species has caused infection in a burn patient (21) and osteomyelitis in a dog (20).

### CASE REPORTS

**Case 1.** The patient was a 50-year-old woman with a 30-year history of hypertension and a 4-year history of hemodialysis, who received a cadaveric renal transplant on 18 August 1990 for end-stage renal disease. She had noted a very limited area of erythema, swelling, and tenderness on her left foot in April 1991. The only trauma that she could recall had occurred earlier in April when someone stepped on this foot during a trip to Virginia. At the time of the injury, she was receiving prednisone, azathioprine, and cyclosporine. She did not seek medical attention until 2 months later when the swelling had progressed from the dorsal to the plantar surface of her foot. At this time, it affected her ability to walk comfortably. A radiograph taken at this time revealed only a small swelling of the soft tissue

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over the dorsum of the middle of the left foot, with no evidence of recent or previous fracture. She was sent home without further diagnostic testing or treatment.

Over the next few months, the lesion enlarged to 2 cm. At that time, she complained of increased pain on walking. The lesion was confined to the plantar surface and appeared cystic. Approximately 1 ml of purulent material was aspirated and cultured. A filamentous fungus subsequently identified as a member of the genus *Phialemonium* W. Gams et McGinnis was recovered from the aspirated pus. Two days later, an incision was made, drainage yielded several milliliters of purulent fluid, and a portion (1.5 by 2 cm) of necrotic tissue was removed. The lesion was then irrigated with an antibacterial solution and sutured loosely. The incision healed, and the patient improved.

Two-and-one-half weeks later, she presented again with recurrence of the mass and increased tenderness on the plantar surface of the left foot. More than 1 ml of purulent material was again drained, and she was begun on oral ciprofloxacin, pending cultures. Hyaline, septate, branching hyphae were seen on direct examination in 10% KOH, in calcofluor white, and by Gram stain. The same fungus was again recovered. An extensive evaluation, including computerized tomography scans, was performed to exclude systemic infection. With negative systemic findings, two areas of necrotic tissue with a possible communication between them were identified, and a tissue mass (2 by 2 by 1 cm) and 10 ml of purulent material were removed. The specimens were again positive for hyphae in 10% KOH-calcofluor white and culture.

The histopathology of the tissue revealed multiple areas of diffuse granulomatous inflammation (Fig. 1A) characterized by aggregates of epithelioid macrophages surrounded by fibroblasts, lymphocytes, and occasional small collections of polymorphonuclear leukocytes. Numerous Langhans-type giant cells were present in the granulomata. Rare-foreign-body-type giant cells containing polarizable foreign material were also seen. The centers of many of the granulomata contained microabscesses and foci of necrotic debris. Dense collagenous tissue with scattered lymphocytic infiltration was seen near the periphery of the lesions. Gomori methenamine-silver staining revealed numerous fragmented fungal elements (Fig. 1B) near the edge of the microabscesses and adjacent to the Langhans-type giant cells. No vascular invasion was noted. The septate hyphae ranged from 1.5 to 3.5  $\mu\text{m}$  in diameter and 3.0 to 70  $\mu\text{m}$  in length. Many hyphae appeared constricted at the septa, and some hyphae had irregular areas of swelling. Branching was primarily at acute angles, although occasional short branches were perpendicular to the main hyphal axis. The hyphae, appearing faintly basophilic with indistinct borders and without evidence of pigmentation, were difficult to visualize by hematoxylin and eosin histopathology. Portions of the hyphae stained positive for melanin as revealed by Fontana-Masson staining (33).

The patient initially did well after surgery, but a small mass near the original lesion recurred 2 months later and required an additional surgical excision. Histopathology again revealed numerous, fragmented fungal elements. A follow-up in April 1992 showed no evidence of recurrence. The patient received no antifungal therapy during this infection.

**Case 2.** A 5½-year-old girl, first diagnosed with focal segmental glomerulosclerosis at the age of 2, was begun on continuous peritoneal dialysis 1 year prior to the mycotic episode. During this period, the patient was admitted to the hospital several times for bacterial peritonitis. Ten days

before the mycotic episode, the patient received a cadaveric renal transplant without operative complications. There was immediate posttransplant diuresis, with a decrease in the serum creatinine level from 7.1 to 0.3 mg/dl and good urinary output. Postoperatively, the patient was started on cyclosporine A, azathioprine, and prednisone. Later, the peritoneal effluent was found to be purulent, but the Gram stain and bacterial cultures were negative. Direct examination of the effluent in 10% KOH and calcofluor white demonstrated the presence of hyaline yeast cells and toruloid hyphae. Fungal cultures grew a yeast-like organism from multiple peritoneal fluid specimens that was identified as *P. obovatum*.

After the first report of a positive fungus culture, the patient was started on antifungal therapy and the dialysate catheter was removed. The patient was given amphotericin B intravenously for a total of 11 doses and ketoconazole orally for a total of 4 days. The ketoconazole was replaced by oral flucytosine for 9 days. Amphotericin B and flucytosine were discontinued after a biopsy of the transplanted kidney showed signs of rejection. The patient was started at that point on oral fluconazole until repeated peritoneal fluid cultures yielded no growth.

## RESULTS

**Case 1.** The first specimen, a small amount of purulent material, was negative by direct examination. The fungus was recovered within 6 days on slants of Sabouraud dextrose agar (SDA), SDA with gentamicin, and Mycosel (Becton Dickinson, Cockeysville, Md.) incubated at 23 to 24°C. In addition, the fungus was recovered on brain heart infusion agar (BHI; Remel, Lenexa, Kans.) with gentamicin and 5% sheep blood agar incubated at 35°C. Specimens from the second drainage and the excisional surgery were positive by both direct examination and culture. Branched, septate hyphae with some swollen cells were detected in the specimens by using calcofluor white, KOH, and Gram stain.

The colony was flat, glabrous, and white, becoming yellow with production of a diffusible yellow pigment after 1 week incubation at 23.5°C (Fig. 2). Subsequently, small areas of the colony became dull gray. Microscopically, small, curved conidia and long, tapering phialides were observed. The initial identification was either an *Acremonium* sp. on the basis of the original yellowish white colony and the production of phialids or a *Phialophora* sp. on the basis of the appearance of areas of gray pigmentation and phialides.

Slide cultures were prepared with glucose-yeast extract-salt sporulation medium, incubated at 23.5°C, and examined after 1, 2, and 3 weeks. Hyphal ropes and aggregates of conidiophores were common. Conidiophores were mostly solitary, lateral, and terminal and consisted of both phialides and adelophialides ranging from 4 to 29  $\mu\text{m}$  (average, 13  $\mu\text{m}$ ) in length (Fig. 3A). Occasionally, conidiophores were branched and clustered. The conidiogenous cells were tapered and terminated in distinct, parallel-walled to V-shaped collarettes, although not all had distinct collarettes. Subsequent slide culture preparations grown for 15 days on potato dextrose agar (PDA) resulted in fewer distinct collarettes. Conidia were cylindrical to allantoid in shape and ranged in size from 3.0 to 8.0  $\mu\text{m}$  (average, 4.7  $\mu\text{m}$ ) by 1.0 to 2.0  $\mu\text{m}$  (average, 1.4  $\mu\text{m}$ ) (Fig. 3B). Hyphae were hyaline to pale yellow-brown. These characteristics compared with those of the three described species of *Phialemonium* (Table 1) were considered most consistent with the description of *P. curvatum* as described by Gams and McGinnis (10).

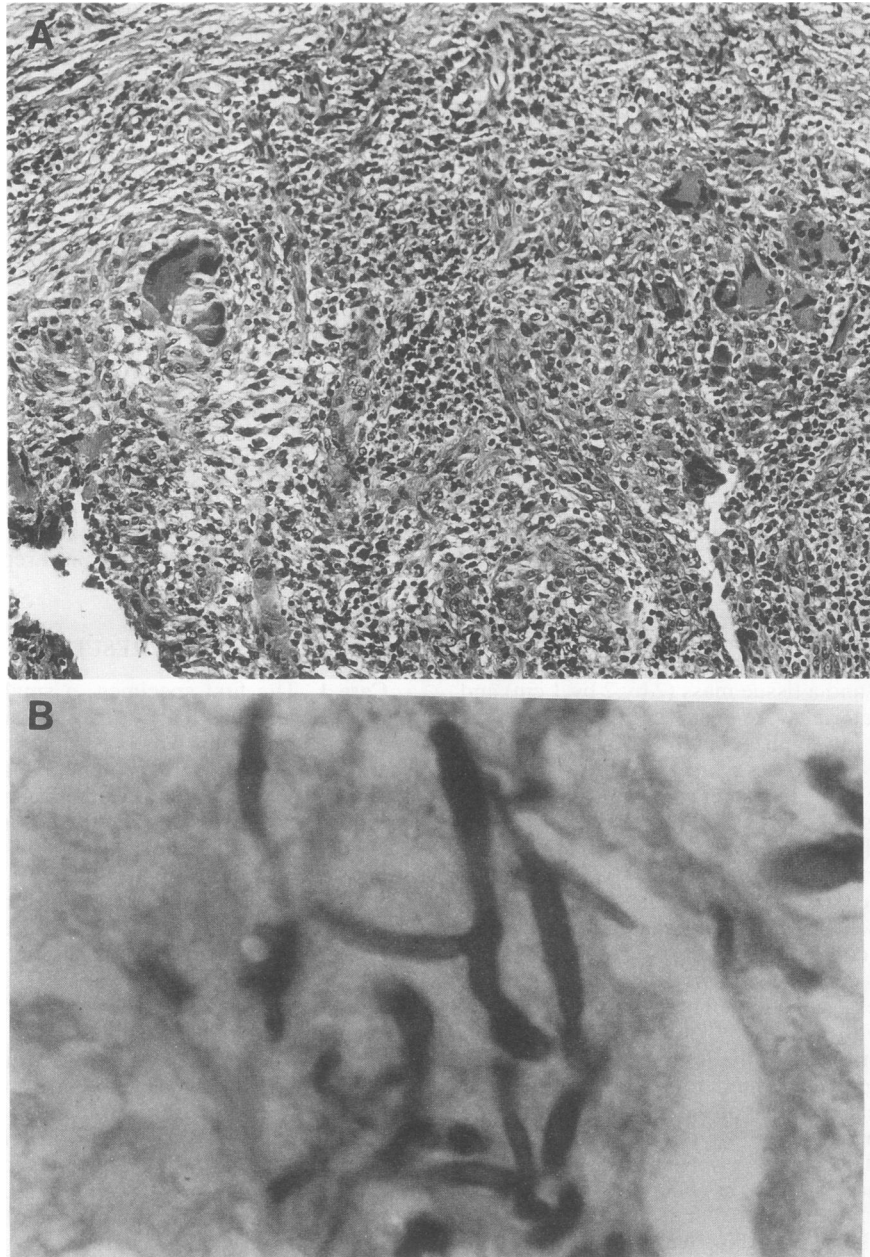


FIG. 1. Sections of subcutaneous granulation tissue excised from phaeohyphomycotic cyst (case 1). (A) Diffuse granulomatous inflammation with microabscesses and numerous giant cells (hematoxylin and eosin; magnification,  $\times 130$ ); (B) fragmented fungal elements (Gomori methenamine-silver staining; magnification,  $\times 1,000$ ).

To confirm this identification, the strain was sent to four reference laboratories. The identifications were *Lecythophora* sp. by two, *P. curvatum* by one, and *P. dimorphosporum* by one. In addition, we compared our isolate with strains of *Lecythophora hoffmannii* (Van Beyma) W. Gams et McGinnis (M185-89, M448-89, and M449-89), *L. mutabilis* (Van Beyma) W. Gams et McGinnis (M922-88, M446-89, and M447-89), *L. lignicola* Nannfeldt (M445-89), and *P. obovatum* (M138-89, M429-89, and M492-89) from the culture collection of the New York State Department of Health, with the type strains of *P. dimorphosporum* (CBS 491.82) and *P. curvatum* (CBS 490.82), and with an additional isolate

of *P. curvatum* (UTMB 1600) provided by M.M. The colonies were compared on BHI, SDA, and PDA after incubation in the dark at 23.5, 30, and 35°C. Significant variation was noted in the growth rates, colony textures, and pigmentations on the three media or at the three temperatures. The case 1 isolate from the present study grew best at 30°C, produced extensive aerial hyphae on PDA compared with glabrous colonies on BHI and SDA, and produced a yellow diffusing pigment on PDA and SDA at 25 and 30°C only. Extensive colony variation was also noted with the 13 reference strains tested. The case 1 isolate was different from all reference strains, was significantly different from the

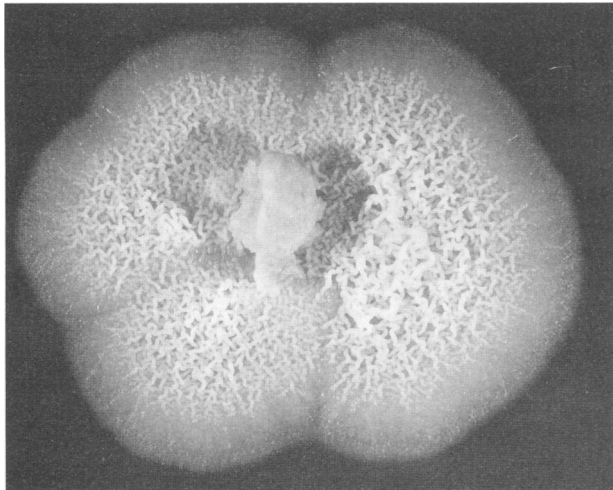


FIG. 2. Colony morphology of *P. curvatum*-*P. dimorphosporum* isolate from case 1 (PDA, 14 days, 25°C).

*Lecythophora* species and *P. obovatum*, but was not consistent with the appearance of either *P. curvatum* or *P. dimorphosporum*.

Microscopic comparisons of our strain were made with isolates of *L. hoffmannii* (M185-89), *P. obovatum* (M138-89), and the type strains of *P. curvatum* and *P. dimorphosporum*. Cultures were grown on sporulation medium and PDA at 23.5°C for 2 weeks. The present strain was distinct from the *Lecythophora* species by the extensive production of very long phialides, although a few short volcano-shaped phialides were noted. It differed from *P. obovatum* because of the lack of obovate-shaped conidia. Our isolate produced a range of conidial shapes and conidiogenous cells, a morphology which overlapped with the descriptions of both *P. curvatum* and *P. dimorphosporum*. Therefore, we could not assign our isolate to either species because it is intermediate between the two taxa. We feel that the isolate more closely resembles *P. curvatum*.

Susceptibility testing was performed as previously described (14, 15). The 48-h MICs were 0.25 µg/ml for amphotericin B, 10 µg/ml for miconazole, and >40 µg/ml for ketoconazole, fluconazole, and itraconazole. The 24-h MIC for 5-fluorocytosine was >250 µg/ml.

**Case 2.** The patient's peritoneal fluid was plated on BHI agar with 10% sheep blood plus gentamicin and chloramphenicol, inhibitory mould agar, and BHI alone. The cultures were incubated at 30°C, and after 48 h of incubation showed many white to cream-colored yeast-like colonies. Tease mounts from the colonies contained a mixed population of yeast cells, hyphae, and conidia and resembled *Hormonema* spp. in that the conidia occurred in clusters along the hyphae and originated from intercalary conidiogenous cells having a single conidiogenous locus.

Slide cultures and subcultures of the organism were grown on PDA for 14 days at 25°C. After 5 days of incubation, a green diffusible pigment was seen on the underside of the PDA in the plates and tubes. The slide cultures were examined with a phase-contrast microscope and compared with the type species of *P. obovatum* UTMB 20 (type strain), *P. curvatum* UTMB 1603 (type strain), and *P. dimorphosporum* UTMB 19 (type strain). Microscopically, the fungus produced cylindrical adelophialides with and without paral-

lel-walled collarettes. Phialides consistently produced obovate, hyaline conidia (3.5 to 6 µm by 1.2 to 1.7 µm) which were one celled, smooth walled, and apiculate at their truncated bases. Chlamydoconidia were present in older cultures, and the thallus became black at its center. These characteristics are typical of *P. obovatum*.

Susceptibility testing was performed as a courtesy by the Fungus Testing Laboratory at the University of Texas Health Science Center in San Antonio. The 72-h MICs were >18.47 µg/ml for amphotericin B, >322.75 µg/ml for 5-fluorocytosine, 0.8 µg/ml for ketoconazole, 40 µg/ml for fluconazole, and 1.25 µg/ml for itraconazole. The minimum lethal concentrations were 3.2 µg/ml for ketoconazole, 80 µg/ml for fluconazole, and >10 µg/ml for itraconazole.

## DISCUSSION

These two cases of *Phialemonium* infection illustrate the increasing significance of dematiaceous fungi in immunocompromised patient populations. These organisms, once thought to be insignificant as a primary cause of infection or merely troublesome contaminants, are now presenting themselves as emerging pathogens. Mycologists and clinicians alike must be aware of the significance of these organisms in selected patients.

*Phialemonium* is a genus erected in 1983 by W. Gams and M. R. McGinnis to accommodate selected hyphomycetes intermediate between *Acremonium* and *Phialophora* species (10). *Phialemonium* spp. can be distinguished from *Lecythophora* spp. by the abundant production of adelophialides (reduced intercalary phialides not delimited from the vegetative hyphae by a basal septum) that may or may not have collarettes and by a colonial morphology consisting of white to tan colonies which, depending upon the species, may produce a green pigment that diffuses into the medium. *Lecythophora* spp. form initially pink to salmon, pasty colonies, from which the pigments do not diffuse into the medium. Microscopically, *Lecythophora* spp. produce abundant inconspicuous adelophialides, with or without collarettes, that resemble the shape of a volcano. It has to be noted that both taxa are capable of producing conspicuous tapering phialides with parallel-walled collarettes and basal septa in older cultures. These phialides are similar to those produced by *Phialophora parasitica* and *Phialophora repens* (10, 18).

Members of the genera *Phialophora* and *Acremonium* typically produce tapering phialides with basal septa and conidia accumulating in balls or in short chains. In general, *Phialophora* species produce distinctive olivaceous colonies that differ from *Acremonium* spp. and from young colonies of *Phialemonium* and *Lecythophora* spp. *P. obovatum*, *L. mutabilis*, and *L. hoffmannii* can become olivaceous with age. Colonies of *P. repens* may be slow to develop the characteristic olivaceous brown pigment, and lavender isolates have been described. In our examination of the type strain for *P. repens* (UTMB227), we observed by microscopic examination that the age of the colony greatly affected the morphology. Conidia in 5-week-old cultures were like those of *P. dimorphosporum*, whereas in 11-day-old cultures they were like those of *P. curvatum*. This emphasizes the importance of consistent and uniform growth conditions when studying these fungi. In general, the phialides and collarettes are more prominent and thick walled in the genus *Phialophora* (8).

Three species of *Phialemonium* have been described previously (10). *P. obovatum* is the most distinctive and can be

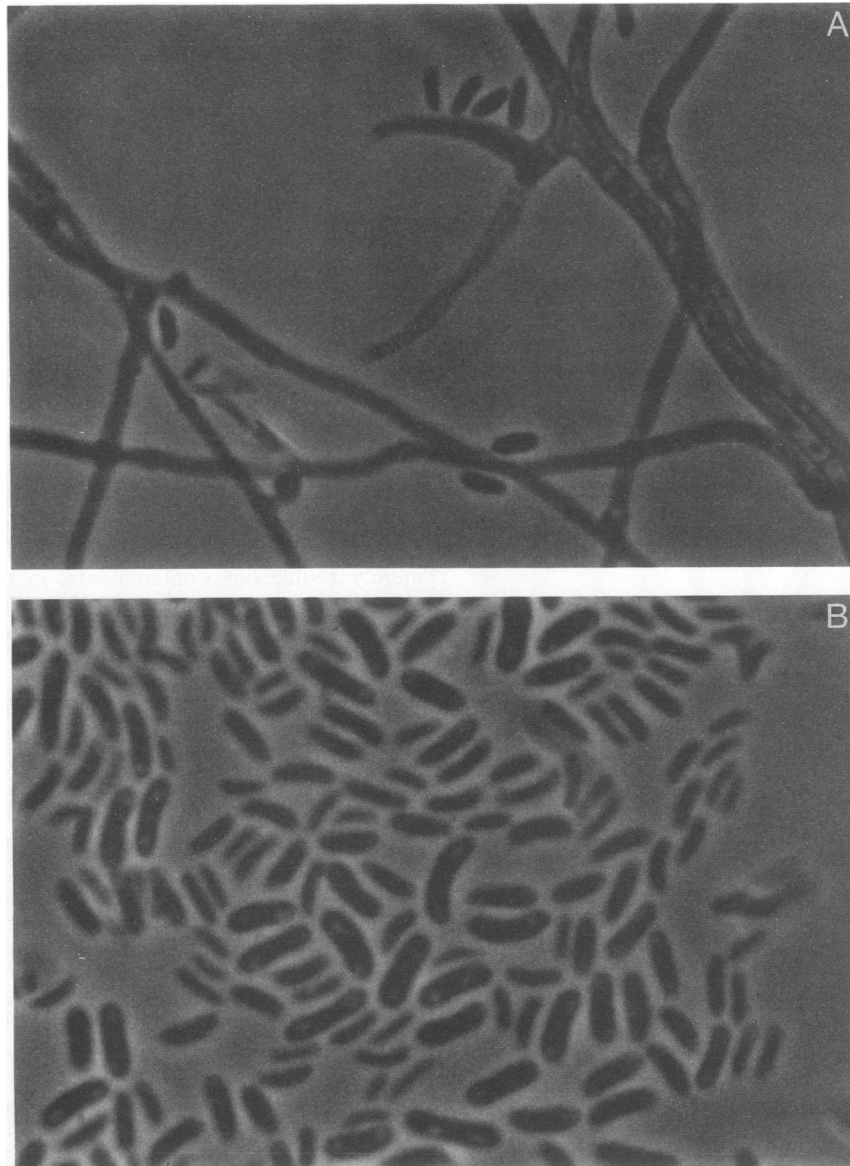


FIG. 3. Microscopic morphology of isolate from case 1. (A) Phialides, adelophialides, and conidia (magnification,  $\times 1250$ ); (B) conidia (magnification,  $\times 1250$ ).

easily recognized by its ability to produce a greenish diffusible pigment and by having obovoid conidia. The differences between the other two species are not as distinct, especially when standardized growth conditions are not used. *P. curvatum* was distinguished on the basis of its cylindrical to allantoid conidia, its colonies that are white, becoming grayish, and its musty, yeast-like, or camphor odor. *P. dimorphosporum* has both allantoid and obovoid conidia that vary in size and shape, colonies similar to those of *P. curvatum* that may become pale vinaceous buff, but no remarkable odor.

The isolate from case 2, with its green colony, diffusible green pigment, and obovoid conidia, was easily identified as *P. obovatum*. However, the isolate from case 1 presented a more difficult problem. It became clear in this study that the isolate from case 1 fit into the genus *Phialemonium* but that its assignment to the level of species was less clear. Since all

isolates of *P. obovatum* that we have examined have produced greenish colonies and since greenish colonies were not produced by this isolate, we were left with the possibility that the isolate was *P. curvatum*, *P. dimorphosporum*, or an undescribed species. The best argument for this strain being a new species would be based on the production of a distinct yellow diffusible pigment and a full range of conidial shapes. The best assignment for this isolate is *P. curvatum* on the basis of its distinct conidiophore morphology, the presence of curved conidia, the absence of chlamydoconidia, and its ability to grow at  $37^{\circ}\text{C}$ . In addition, the isolate did produce a vinaceous pigment under certain conditions and a wide range of conidial sizes and shapes that is characteristic of *P. dimorphosporum*. This strain yielded a range of phenotypic variation that was consistent with the range of variation seen with type cultures and descriptions of both *P. curvatum* and *P. dimorphosporum*. Additionally, within a given slide cul-



TABLE 1. Characteristics of *Phialemonium* species

Isolate	Morphology			Growth temp (°C)
	Colony	Conidia	Conidiophores	
Case 1	White, becoming yellow with production of a diffusible yellow pigment; later becoming gray (a vinaceous pigment was produced under some conditions)	Cylindrical to allantoid, 3.0–8.0 by 1.0–2.0 $\mu\text{m}$ ; no chlamydoconidia	Phialides, often with distinct collarettes; pegs 1.8–4.4 $\mu\text{m}$ long by 1.5 $\mu\text{m}$ wide; discrete phialides 4.5–29 $\mu\text{m}$ long by 1.5 $\mu\text{m}$ wide	15–37
<i>P. curvatum</i>	White, becoming yellow or grayish	Uniformly cylindrical to allantoid, 3.5–6.0 by 1.0–1.4 $\mu\text{m}$ ; no chlamydoconidia	Phialides, usually without collarettes; pegs 1–7 $\mu\text{m}$ long by 0.5–1.0 $\mu\text{m}$ wide; discrete phialides up to 12–22 $\mu\text{m}$ long by 1.5–2.0 $\mu\text{m}$ wide	10–36
<i>P. dimorphosporum</i>	White, becoming cream-colored and pale vinaceous buff on reverse and near margin	Allantoid or ellipsoidal to obovate, 4.0–5.5 by 1.0–1.5 $\mu\text{m}$ ; rare swollen cells in old cultures	Phialides, usually without collarettes; pegs 2.0–9.0 $\mu\text{m}$ long by 0.6–1.0 $\mu\text{m}$ wide; discrete phialides, 10.0–30.0 $\mu\text{m}$ 1.5 $\mu\text{m}$ wide	10–34
<i>P. obovatum</i>	White, becoming pale yellow or greenish with production of a green diffusible pigment; later becoming black at center	Obovate, consistently straight, with an apiculate and minutely truncated base, 3.5–6.0 by 1.2–1.7 $\mu\text{m}$ ; chlamydoconidia in very old cultures	Phialides, usually without collarettes; pegs, 1.0–9.0 $\mu\text{m}$ long by 0.5–1.0 $\mu\text{m}$ wide; discrete phialides up to 15 $\mu\text{m}$ long by 1.0–2.0 $\mu\text{m}$ wide	15–40

ture, there was considerable variation in the morphology of both the conidiogenous cells and the conidial shape. Our observations illustrate that a continuum exists for our strain, *P. curvatum*, and *P. dimorphosporum*. Therefore, this group may best be viewed as a complex.

The variability seen with these fungi on different media and at different temperatures complicates the taxonomy of the pleomorphic fungi. Two suggestions are offered for improvement. The first is to determine a set of standardized growth conditions to stabilize the morphology for standard taxonomic descriptions. This should include a specific formulation of sporulation media for inoculation and incubation under specified conditions of temperature and duration. The use of PDA and cultures incubated at 24 to 25°C for 2 weeks is a reasonable starting point towards developing standardized growth conditions for these and similar fungi. The second is the use of molecular based comparisons of strains followed by correlation to the morphological determinants produced from the above. In this way, it should be possible to define stable morphological indicators of genetically related entities. The fundamental taxonomic question is then raised as to whether a fungal species should be based upon purely morphological determinants, purely molecular based methods, such as similarities in DNA sequences, or a best fit comparison of the two. This complex, with its inherent phenotypic variation, could be used as a model to address this issue. Studies directed at determining relatedness through similarities in DNA sequences from isolates of this complex are planned. We feel it prudent to classify the case 1 strain as a member of a *P. curvatum*-*P. dimorphosporum* complex; however, weighing the present evidence, this isolate more closely resembles *P. curvatum*.

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