

1                   **Cutaneous Phaeohyphomycosis caused by *Paraconiothyrium cyclothyrioides***

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43 **Abstract**

44 *Paraconiothyrium cyclothyrioides* is a recently-described coelomycetous fungal species. We  
45 present a case in a renal transplant patient with chronic skin lesions of the lower extremities  
46 caused by *P. cyclothyrioides*. Treatment with posaconazole led to complete resolution of the  
47 lesions. *P. cyclothyrioides* should be considered as an opportunistic human pathogen in  
48 immunocompromised patients.

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**Case Report**

51 A 49-year-old Latin American man with end stage renal disease and cadaveric renal transplant  
52 (two years prior to the current admission) was hospitalized with cutaneous lesions in his lower  
53 extremities. The patient had history of type II diabetes, hypertension and atrial fibrillation. One  
54 and half years previous to admission, the patient received thymoglobulin for acute T-cell  
55 rejection with improvement of the episode. Approximately 6 months before current admission,  
56 the patient first noticed a painless, non-pruritic, mildly tender scaly papule on the left tibia just  
57 inferior to the knee joint and applied a local adhesive band to prevent friction injury. Over the  
58 next few months, other lesions appeared on both tibial surfaces, which then began to coalesce  
59 into violaceous, necrotic, plaque-like lesions with some areas of ulceration and crusting and mild  
60 self-limiting sanguineous discharge without accompanying fevers. The patient lives in  
61 Brownsville, TX , works in an office as a manager and denied any travel outside Texas, trauma,  
62 animal or insect bites. Patient's hobby included performing mechanical work on car engines.  
63 Medications included leflunomide, prednisone, tacrolimus, doxazosin, clonidine, carvedilol,  
64 minoxidil, amlodipine, trimethoprim-sulfamethoxazole, insulin, famotidine, gemfibrozil and  
65 pravastatin. Complete blood count was notable for platelet count of 73 and creatinine of 2.4

66 mg/dL. On physical exam he was afebrile and vital signs were stable. Cardiac exam evidenced  
67 an irregular rate and a II/VI murmur loudest in the apex. The lungs were clear and the rest of the  
68 examination was unremarkable except for the lower extremities. He had a crusted, ulcerated  
69 plaque on the anteromedial aspect of the left knee (measuring 6 x 3 cm), and several similar-  
70 appearing lesions of smaller size on the same area (**Figure 1A**). The patient was started on  
71 vancomycin (1.25 gm every 24 h), radiographs of the knee did not reveal any bone abnormality.  
72 A skin biopsy yielded a presumptive diagnosis of pyoderma gangrenosum, antibiotics were  
73 stopped and the patient was discharged with wound care instructions and silver sulfadiazine.

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75 Two months prior to the current admission a fungal culture from the skin tissue, was positive for  
76 a single mold which grew on Sab Dex agar but was not identified at the time. Oral voriconazole  
77 (200 mg orally every 12 h) was initiated. A month later after his discharge, he presented to the  
78 dermatology clinic with evidence of progression of his skin lesions and was again admitted to the  
79 hospital. He denied new recent trauma, travel history and reported compliance with  
80 voriconazole. New punch biopsies revealed pseudo-epitheliomatous hyperplasia, suppurative  
81 granulomas and fungal elements were seen invading the dermis (**Figure 1B**). Cultures yielded a  
82 mold (only one colony type) with the same morphology of the previous culture in Sab Dex agar,  
83 that was subsequently identified as *Paraconiothyrium cyclothyrioides* (see below). The patient  
84 was started on amphotericin B lipid complex, 400 mg IV every 24 h and micafungin 100 mg IV  
85 every 24 h. Due to the elevation in the creatinine level, amphotericin B was stopped and  
86 posaconazole (400 mg orally every 12 h with meals) was initiated. Micafungin was discontinued.  
87 The skin lesions markedly improved 10 days after initiation of posaconazole therapy. Follow up  
88 at three weeks after hospital discharge showed complete resolution of the skin lesions with only

89 some remaining scaling of the skin in the area. Posaconazole was continued for an additional  
90 four weeks.

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92 Extensive morphological and molecular characterization of the isolate was required to obtain a  
93 definitive identification. Accessioned into the Fungus Testing Laboratory culture collection as  
94 UTHSC 11-2294, the isolate was subcultured onto potato flakes agar (PFA), V-8 agar, and  
95 carnation leaf agar (CLA), all prepared in-house, at 25°C. A photomicrograph of the  
96 macroscopic morphology on PFA after 6 days incubation at 30°C shows a woolly, olivaceous-  
97 gray colony with a white periphery (**Figure 2A**). A brown diffusing pigment developed after  
98 three weeks incubation at 25 °C . Temperature studies on PFA indicated optimal growth at 27°C  
99 and minimal growth (1+) at 35°C. After 4 weeks incubation on CLA at 25°C thick-walled,  
100 submerged, reddish-brown conidiomata were present on CLA (**Figure 2B**). The hyaline, discrete  
101 or integrated, phialidic conidiogenous cells (**Figure 2C**) producing brown, cylindrical, 1-celled  
102 conidia measuring approximately 1.5 x 3-4 µm (**Figure 2D**) were suggestive of taxa known in  
103 the pycnidial coelomycete genera *Microsphaeropsis/Paraconiothyrium/Coniothyrium*. In order  
104 to accurately identify the isolate, molecular characterization by DNA sequencing was performed  
105 and the sequence was deposited under accession number R-4779 at University of Texas Health  
106 Science Center Fungus Testing laboratory culture collection. Briefly, DNA was recovered from a  
107 24 hour culture grown on potato dextrose agar (Difco, Detroit, MI) and amplified by PCR using  
108 the ITS1 and NL4 primers as previously described (2). The amplicon was sequenced in the  
109 UTHSCSA Advanced Nucleic Acids Core facility to yield ITS and D1/D2 sequences (2). A  
110 BLASTn search of GenBank was performed using the ITS sequence and cutoff values of  $\geq 97\%$   
111 identity at a query length  $\geq 90\%$  were used for consideration of conspecificity. The results of this

112 search identified *Microsphaeropsis arundinis* (accession # JN851034.1; 99% identity over 551  
113 nucleotides), *Bipolaris sorokiniana* (accession# DQ337383.1 99% identity over 541 nucleotides)  
114 and *Paraconiothyrium cyclothyrioides* (accession# AY642529.1, 99% identity over 505  
115 nucleotides). The D1/D2 sequence was not informative because there is no Genbank deposit for  
116 *Paraconiothyrium cyclothyrioides*. As ITS and D1/D2 sequencing failed to discriminate between  
117 *P. cyclothyrioides* and *M. arundinis*, both of which showed 99% nucleotide identity to the case  
118 isolate, our sequence data was forwarded to the Centraalbureau voor Schimmelcultures (CBS)  
119 for further analysis by G.J.M. Verkley and J.B. Stielow and subsequent comparison with their  
120 nucleotide sequence database of known isolates (3). With additional sequencing of  $\gamma$ -actin and  $\beta$ -  
121 tubulin loci (primers ACT512f/ACT783r and Btub4rd/Btub4fd , respectively) (3), the sequence  
122 identity of R-4779 with the type strain of *P. cyclothyrioides* (CBS 972.95) was 100%, 97%, and  
123 99.1% for ITS, actin, and  $\beta$ -tubulin, respectively; suggesting that the case isolate is *P.*  
124 *cyclothyrioides*. The ITS and D1/D2 sequences were deposited in GenBank under accession  
125 numbers JQ681303 and JQ681304, respectively. The case isolate has also been deposited in the  
126 University of Alberta Microfungus Collection and Herbarium under the accession number  
127 UAMH 11641.  
128  
129 Coelomycetous fungi are common, anamorphic (asexual), plant and soil organisms that produce  
130 conidia within some type of -closed (pycnidial) or semi--closed (acervular) structure.  
131 The coelomycete genus *Paraconiothyrium* was proposed to accommodate species resembling  
132 those in *Microsphaeropsis* and *Coniothyrium* that do not match species in these genera  
133 morphologically and phylogenetically (2). Due to the variability and plasticity in morphological  
134 characters, especially in culture, it is difficult to distinguish members of these genera

135 morphologically, but generally *Coniothyrium* and *Microsphaeropsis* mostly include species with  
136 true pycnidia (conidiomata with an opening) while most species of *Paraconiothyrium* form  
137 more complex conidiomata lacking a distinct ostiole. *Coniothyrium* spp. exhibit annellidic  
138 conidiogenesis, while those of the other two genera mostly show phialidic conidiogenesis,  
139 sometimes with percurrent proliferations. The species thus far included in *Paraconiothyrium*  
140 were formally described based on their anamorph or asexual reproductive stage in vitro and  
141 currently include *P. cyclothyrioides*, first isolated from a soil sample collected in Papua New  
142 Guinea in 1995, *P. estuarinum*, *P. brasiliense* and *P. fungicola* (3). Molecular phylogenetic data  
143 places all three genera within the order Pleosporales and resolves them as three distinct clades  
144 (3).

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146 To the best of our knowledge, this is the second reported case of infection with  
147 *Paraconiothyrium cyclothyrioides*. We present unequivocal evidence that the organism was the  
148 pathogen in this case since it invaded the skin of the patient and lesions resolved with appropriate  
149 antifungal therapy. Balajee et al. (1) described a case in which a three-year-old child presented  
150 with crusted nodules on the lower extremity one year after receiving a liver transplant. ITS  
151 sequencing and comparison with a large coelomycete database at the United States Department  
152 of Agriculture (accessible at <http://nt.ars-grin.gov/sbmlweb/fungi/index.cfm>) identified the  
153 isolate as *P. cyclothyrioides*. Localization of the lesions (lower extremities) was very similar to  
154 our case but no information was provided in terms of the clinical evolution or treatment of the  
155 infection (1). Interestingly, a fungus isolated from a skin lesion of a 56 year old  
156 immunocompromised patient was recently identified as *Paraconiothyrium maculiculis*, (4) a

157 close relative of *P. cyclothyrioides*, supporting the fact that *Paraconiothyrium* spp. are potential  
158 human pathogens.

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160 Our case highlights the importance of obtaining tissue samples for culture when  
161 immunocompromised patients present with chronic indolent skin lesions since the differential  
162 diagnosis is broad and includes fungi, mycobacteria and *Nocardia* spp., among others. In fact,  
163 several biopsies were taken during the course of this infection which increased the likelihood of  
164 isolating the etiologic agent. In our case, although susceptibilities were not performed, the  
165 clinical course of our patient suggest that *P. cyclothyrioides* has important differences in  
166 susceptibility to azole antifungals; indeed, our patient did not respond to voriconazole but  
167 showed a remarkable improvement when switched to posaconazole with resolution of the  
168 lesions. We were unable to evaluate the response to amphotericin B since this compound had to  
169 be stopped due to renal toxicity.

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171 In summary, the rare soil fungus *P. cyclothyrioides* has pathogenic potential in chronic, crusted  
172 and ulcerated skin lesions in transplant patients and should be considered in the differential  
173 diagnosis of invasive fungal infections in immunocompromised patients. The most accurate  
174 identification among *Coniothyrium* like fungi and allied genera can be achieved by  $\beta$ -tubulin  
175 sequencing using the suggested primer pair. Posaconazole appears to be effective in treating  
176 these infections.

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204 **Legends to Figures**

205

206 **Figure 1. Panel A**, Crusted, ulcerated skin lesions of the left knee; **Panel B**, Periodic acid-schiff  
207 (PAS) stain showing fungal elements (hyphae, black arrows) invading the dermis (60 X  
208 magnification).

209 **Figure 2. Macroscopic and microscopic features (lactophenol cotton blue mounting**  
210 **medium) of case isolate of *Paraconiothyrium cyclothyrioides*. Panel A**, Colony after 6 days  
211 incubation at 30°C on potato flakes agar; **Panel B**, thick-walled conidioma showing conidia that  
212 are reddish-brown, in mass, within the cavity; **Panel C**, Phialidic conidiogenous cells protruding  
213 into the cavity of the conidioma; **Panel D**, Small, 1-celled, brownish cylindrical conidia.

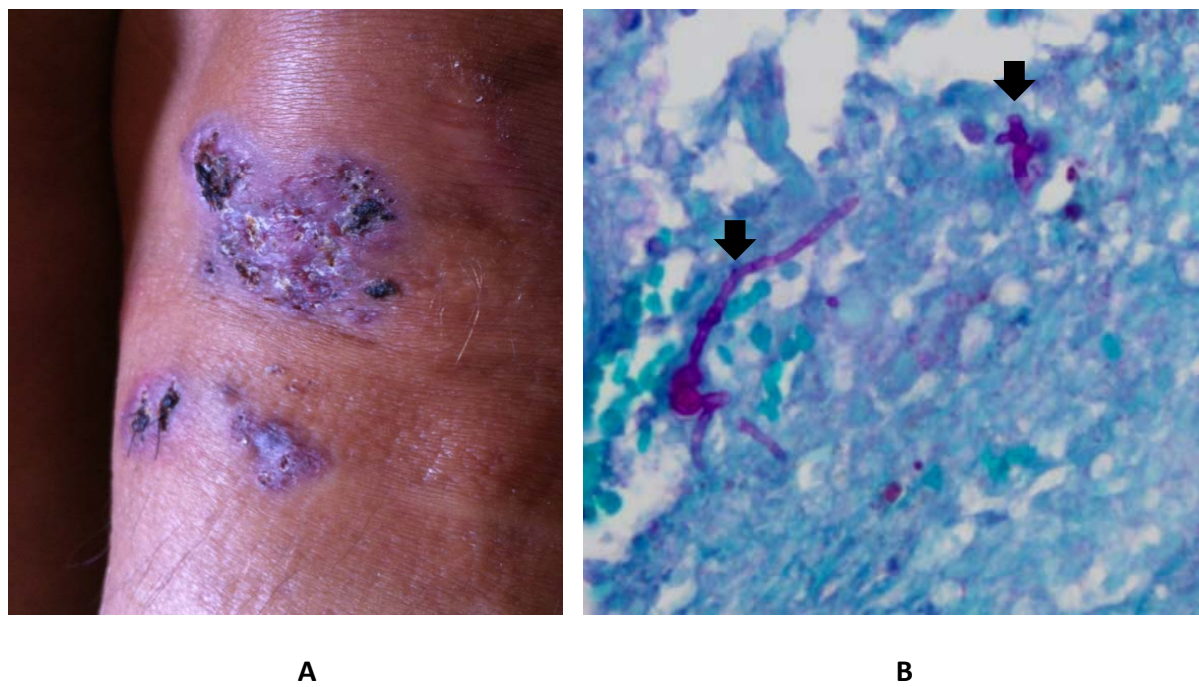
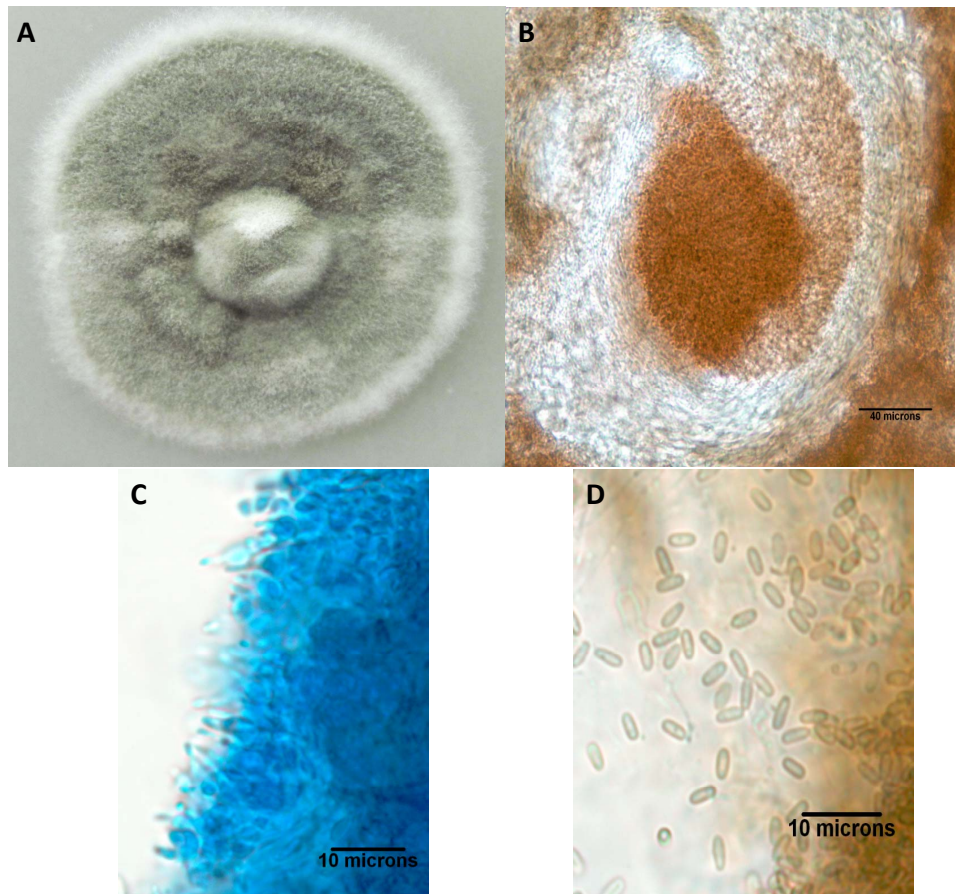


Fig. 1

Figure 1. **Panel A**, Crusted, ulcerated skin lesions of the left knee; **Panel B**, Periodic acid-schiff (PAS) stain showing fungal elements (hyphae, black arrows) invading the dermis (60 X magnification).



**Figure 2.** Macroscopic and microscopic features (lactophenol cotton blue mounting medium) of case isolate of *Paraconiothyrium cyclothyrioides*. **Panel A**, colony after 6 days incubation at 30°C on potato flakes agar; **Panel B**, thick-walled conidioma showing conidia that are reddish-brown, in mass, within the cavity; **Panel C**, phialidic conidiogenous cells protruding into the cavity of the conidioma; **Panel D**, small, 1-celled, brownish cylindrical conidia.