Clinical, morphological, and molecular characterization of *Penicillium canis*, sp. nov., isolated from a dog with osteomyelitis

Running title: Characterization of *Penicillium canis*, sp nov.

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Abstract:

Infections caused by *Penicillium* spp. are rare in dogs, and the prognosis in these cases is poor. An unknown species of *Penicillium* was isolated from a bone lesion in a young dog with osteomyelitis of the right ilium. Extensive diagnostic evaluation did not reveal evidence of dissemination. Resolution of lameness and clinically stable disease were achieved with intravenous phospholipid complexed amphotericin B initially, followed by long-term combination therapy with terbinafine and ketoconazole. A detailed morphological and molecular characterization of the mold was undertaken. Sequence analysis of the internal transcribed spacer revealed the isolate to be closely related to *Penicillium menonorum* and *Penicillium pimiteouïense*. Additional sequence analysis of Beta-tubulin, calmodulin, mini-chromosome maintenance factor, DNA dependent RNA polymerase, and pre-rRNA processing protein revealed the isolate to be a novel species; the name *Penicillium canis*, sp. nov., is proposed. Morphologically, smooth ovoid conidia, greenish-gray colony color, slow growth on all media, and failure to form ascomata distinguish this species from closely related *Penicillium* spp. The type strain (NRRL 62798) has been deposited in the culture collection (UTHSC DI13-196) at the University of Texas Health Science Center Fungus Testing Laboratory.
Introduction:  

*Penicillium* is a genus of ubiquitous saprobic fungi with over 300 known species, but few are recognized as pathogens in dogs or people (1, 2). *P. marneffei*, the only dimorphic member of the genus, is an emerging pathogen in immune-compromised humans, and infections also have been reported in immune-competent patients (3, 4). Dogs are potential reservoirs for *P. marneffei* in some regions (5). Infections caused by other species are exceedingly rare in humans.

Opportunistic filamentous fungal infections are infrequently reported in the dog (2, 6-12). Clinical manifestations often include some combination of bone or back pain, respiratory disease, neurologic abnormalities, renal disease, hepatic disease, draining wounds, and uveitis. Cases incited by *Geosmithia argillacea*, subsequently characterized in the genus *Rasamsonia* and reidentified by Houbraken *et al.* as *R. piperina* (13, 14), *Paecilomyces* spp., *Phialosimplex* spp., *Aspergillus terreus*, and other unclassified species in the genera *Penicillium* and *Aspergillus* have been reported, with infections caused by aspergilli being the most common (2, 6-12, 15-18). The exact species is questionable in some reports as isolates were identified on the basis of morphologic features without concurrent molecular sequencing (2, 6-9). The majority of infections were reported in German Shepherd Dogs (GSD), a breed presumed to have a hereditary immunologic defect (2, 7, 11, 12, 18, 19). Both systemic disease and osteomyelitis due to *Penicillium* spp. are rare in dogs. *P. verruculosum*, *P. purpurogenum*, and several penicillia not identified to the species level have been reported in dogs with fungal osteomyelitis (2, 6, 7, 10-12). The prognosis for these cases is poor, with most dogs succumbing to disease or undergoing euthanasia shortly after diagnosis (2, 7, 9-12). Although clinically stable disease during aggressive combination antifungal therapy has been rarely reported, cures are unlikely...
Newer antifungals have improved response rates in humans with opportunistic fungal infections; however, limited species-specific information and great expense have limited their use in veterinary medicine (20).

This report documents the clinicopathologic findings, treatment, and outcome of a young Rhodesian Ridgeback dog with fungal osteomyelitis caused by a novel _Penicillium_ species, _P. canis_. The morphological and molecular sequencing features of _Penicillium canis_ are characterized in this report.

**Case Report:**

A 3-year-old, 44 kg female spayed Rhodesian Ridgeback dog was referred to the Michigan State University Veterinary Teaching Hospital (MSU-VTH) for further evaluation of a painful, proliferative bony lesion on the right ilium causing clinically apparent lameness (day 0). The onset of lameness was approximately two weeks prior to evaluation at MSU-VTH, and the patient was otherwise normal. Empiric therapy with carprofen (100 mg per os [PO] every twelve hours [q12h]) and tramadol (100 mg PO q8-12h) had been initiated for pain control prior to evaluation. On general physical examination, the dog had significant toe touching lameness of the right hind limb. Palpation of the right ilium elicited severe pain and vocalization.

Initial diagnostic testing included a complete blood count, serum biochemical profile, and urinalysis performed at the Michigan State University Diagnostic Center for Population and Animal Health (MSU DCPAH), Clinical Pathology Laboratory. Hematologic evaluation revealed a leukocytosis (14.6 × 10⁶/μL; reference range, 6.1-12.0 × 10⁶/μL) characterized by a marked lymphocytosis (6.8 × 10³/μL; reference range, 1.1-3.1 × 10³/μL) with a normal neutrophil concentration. Serum biochemical and urinalysis abnormalities included mild hypoalbuminemia (2.6 g/dL; reference range, 2.8-4.0 g/dL), hyperglobulinemia (4.6 g/dL;...
reference range, 2.2-4.1 g/dL), and proteinuria. Three view thoracic radiographs did not reveal any evidence of pathology. Pelvic radiographs revealed a proliferative bony lesion of the right ilium (Fig. 1A) similar to radiographs obtained by the referring veterinarian. Fine needle aspirates of the lesion revealed marked neutrophilic and moderate macrophagic inflammation; fungal culture was recommended. A blastomyces urine antigen test (MiraVista Diagnostics Laboratory, Indianapolis, IL) was negative. Results of flow cytometric evaluation and PCR clonality testing for antigen receptor rearrangements to further characterize the lymphocytosis were supportive for a heterogenous and reactive lymphoid population.

Seven days after initial evaluation, a computed tomography scan and guided biopsies were performed. The CT revealed a mixed proliferative and lytic lesion predominantly involving the right ilial body, but also involving the ventral aspect of the adjacent sacrum. A whole body bone scan was performed using intravenously administered Technetium 99m-MDP to further evaluate the dog for systemic involvement. In addition to the known iliac lesion, there was focal uptake at the left fourth costochondral junction. The costochondral region was fine needle aspirated, but samples were hemodiluted and lacked cytologic evidence of disease. Four core biopsies of the ilium were obtained using a trephine bone biopsy needle. Ultrasound guided fine needle regional lymph node (sub-lumbar) aspirates were obtained. Biopsy samples were submitted to MSU DCPAH Microbiology and Anatomic Pathology for microbial culture and histopathologic evaluation, respectively. Biopsy samples were routinely prepared and examined via light microscopy (Fig. 2). There were myriad non-pigmented septate fungal hyphae; 3-4 μm in width and 7-40 μm in length with non-parallel walls, and occasional 45° angle branching throughout all sections of bone. Cytologic examination of lymph node samples revealed reactive lymphoid hyperplasia with no evidence of fungal hyphae. Routine aerobic and anaerobic
bacteriologic cultures were negative for growth. However, mycologic cultures incubated on Sabouraud dextrose (SDA) (Remel, Lenexa, KS) and Mycosel™ (Becton, Dickinson, and Company, Sparks, MD) agars at 25C and 35C resulted in heavy growth of a non-pigmented mold with white/brown colony morphology (surface/reverse) within 4-5 days of initial incubation. The hyphae were septate and had branched “Penicillium-like” conidiophores. No other agents were isolated. The conidiogenous cells appeared to be annellated. Although the morphologic features resembled those of a moniliaceous (hyaline) hyphomycete, in particular, a *Scopulariopsis* sp., definitive identification was not possible. Partial 28S rDNA sequence of DNA extracted from the sample showed the organism belong to the genus *Penicillium*. Given the uncertain identity, the isolate from the SDA culture was submitted to the Fungus Testing Laboratory at the University of Texas Health Science Center in San Antonio for molecular characterization, identification, and *in vitro* antifungal susceptibility testing. The isolate was accessioned into their culture collection as UTHSC DI13-196. Antifungal susceptibility testing was performed by broth microdilution according to CLSI guidelines for filamentous fungi (M38-A2) (21). Minimum inhibitory concentrations (MIC) were determined for amphotericin B, fluconazole, itraconazole, posaconazole, voriconazole, ketoconazole, and terbinafine as the lowest concentration of each agent that resulted in 100% inhibition of growth compared to the growth control after 48 hours of incubation. Posaconazole and terbinafine had the most potent *in vitro* activity (MIC 0.06 μg/mL for each), followed by amphotericin B, itraconazole, ketoconazole (0.125 μg/mL for each), and voriconazole (0.25 μg/mL), while fluconazole was the least active of the agents tested (64 μg/mL). The DNA was isolated, and the internal transcribed spacer (ITS), beta-tubulin, and calmodulin loci were amplified and sequenced (22-24). Sequence analysis of ITS results showed the highest matches with *Penicillium menonorum* (Genbank
Accession No. HQ646591, 97.6% identity) and Penicillium pimiteouiense (Genbank Accession No. FJ624254, 97.3% identity) (25). Due to the relatively low sequence match, the isolate was referred to the Bacterial Foodborne Pathogens and Mycology Research Unit, National Center for Agricultural Utilization Research, United States Department of Agriculture for further analysis. The methods used to further characterize the isolate and the results are described below.

Multiple treatment options including aggressive surgical resection followed by medical therapy, oral antifungal therapy using an azole agent, intravenous antifungal therapy with amphotericin B, or a combination of these therapies were discussed with the owners. Given the possibility of systemic disease combined with the challenging and invasive nature of surgical resection (due to sacral involvement), the owners elected aggressive multimodal medical therapy, and carprofen therapy was discontinued (day 19).

On day 23, hematologic and biochemical re-evaluations were similar to initial findings. Therapy was initiated with 75 mg of phospholipid complexed amphotericin B (AMB, Abelcet, Sigma-Tau Pharmaceuticals, Gaithersburg, MD) administered intravenously (I.V.) over 1 hour. A two hour diuresis with 0.9% saline I.V. was administered before and after the AMB infusion. A cumulative dose of 975 mg (23 mg/kg over 4 weeks) was given at a frequency of 75 mg three times per week. During the second week of AMB therapy, combination oral antifungal therapy consisting of ketoconazole (1 g/day) and terbinafine (1 g/day) was initiated. Therapy with posaconazole was recommended, but declined due to financial restrictions. Therapy with a combination product of SAM-e and silymarin (Denamarin®, Nutramax Laboratories, Lancaster, SC) also was initiated to minimize potential hepatotoxicity of high dose ketoconazole. Upon cessation of AMB therapy (day 51), the dog was clinically sound and non-painful. Physical examination findings and owner-reported activity levels were normal.
During the second month of ketoconazole and terbinafine therapy, the dog developed diffuse, progressive hyperpigmentation and truncal alopecia. Skin cytology samples were obtained and reported to show no cytologic abnormalities. Therapy with oral cephalexin (100 mg q12h) and topical chlorhexidine shampoo failed to improve the pigmentation changes and alopecia. An adverse drug reaction was considered, but was not pursued as dose reductions or drug withdrawal could have led to relapse and progression of clinical disease. Aside from dermatologic changes, the dog remained normal.

On day 83, repeat pelvic radiographs documented remodeling of the right ilial lesion with overall improvement (Fig. 1B). Periodic re-evaluations were performed at MSU-VTH over the subsequent 8 months. Although serum globulin concentrations returned to normal, only a mild improvement in reactive lymphocytosis ($5.8 \times 10^3/\mu L$; reference range, $1.1-3.1 \times 10^3/\mu L$) was observed. Repeat pelvic radiographs on day 150 revealed further mild improvements of the right ilial lesion. The ketoconazole dose was reduced to 800 mg/day while the terbinafine dose remained unchanged. At the time of manuscript submission (day 250), the dog was continuing to receive ketoconazole and terbinafine and was clinically normal. Both drugs were continued for long-term maintenance with no intent to modify therapy unless side effects or disease progression were to be observed.

Materials and Methods:

Cultures used in this study and their corresponding GenBank deposit numbers are listed in Table 1 (supplemental material). *Penicillium canis* z-384 was isolated and sequenced in 2008, but not characterized (S. W. Peterson, unpublished data), and the culture died during the intervening period.
Isolates were grown on Czapek’s yeast autolysate agar (CYA, 26), Blakeslee’s malt extract agar (MEA, 26), CYA amended with 5% NaCl, CYA amended with 20% sucrose (26), and mixed grain baby food agar (MGA) composed of 50 g Gerber mixed grain baby cereal and 15 g agar in 1 L deionized water. All media were prepared in-house. Nine cm diameter Petri plates were inoculated at three points on each plate for each medium and incubated at 25°C. CYA cultures also were prepared and incubated at 5 and 37°C for 7 days in darkness. Mature colonies were photographed with a digital camera and, bits of mycelium were teased apart in a drop of 1% Triton X-100 for microscopy and digital photography. Images were cropped, sized and positioned into plates using Adobe Photoshop LE ver. 10.

Cultures were grown for 2–3 days on ME broth in gently agitated flasks to accumulate 1–2 g biomass. Mycelia were harvested by vacuum filtration over Whatman #1 paper, placed in micro centrifuge tubes, frozen, and freeze-dried. Dry mycelium was ground to powder and rehydrated using CTAB buffer, proteins were extracted with chloroform, and nucleic acids were precipitated using isopropanol (27).

Beta-tubulin (BT2), calmodulin (CF), nuclear internal transcribed spacer region (ITS), mini-chromosome maintenance factor (Mcm7), DNA dependent RNA polymerase (RPB2) and pre-rRNA processing protein (Tsr1) were amplified using the primers and conditions specified by Peterson and Jurjevic (27). Amplified DNA was prepared for sequencing using ExoSapit (Affymetrix), sequencing reactions were carried out using BigDye ver. 3.2 (Applied Biosystems), and sequences were read on an ABI 3730 genetic analyzer (Applied Biosystems). Sequencing was bidirectional. Raw sequences were compared and corrected using Sequencher 5.1 (Gene Codes).
Sequences were aligned using CLUSTALW or MUSCLE, as implemented in MEGA 5.2 (28). Data sets containing sequences from each single locus were aligned and analyzed under MEGA5.2 using the maximum likelihood estimation with the GTR+G+I model, as suggested by MODELTEST (29). Bootstrapping was performed using 500 iterations of the data and the criterion detailed above. The trees were formatted for publication using CorelDraw.

Results:

The maximum likelihood trees (Fig. 3) were based on, 442 aligned BT2 nucleotides, 750 aligned CF nucleotides, 565 aligned ITS nucleotides, 616 aligned Mcm7 nucleotides, 1014 aligned RPB2 nucleotides, and 817 aligned Tsr1 nucleotides. Initial sequence analysis used *Penicillium lassenii* as the out-group (30). In the tree, *P. canis* is basal among the examined species and sister group to *P. erubescens*, although with a low bootstrap proportion. The same isolates formed terminal groups at each locus (Fig. 3), but the relative positions of the taxa in the tree differed, with low bootstrap support for some of the branches. In the ITS barcode analysis, *P. parvum* and *P. pimiteouiense* are indistinguishable from each other, but other species in this clade can be identified.

Taxonomy

*Penicillium canis* sp. nov. S. W. Peterson (Fig. 4)

Mycobank number: MB 807056

*Etymology*: canis refers to isolation of the fungus from the ilium of a dog.

*Holotype*: BPI 892763, a dried colony of NRRL 62798 grown for 10 days on mixed-grain baby food agar. Culture was isolated from an ilial bone lesion on a Rhodesian Ridgeback dog, residing in Ann Arbor, Michigan on 3 April 2013 by Daniel K. Langlois.

**Phenotypic diagnosis:** The smooth ovoid conidia along with greenish-gray colony color and slow growth on all media distinguish this species from *P. pimiteouiense* and *P. menonorum*.

**Description:** Colonies on CYA attained 6–7 mm diameter after 7 days incubation at 25°C, were white, forming a 2 mm high cushion of loose vegetative hyphae. Sporulation was sparse and basal, with no exudate, soluble pigments, sclerotia, or ascomata. Reverse was yellowish near chamois color. Colonies on MEA attained 7–8 mm diameter after 7 days incubation at 25°C, were white, and formed a 2–3 mm raised cushion of largely vegetative hyphae. Sporulation was sparse, with no exudate or soluble pigments, no sclerotia or ascomata, and reverse was a pale drab. Colonies on MG agar incubated 7 days at 25°C attained 5–6 mm diameter, formed a 2–3 mm raised cushion, sporulated heavily in greenish-gray color, with no exudate, soluble pigments, sclerotia or ascomata. Colony reverse was not visible on this medium. There was no growth on CYA at 5°C; at 37°C small white 2–3 mm diameter colonies were formed after 7 days. Growth at 25°C on CYA-5% NaCl and CYA-20% sucrose was 2 mm and 5 mm diameter, respectively.

Conidiophores were simple, arising from basal and aerial hyphae, smooth-walled, hyaline, 5–25 × 1.5–2.5 µm, non-vesiculate, bearing an apical whorl of 2–5 ampuliform phialides 5–7 (10) × 2–3 µm with a 1–2 µm collula, bearing ovoid smooth walled conidia 4–5 × 2–3 µm.

Phenotypically *P. canis* resembles *P. pimiteouiense* and *P. menonorum* at the microscopic level with these species all possessing short, unbranched, non-vesiculate conidiophores with a whorl of 2–5 phialides. Conidia of *P. pimiteouiense* are spherical and...
finely roughened, and conidia of *P. menonorum* are spherical to subspherical and rugose, while conidia of *P. canis* are elongate-ovoid and smooth. Colony diameters on CYA at 25°C after 7 d are 5–6 mm for *P. canis*, 17–18 mm for *P. pimiteouiense*, and 17–20 mm for *P. menonorum*, and conidial colors en masse are greenish-gray, olive-gray and bluish-gray respectively. *P. canis* produces CYA colonies of 2–3 mm diameter when grown 7 d at 37°C, while *P. pimiteouiense* and *P. menonorum* colony diameters are 20–21 mm and 29–32 mm, respectively. *P. canis* is easily distinguished from *P. erubescens*, *P. parvum* and *P. rubidurum* by its failure to form ascomata as the latter three species do. *Penicillium vinaceum* produces vinaceous purple pigments in the growth agar which is quite distinct from the appearance of *P. canis*. *Penicillium dimorphosporum* produces conidia with two quite distinct phenotypes aiding differentiation from *P. canis*.

**Discussion:**

Similar to the present case, lameness and pain are common signs for dogs with fungal osteomyelitis and typically the primary reason for evaluation (10, 11). However, most dogs with osteomyelitis caused by opportunistic molds have evidence of disseminated disease and often present with additional clinical signs including anorexia, weight loss, pyrexia, lameness, back pain, lymphadenomegaly, ocular disease, epistaxis, and neurologic signs (2, 10, 16, 18, 19, 31, 32). Rapid dissemination commonly occurs and the short term prognosis is grave. Reported cases of infections by *Penicillium* spp. have involved the appendicular and axial skeletal systems, liver, spleen, kidney, pancreas, heart, lungs and lymph nodes (2, 7, 11, 12). Although a thorough systemic evaluation was performed in the dog of our report (radiographs, ultrasound, CT scan, bone scan, and hematologic evaluations), only evidence of osteomyelitis was found. Whether the costochondral junction lesion observed on bone scan represented fungal disease, or
another lesion such as a healing fracture, was unknown. Radiopharmaceutical uptake was modest
and cytological examination of aspirates was inconclusive.

The possibility of sample contamination must be considered for fungal cultures obtained
on a single occasion because penicillia are common laboratory contaminants. Fungal hyphae
clearly documented within histopathological lesions from the present case had morphological
features similar to cultures of these samples, and bacterial cultures were negative. Fungal
cultures and preliminary molecular characterization performed at the initial institution resulted in
growth of a solitary identical fungus on multiple plates that belonged to the genus *Penicillium*.
Original material and subsamples thereof exhibited pure growth of the same single fungus at all
three laboratories. Moreover, contamination of multiple plates with the same previously
uncharacterized fungus is highly improbable. In fact, of over 500 fungal cultures performed at
the initial laboratory in the year 2013, only 5 resulted in growth of a *Penicillium* spp. and none
were identified within 4 months of the isolate in our report. Given the above evidence, the
authors believe that *Penicillium canis*, sp. nov., was the cause of osteomyelitis in the dog
reported herein.

*Penicillium* is a genus of ascomycetous fungi that are saprobic, filamentous, and typically
monomorphic. *Penicillium* spp. have septate hyphae (2–5 µm diam) that give rise to branched or
un-branched conidiophores with secondary branches that give *Penicillium* a brush-like
appearance (2, 33). *Scopulariopsis* spp. also have septate hyphae with either single unbranched
conidiophores or branched “penicillus-like” conidiophores and colony morphology can be
similar. Although *Scopulariopsis* spp. may resemble *Penicillium* spp., they generally have
shorter and sometimes simpler conidiophores. In addition, their conidia-bearing (conidiogenous)
cells are annellidic rather than phialidic (34). Based on morphology alone, the isolate was
originally thought to be a Scopulariopsis sp. However, initial sequencing of ITS revealed the isolate reported herein to be a novel Penicillium species closely related to, but not conspecific with P. menonorum and P. pimiteouiense (25). These sequencing results revealed only 76% similarity with a Scopulariopsis sp. As definitive species identification was not possible using the above target, the isolate was subsequently referred to and characterized as a new species by one of us (SWP).

Fungal systematics most commonly involves phylogenetic species concepts (35) and analysis of multiple loci in order to apply the genealogical concordance test of species boundaries (36). In this case the five protein coding loci are in complete agreement (Fig. 3) about the strongly supported terminal grouping of the isolates leading to clear identification of species including Penicillium canis. The multilocus analysis validates the species concept and allows confident use of the barcode locus (ITS) in future investigations. These findings emphasize the importance of using the genealogical concordance phylogenetic species concept to define species and understand intraspecific ITS variability and thus barcode species identification. The ITS barcode sequence analysis can point out potential cryptic species even when a presumed morphologic diagnosis is possible.

The pathogenic potential of the newly discovered Penicillium canis is unknown. Overall, Penicillium spp. are rare opportunists affecting individuals with compromised immunologic function (1). However, there is recent evidence that some species may be more pathogenic than others. P marneffei is a well-recognized emerging public health concern, endemic in southeastern Asia (3). It is considered a disease defining illness in HIV infected patients with mortality rates as high as 50% (4). The isolate in our report caused disease in a seemingly immunocompetent
host. The long term survival of the dog reported herein was surprising compared to previously reported infections in dogs incited by *Penicillium* species.

Antifungal susceptibility testing was performed to guide treatment; however, some reports suggest that *in-vitro* activity does not always correlate with *in-vivo* activity (37, 38).

Nonetheless, these results were used to support therapeutic decision-making. Amphotericin B was chosen as the initial therapeutic agent based on its broad spectrum antifungal activity and *in-vitro* activity as well as its documented efficacy in treating human *P. marneffei* infections (3, 39).

Posaconazole was the recommended treatment of choice to follow AMB therapy, but financial limitations precluded its use. Terbinafine and ketoconazole were chosen as alternatives for long-term maintenance therapy. It is well recognized that combination antifungal therapy has potential synergistic effects (40-43). Furthermore, the MIC for both drugs were suggestive of drug efficacy as concentrations should be readily achieved based on canine pharmacokinetic studies (44, 45). Clinical and radiographic improvements observed in the dog in our report substantiated the efficacy of this multimodal therapy.

It is noteworthy that the dog in the present report was not a GSD as the majority of disseminated opportunistic fungal infections have been reported in this breed, possibly as a heritable immune deficiency (2, 7, 11, 12, 15, 18, 21). Similar to opportunistic fungal infections in non-GSD breeds, the Rhodesian Ridgeback in our report did not have evidence of underlying immune dysfunction and had been healthy prior to the onset of lameness (10). There was no evidence of dissemination during the follow-up period. Nonetheless, lymphocyte proliferation assays and measurement of immunoglobulin levels would be needed to provide more information on the immune competence of this dog.
This report characterizes morphological and histopathological features of a newly discovered species of *Penicillium* with potential to cause disease in otherwise apparently healthy dogs. Identification of future cases will provide additional insight regarding the ecological niche and pathogenic potential of this species. Although the overall prognosis for opportunistic fungal infections in dogs is poor, aggressive multi-modal antifungal therapy provided long-term disease stability in the present case of a dog infected with *Penicillium canis*.

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The authors declare no conflict of interest.
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Figure Legends:

**FIG 1** Ventrodorsal projections of the pelvis at presentation (A) and three months later (B). There is extensive mixed periosteal proliferation and permeative lysis along the right ilial body and wing on initial radiographs, which is smoother and less severe on subsequent images.

**FIG 2** Photomicrographs of sections from the bone biopsy. (A) H&E stain 40×: Bone segments were surrounded by marked granulomatous inflammation and small regions of necrosis. Intrahistiocytic and extracellular non-pigmented fungal organisms were documented throughout the section. Small numbers of lymphocytes, plasma cells, and neutrophils also were present throughout the section. (B) Periodic-acid Schiff stain 40×: Note the large number of organisms within the cytoplasm of macrophages and free throughout the section. Intracellular and extracellular fungal organisms were non-pigmented, septate, (3-4 µm diameter by 10-20 µm length) and exhibited occasional dichotomous branching. There also were round, 5-7 µm diameter, fungal structures that likely represent cross-sections through conidia.

**FIG 3** Phylogenetic trees showing the relationship of *Penicillium canis* and closely related *Penicillium* species. The trees were calculated for each locus sequenced using maximum likelihood in MEGA5.2. Bootstrap support above 50% is listed at the tree nodes; superscript T indicates type strain. Branch lengths are proportional to phylogenetic distance.

**FIG 4** *Penicillium canis* NRRL 62798. (A) 7d colonies grown on CYA at 25C. (B) 7d colonies grown on MGA at 25C. (C) Typical conidiophore with two apical phialides. (D) Characteristic elongate ovoid, smooth-walled conidia. Bar=10 µm for C and D.