71 Coprinopsis/Hormographiella

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71.1 INTRODUCTION

71.1.1 CLASSIFICATION AND MORPHOLOGY

The genus Coprinopsis is a basidiomycete belonging to the family Psathyrellaceae, order Agaricales, class Homobasidiomycetes, phylum Basidiomycota, kingdom Fungi. The family Psathyrellaceae currently consists of five genera: Coprinellus, Coprinopsis, Parasola, Psathyrella, and Psathyrellaceae incertae sedis [1]. In turn, the genus Coprinopsis covers 36 recognized species and 11 unas­signed species. Of these, Coprinopsis cinerea (anamorph: Coprinus cinereus; obsolete synonym: Coprinus cinerea; anamorph: Hormographiella aspergillata) is a mushroom species, whose involvement in human disease process is through its anamorph H. aspergillata.

The anamorphic genus Hormographiella (class Asco­mycetes, phylum Ascomycota) is composed of four mold species: H. aspergillata, H. candelabra, H. candelabra, and H. verticillata [2]. As the anamorph of Coprinopsis cinerea (formerly Coprinus cinereus), H. aspergillata is associated occasionally with diseases in humans and animals [3].

Hormographiella aspergillata shows moderate growth on mycological media (e.g., Sabouraud glucose agar, potato dextrose agar, and chocolate agar). Colonies measure 3 cm in 10 days at 30°C and are dense, velvety with cottony tufts and irregular margin, white to slightly cream in color, and pale on reverse. Hyphae are hyaline and septate; conidiophores are simple and well-differentiated; arising from the end of conidiophores either in clusters or in irregular groups. Arthroconidia are catenate, hyaline, smooth-walled, single-celled, and rectangular or cylindrical with truncate ends but with terminal cells rounded at the tip [4,5].

71.1.2 CLINICAL FEATURES AND PATHOGENESIS

Coprinopsis cinerea (formerly Coprinus cinereus) is the inky cap mushroom. It is observed growing on lawns. Hormographiella aspergillata has been isolated from human lung tissue, bronchoalveolar lavage fluid, brain tissue, cerebrospinal fluid, and eye and skin scrapings, and was responsible for pneumonia, lung abscess, endocarditis, and keratitis in both immunocompetent and immunosuppressed individuals [6–9]. The fungus is responsible for keratoconjunctivitis in dogs [5].

Nenoff et al. [8] described a fatal case of pulmonary infection with Hormographiella aspergillata in a 40 year old woman, who underwent autologous bone marrow transplantation for acute lymphoblastic leukemia. Coprinopsis (Coprinus) sp. was isolated repeatedly from bronchial secretions and bronchoalveolar lavage. Histopathological examination of the lung tissue demonstrated septate hyphae characteristic of Aspergillus and Coprinopsis (Coprinus) sp.

Verweij et al. [9] documented another fatal case of pulmonary infection due to H. aspergillata involving a 24 year old man receiving intensive cytotoxic treatment for acute lymphoblastic leukemia. The patient became neutropenic with fever and headache and complained of right-sided pleuritic chest pain. Chest radiographs showed focal pulmonary infiltrates in the right lower lobe, progressing to the left upper and lower lobes of the lung. The patient was treated with amphotericin B and then itraconazole. However, the patient died from respiratory failure. A necrotizing bronchopneumonia was noted at autopsy. Periodic acid-Schiff and Gomori methenamine silver stains revealed a large mass of hyphae in the cavity of the lung abscess. The hyphae measuring between 1.9 and 4.6 μm in diameter were septate, showing...
irregularly shaped swellings and branching at an acute angle or dichotomously. Cultures of the left and right lungs grown for 2 days yielded white to cream-colored, cottony and dense fungal colonies, while cultures from all other organs were sterile. Microscopic examination of the fungus showed hyaline, septate hyphae, macronematous conidiophores with no clamp connections, and straight or curved, thin-walled conidia accumulating around the conidiophore. As the isolate displayed undiagnostic arthroconidial shape and failed to sporulate, its identity was confirmed as *H. aspergillata* (*C. cinereus*) on the basis of small subunit (SSU) and internal transcribed spacer (ITS) restriction fragment length polymorphism (RFLP) patterns.

There was also a report of a lung abscess due to *H. aspergillata* (stat. anamorph. *Coprinus cinereus*) in a patient with non-Hodgkin’s lymphoma [10]. In addition, Lagrou et al. [11] described the involvement of *Hormographiella aspergil­lata* in lung tissues of a 34 year old man, who underwent an uncomplicated allogeneic peripheral blood stem cell transplantation from a human leukocyte antigen (HLA)-identical sibling for acute myeloid leukemia. The patient presented with serious capillary leak syndrome, hepatic and renal failure, progressive encephalopathy, neutropenic fever, bilateral pulmonary infiltrates, dyspnea, and cough. Microscopic examination of bronchoalveolar fluid (BAL) samples with Grocott staining showed *Aspergillus*-like molds, and cultures of the BAL samples yielded fast-growing, white to slightly cream-colored dense fungal colonies. Clusters of rectangular, cylindrical arthroconidia with truncate ends were observed but with terminal cells rounded at the tip produced from conidio­phores. The patient died from respiratory failure and refractory septic shock. At autopsy, multiple intraparenchymal and subpleural hemorrhagic nodules were found in both lungs. Hematoxylin and eosin staining of the lung tissues confirmed the presence of septated hyphae. The fungus was identified as *Coprinus cinereus* by amplification and sequencing of the ITS 2 region. It is interesting that the teleomorph was identi­fied here rather than the anamorph, and further verification may be required.

Conen et al. [12] also documented three cases of invasive pulmonary infections with *Hormographiella aspergillata* in patients undergoing treatment for acute leukemia. The fungus was detected in BAL, and despite systemic antifungal therapy with voriconazole and amphotericin B, all three patients died. Greer et al. [13] presented a case of *H. aspergil­lata*-related endocarditis involving a 77 year old female with a bioprosthesis for symptomatic mitral valve stenosis. The patient developed an unusual clot-like mass on the atrial side, due to extensive colonization by a thick filamentous fungus with true hyphae, pseudohyphae, and yeast forms. The fungus was identified as *Hormographiella aspergillata*, which is perhaps surprising as yeast forms are not associated with the fungus in question.

Abuali et al. [4] described the isolation of *Hormographiella aspergillata* from a skin lesion of a 14 year old female patient with acute myelogenous leukemia. The patient underwent an allogeneic HLA-matched bone marrow transplant and later developed odynophagia and persistent febrile neutropenia. A computed tomography (CT) scan of the lungs revealed pulmonary nodules. The patient had white plaques involving the soft palate and pharynx. A smear from a throat culture showed hyphal elements and conidiophores, and a biopsy of the palate lesion revealed submucosa and mucosa infiltrated with hyphal forms with sparse septation, rare branching, and chlamydoconidia, which was confirmed by ITS 2 sequencing as *Rhizomucor variabilis*. Following treatment with posaconazole and caspofungin, the palate lesion had decreased in size, with improved symptoms. However, the patient had persistent severe refractory pancytopenia and developed an altered mental status and a generalized seizure. A CT scan of the brain showed multiple hypodense lesions of the cerebral hemispheres and cerebellum and a repeat CT scan of the lungs uncovered cavitating lesions in the right upper lobe/right middle lobe. A modified therapy consisting of liposomal amphotericin (Ambisome) and caspofungin was initiated. The patient developed high fever and new small erythematous skin papule on the right knee and the left arm. Cultures of the skin biopsy yielded a white mold, which was identified as *Hormographiella aspergillata*. The patient died from respiratory failure 2 weeks after the appearance of the initial skin lesion.

*Hormographiella* spp. are considered sensitive to some azoles such as miconazole but resistant to fluconazole or itraconazole; they are variably sensitive to amphotericin B and are resistant to flucytosine [3].

### 71.1.3 Diagnosis

Infections caused by *Hormographiella aspergillata* are reported increasingly, especially in patients with acute leukemia or other underlying diseases. It is important to identify *Hormographiella* from other molds as this fungus displays varied sensitivity to antifungal drugs.

Filamentous basidiomycetes are notably difficult to identify because they do not always show key diagnostic features and tend to only form undifferentiated arthroconidia. For example, some *Hormographiella* isolates fail to sporulate, and others do not display diagnostic clamp connections or arthroconidial shape. Other common fungi forming arthroconidia include *Geotrichum candidum*, *Trichosporon* species, *Geomyces pannorum*, *Malbranchea* species, *Arthrographis kalrae*, and *Scytalidium* species. *Arthrographis*, *Geomyces*, and *Hormographiella* also produce conidiophores. However, the *Hormographiella* conidiophore is clearly broader than the conidiogenous hyphae, which is sometimes useful [2].

Definitive determinations of *Hormographiella* are only possible with molecular techniques using RFLP patterns of PCR-amplified ITSs (~600 bases) and SSU rRNA (~1700 bases). Alternatively, sequencing analysis of ITS 2 and the D1/D2 variable domains (~600 bases) at the 5’ end of the large subunit rRNA gene (D1/D2) is required [14–16].
71.2 METHODS

71.2.1 SAMPLE PREPARATION

Clinical specimens (e.g., BAL fluid) are first examined by microscopy for mycotic elements with KOH and other stains. Tissue samples (e.g., skin, lung, liver, spleen, and kidney tissues) are fixed in 10% formalin, embedded in paraffin, sectioned at 5μm, and stained with periodic acid-Schiff. The fungal material is ground with a mini-grinder (Tissue-Aid). The ground material is then transferred to a 2 mL Eppendorf tube containing a 2:1 (wt/wt) mixture of silica gel and Celite (silica gel 240, Merck; Celite 545, Machery) and 300 μL of TES buffer (2 g Tris (hydroxymethyl)aminomethane, 0.38 g Na-EDTA, and 2 g sodium dodecyl sulfate in 80 mL of ultrapure water (pH 8)). The fungal material is ground with a micropestle for 1–2 min. The volume is adjusted by adding pure water (pH 8). The fungal material is ground with a mini-grinder (Tissue-Aid) and mixed by inverting the tube for 30 min at 65°C. One volume (~700 μL) of 5 M NaCl solution, the mixture is incubated for at least 30 min (in iced water) and centrifuged. The supernatant is decanted. The pellet is washed once with 1.5 mL of ice-cold isopropanol. After incubation for 30 min at 0°C (in iced water) and centrifugation at 14,000 rpm at 4°C for 10 min, the top layer is transferred to a clean Eppendorf tube. To the sample is added 225 μL of 5 M NH₄-acetate and it is incubated for at least 30 min (in iced water) and centrifuged. The supernatant is transferred to a clean sterile Eppendorf tube and mixed with a 0.55 volume (~510 μL) of ice-cold isopropanol. After centrifugation for 7 min at 14,000 rpm at 4°C (or room temperature), the supernatant is decanted. The pellet is washed twice with ice-cold 70% ethanol and dried by using a vacuum dryer. The powder is resuspended in 48.5 μL of Tris-EDTA buffer with 1.5 μL of 10 mg/mL RNase/mL, incubated at 37°C for 15–30 min, and stored at −20°C until use [17].

71.2.2 DETECTION PROCEDURES

71.2.2.1 Sequencing Analysis of the ITS 2 Region

Rampazzo et al. [5] utilized universal primers ITS 3 (5’TCTCTCGGTATTGATATGTC-3’) and ITS 4 (5’-TCATCGGATGAAACGCAGC-3’) to amplify a 350 bp fragment of the ITS 2 region, interspacing the 5.8S rRNA and 28S rRNA of fungal ribosomal RNA genes for identification of Hormographiella.

Procedure

1. The PCR mixture (30 μL) is composed of 12 pmol of each primer, 0.25 mM each of dATP, dCTP, dGTP, and dTTP, 5 mM MgCl₂, 1× reaction buffer, 2.5 U Taq DNA polymerase, and 3 μL paraffin extract.
2. Amplification is performed with an initial 94°C for 3 min; 35 cycles of 94°C, 55°C, and 72°C for 30 s each; and a final 72°C for 7 min.
3. PCR products are analyzed on a 1.5% agarose gel stained with ethidium bromide. Fragments are purified with the high pure PCR purification kit (Roche Diagnostics) and 30 ng of purified PCR product is sequenced with the BigDye v3.1 terminator sequencing kit (Applied Biosystems) using the same primers used for PCR. Reactions are run on an ABI 3130xl (Applied Biosystems) and sequences are edited using Sequencher 4.6 (GeneCodes, MI). Sequences proofread in both directions are compared against GenBank using BLAST.

Note. The primer combination ITS 3/ITS 4, covering the ITS 2 region, amplified a product of about 350 bp from the clinical sample. Sequence analysis of the fragment gave 343 bp of sequence information after trimming of the primer sequences. Comparison against GenBank showed a 99.7% identity (one mismatch over the 343 bp) to the corresponding sequence of C. cinerea, the anamorph of which is H. aspergillata (see also Lagrou et al. [11] above). Interestingly, other primer combinations covering ITS 1 or parts of 18S rRNA gene did not result in any amplification products in this case.

71.2.2.2 PCR-RFLP Analysis of SSU and ITS 2 Regions

Verweij et al. [9] described the use of primers NS1 and NS24 (for amplification of SSU) and primers ITS 1 and ITS 4 (for amplification of ITS 1 and 2, including 5.8S ribosomal RNA [rRNA]) for identification of C. cinerea and other molds. Amplicons are digested with the restriction enzymes HinfI, HaeIII, RsaI, and DdeI and electrophoresed on 1.4% agarose gels. Patterns are compared by using the Image Master (Pharmacia) software package for evaluation of molecular weights. The fungus had different patterns to the other fungi analyzed.

71.3 CONCLUSION

Hormographiella aspergillata is the anamorph of Coprinopsis cinereus (Coprinus cinereus), which is a basidiomycete with a typical mushroom form normally occurring in compost and sewage [2]. H. aspergillata has been associated with...
human infections such as pneumonia, endocarditis, and endophthalmitis. Due to the difficulty of identification on the basis of macroscopic and microscopic features, filamentous basidiomycetes such as *Hormographiella* species are poorly recognized in the clinical laboratory. Application of PCR and sequencing analysis of the ITS2 and other gene regions permits rapid and accurate identification of *H. aspergillata*, shortening the time needed for its diagnosis from as much as 4–6 weeks to a day, and hence facilitating early antifungal therapy.

**REFERENCES**


**AUTHOR QUERIES**

[AQ1] Please check the sentence starting “Hyphae are …” for completeness.

[AQ2] Please provide access date for Ref. [1].