Mold Removal and Rehousing of the Ichthyology and Herpetology Skeletal Collections at the Natural History Museum of Los Angeles County

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Infection by fungal spores and hyphae is an acute problem that may cause damage or loss of specimens in natural history collections. Most ichthyology and herpetology collections are fluid-preserved whole animals, stored in glass jars, but collections frequently also maintain dried specimens of skin or bones, which are vulnerable to fungus. An infection of *Aspergillus* fungus was discovered in the ichthyology and herpetology skeletal collections at the Natural History Museum of Los Angeles County (LACM) in October of 2003. Within our collections, 12% of fish and 4% of herpetological skeletons were visibly infected. We elected to use 70% ethanol as a fungicide because it is non-toxic, effective, inexpensive, and produces minimal damage. A total of 688 infected specimens were cleaned, and all 7,987 specimens were rehoused between June 2005 and May 2007. Treatments were carried out by a commercial fungus remediation firm, and the process was monitored by an environmental consultant. Treated specimens were stored in new plastic boxes, housed in one of four ways: sealed bags; sealed bags with desiccant; desiccant only; or no bag or desiccant. Skeletons not visibly contaminated were brushed clean, catalogued, and rehoused in sealed plastic bags and plastic boxes. Periodic agar plate sampling showed no fungal growth in a subset of the four rehousing groups over the course of two years. Among all disinfected specimens, only one displayed a recurrence of fungal growth two years after treatment. We recommend treatment of fungus-infested natural history collections with 70% ethanol, and storage in polyethylene boxes and polystyrene or polypropylene bags, to prevent infection and to contain the spread of infection if it does occur.

NATURAL history collections encompass a variety of specimens and types of storage, including pressed plants on paper in herbaria, dried bones, skins, or insects, whole organisms preserved in formalin and stored in ethanol, and anthropological collections ranging from bones to artifacts to textiles and pottery. All of these collections serve communities of researchers, policy makers, conservation workers, and the general public. Collections are kept with the intention that they will be safeguarded and available for use in perpetuity, or at least into the forseeable future. Specimens of the types described above are relatively stable, but must still be held under appropriate conditions (regulated light, temperature, and humidity) and monitored for degradation or injury. Maintenance of collections includes ongoing monitoring and adjustments, and may also encompass remediation of rare acute problems such as damage from fire, earthquake, flood, insects, or fungus.

In the case of an acute collection problem, the first step is to stop the cause of the problem and prevent further damage, followed by remediation of damage that has occurred and prevention of future damage (Waller, 1995). In this contribution we describe the identification and treatment of an infection of *Aspergillus* fungus in collections of dried skeletal specimens in the sections of Ichthyology and Herpetology at the Natural History Museum of Los Angeles County (LACM). Thousands of species of microscopic fungi are common in our environment; these fungi feed on dead organic matter, including natural history collection materials such as dried plants, skins, bones, paper, or textiles. Potential damage from fungus infection ranges from stains to complete destruction of specimens as the hyphae actually consume their substrate (Strang and Dawson, 1991), with cellulose and proteinaceous material most vulnerable to fungal attack. Fungi may also present a health hazard to those who use the collections (Merritt, 1993). Temperature and humidity are the major external factors influencing fungal growth; most fungi do not grow or germinate when humidity is less than 65% (Strang and Dawson, 1991). Because fungi are nearly ubiquitous, there is no practical way to eliminate them completely. Treatment of a fungus outbreak in a natural history collection involves adjusting collection conditions to prevent growth of fungus, removing fungal hyphae from infested specimens, and providing appropriate specimen containers to retard and contain any future fungal growth.

The sections of Ichthyology and Herpetology together maintain approximately 8,000 disarticulated and articulated fish and herpetological skeletons (5,000 fish and 3,000 amphibians and reptiles). These specimens include both freshwater and marine fishes, including elasmobranch jaws and skeletons, as well as frogs, snakes, turtles, and lizards. The collection serves as a valuable resource for morphological, evolutionary, archaeological, and paleontological studies (Fierstine and Ray, 2001; Porcasi and Andrews, 2001; Wake, 2004). In October of 2003 we noticed that some of the skeletons were infected with fungus, manifesting as a

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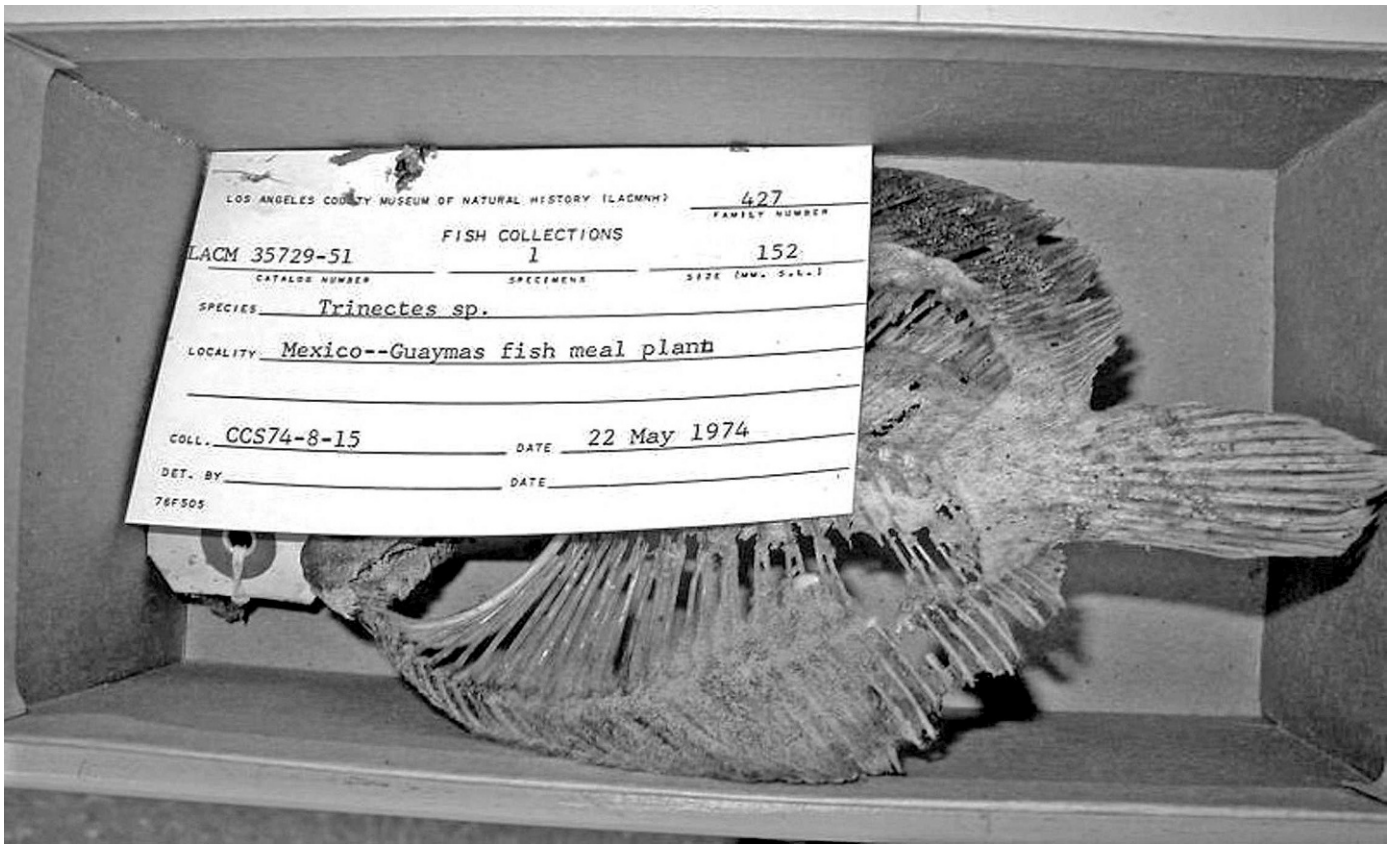

Fig. 1. Specimen of flatfish (*Trinectes* sp.) stored in non-archival quality cardboard box and infested with *Aspergillus* on the anal-fin region.

white or greenish velvety growth on specimens (Fig. 1). We believe the infection was caused by especially wet weather conditions in 2002 and 2003, which caused fluctuations in temperature and humidity in the collection area, resulting in humidity as high as 85% for at least three months, as recorded by hydrothermograph. The fungus was identified as *Aspergillus fumigatus* (Phylum Ascomycota). This common species may cause respiratory illness in humans (aspergillosis). Identification of the fungus was confirmed by culturing swabs from infected specimens and examining fungal hyphae with both light and scanning electron microscopes (Fig. 2). Our first priority was to repair and adjust the climate control and venting systems in the collections, reducing the humidity to 50–55%, with a mean temperature of 72 degrees Fahrenheit. Maintenance of humidity at less than 65% is desirable to retard the growth of fungus (Strang and Dawson, 1991; Simmons, 1995, 2002). Our humidity and temperature regulation systems are interdependent, such that we could not reduce the humidity any further without raising the temperature. Once that was accomplished, we began to investigate the best method for removing fungus from infected specimens and preventing future growth.

We considered a range of treatments for our skeletons, with the goal of removing fungus while preserving the integrity of the specimen, including possible future extraction of DNA. There are many chemical solutions that can be used to eradicate molds including chlorine dioxide, ethanol, formalin, and hypochlorite bleaches. Chemical fungicides may cause health problems and do not kill all spores (Southwell, 2003). Hypochlorite bleaches contain reactive chlorine and may damage specimens (Strang and Dawson, 1991). Formalin is a known carcinogen and impedes extraction of DNA from fixed tissues, including bones (Miething et al., 2006; Stuart et al., 2006). Diluted ethanol (70%) has been used previously on other museum objects and was determined to be the safest and most readily available fungicide for our project (Strang and Dawson, 1991). Once in contact with the hyphae, alcohol kills mold by dehydration and protein denaturation. We conducted a

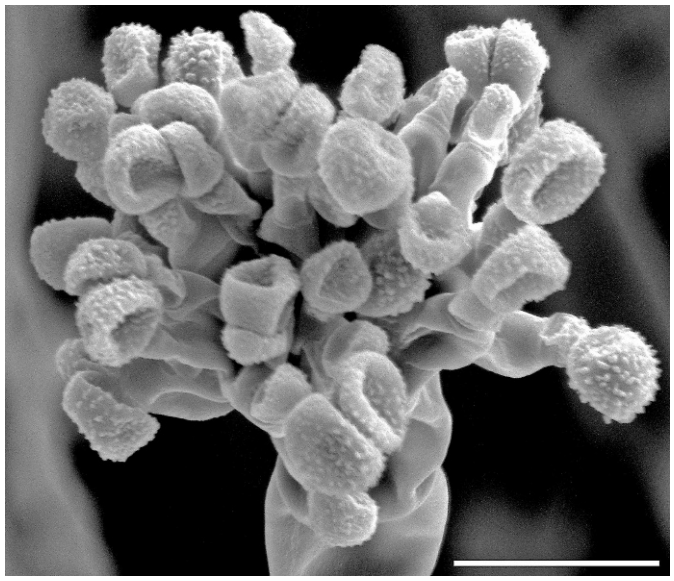

Fig. 2. Scanning electron micrograph of conidiophore of *Aspergillus fumigatus* with new conidia forming, cultured on potato dextrose agar from an infected LACM fish skeleton. Scale bar = 10 μm.

Fig. 3. Treatment of *Ctenopharyngodon idella* specimen in pilot study of fungus remediation. (A) Specimen in cardboard box, with fungus present on vertebrae and caudal fin. (B) Specimen and associated labels soaking in 70% ethanol. (C) Specimen (left) drying on tray. (D) Specimen stored in sealed plastic bag and plastic box, with dessicant packet visible at upper left.

pilot study with several of our infected specimens, soaking them in 70% ethanol for 24 hours, drying the specimen, and manually removing any remaining particulates (Fig. 3). Specimens were immersed rather than swabbed with ethanol so that the solvent could penetrate specimens thoroughly. Skeletons were carefully observed before and after immersion to determine whether or not bones would warp as a result of undergoing alcohol dehydration and air drying. The method appeared to remove mold effectively, as confirmed by swabbing the specimen and culturing the swabs on agar plates, with negative results. Skeletons were not visibly warped by the procedure.

We then considered how to carry out a large-scale treatment project of our specimens. A primary concern was that exposure to high concentrations of *Aspergillus* spores may cause respiratory illness, particularly in individuals that are immunocompromised (Florian, 2002). We were conscious that any concentrated treatment effort would have to be well-contained, away from public areas. We consulted with Los Angeles County Occupational Health and Safety officials who tested the air quality in and around our collection areas, and found that fungal spores and hyphal levels were not at dangerous levels. There are no specific health regulations or limits on *Aspergillus* exposure; the testing procedure merely ensures that airborne fungal levels in a target area are not elevated relative to other areas, and to the ambient external air. In our case, levels in the collections were lower than outside, but the county officials stressed to us that a large-scale decontamination project would have to be carried out by contractors with appropriate experience, training, safety equipment, and insurance.

Exposure to fungus contamination in buildings and homes is well-recognized as a health hazard, and many commercial firms were available that had experience with *Aspergillus* mitigation. However, our situation was unique in that we wanted to have mold removed from delicate, generally small, skeletons rather than from walls or ductwork. We interviewed several contractors and reviewed with them the results of our pilot study and planned treatments, and chose Zenco Engineering (Camarillo, CA) to perform the decontamination. A fungus mitigation project requires not only the contractors who perform the actual cleaning work, but also the services of an environmental consultant whose function is to monitor airflow and air quality in the workspace. We chose Criterion Environmental, Inc. (Ventura, CA) to perform the monitoring. We reviewed our treatment plans with the contractor and consultant and identified a room where a containment

zone could be established, with an isolated airflow circuit that vented directly to the exterior via a fume hood duct. From a survey of our specimens, we estimated that roughly 1,000 would require decontamination. Our pilot study allowed us to estimate that one person could decontaminate 20 skeletons in a day, and that we had space and facilities for two people to do decontamination work. Thus, we estimated that the job would take 25 days, with two days to set up the clean space and establish airflow, and one day to break down the facility, for 28 working days or approximately six weeks. The estimates we received were for a cost of $33,500 for the remediation team and $6,500 for environmental consultant services, for a total of $40,000. We then applied for and received a grant from the National Science Foundation's Biological Resource Collections program. The timeframe for the remediation project covered under the grant extended from June of 2005 to May of 2007. This two-year period encompassed the time during which the decontamination was done by the remediation firm, as well as the remainder of the two years, in which a curatorial assistant (hired for the project) cleaned and rehoused all the uninfected skeletons.

MATERIALS AND METHODS

Each of the nearly 8,000 skeletal specimens was removed from the collections and examined for visible fungal growth. If fungus was present, the specimen was set aside for treatment by the contractors. Other specimens were stored in a room designated for work on non-infected skeletons. Specimens were completely immersed in 70% ethanol for 24 hours and then dried in trays on racks overnight. Any labels that were included with specimens were treated along with the bones and preserved (Fig. 3). Skeletons not cleaned completely by immersion were cleaned by hand using a vacuum equipped with a high efficiency particulate air (HEPA) filter and brush attachments, forceps, and soft paintbrushes. All specimens were examined after treatment and very few were damaged (distorted or broken) during handling and treatment.

Once cleaned, treated skeletons were split equally into four storage categories, to evaluate performance of different storage types, and to gauge if storage modality affected fungal contamination or re-growth. The treatments were: (1) bagged in a heat-sealed 4 mm clear polyethylene bag (Bradley's Plastic Bag Co., Downey, CA); (2) bagged in a heat-sealed 4 mm clear polyethylene bag with desiccant packet (Desiccare Inc., Pomona, CA); for small specimens, the 14 gram ''Pillow Pak'' size was used, for larger specimens the one unit (approximately one ounce) ''Unit Pak'' size was used. (In both cases, the dessicant was silica gel.); (3) no bag, desiccant only; or (4) no bag or desiccant. All were placed into clear polypropylene or clear polystyrene plastic boxes (Rubbermaid boxes purchased from Durphy Packaging Co., Ivyland, PA). Among treated skeletons, a subset of three specimens from each of the four categories was chosen for visual inspection every three months and agar plate sampling every six months. Agar cultures were prepared by dissolving 6 g of potato dextrose agar in 150 ml distilled water and heating in a household microwave oven. This amount of medium was sufficient to produce thirteen 55 mm diameter culture plates: 12 to test treated specimens, plus a control. Samples were taken with individual sterile swabs, brushed onto the plates, and incubated at room temperature for 24 hours. Presence or absence of fungal growth was evaluated with a dissecting microscope. Ideally, the reduced humidity should have prevented any re-growth of fungus; thus, our evaluation of the different types of storage is relevant only to performance under these adjusted conditions.

Specimens not visibly contaminated with fungus were not treated with ethanol but carefully cleaned of any adherent material with forceps and soft brushes. Some specimens, such as the billfishes (Istiophoridae), were greasy and required additional treatment. These skeletons were immersed in a mixture of 70% ethanol and liquid dishwashing detergent for 24 hours, then soaked in standing tap water, then rinsed in running tap water and allowed to dry for an additional 24 hours (Bemis et al., 2004). All skeletons were boxed, bagged, and catalogued (if necessary). Old cardboard boxes were replaced with new plastic boxes, and new archival-quality (100% cotton rag, acid-free, produced by Byron Weston Co.) labels were added; the exterior of the box was also tagged with foil-backed laser labels (University Products, Inc.). Any labels originally included with specimens were cleaned following the same ethanol protocol as the bones; cleaned labels were included with the specimens in their new packaging.

RESULTS

A total of 688 infected specimens (172 in each of the four storage categories) were treated with 70% ethanol. Of that total, 584 were fishes and 104 were herpetological skeletons. Visual inspection of most of the treated skeletons showed no fungal growth re-appearance. The subset of treated specimens tested periodically over a two-year period for fungal re-growth by culturing sterile swab samples on agar plates showed no fungal re-growth. A total of 7,987 specimens (5,048 fish and 2,919 herpetological skeletons), infected and non infected, were cleaned, bagged, and boxed. At the end of the two-year period, all specimens were visually examined before reintegration into the collections. Recurrence of fungal growth was observed on only one specimen, a large snakehead (Channidae) from Pakistan, which had not been thoroughly cleaned when prepared, maintained traces of organic material on the bones and, during remediation, was not housed in a plastic bag or exposed to a silica dessicant. This specimen was subjected to a second cleaning during which it was soaked in 70% ethanol, dried, brushed, bagged, and boxed. As this specimen was the only one that suffered re-growth, we could not demonstrate any significant difference among storage modalities (with/without plastic bags or dessicant). At the end of the treatment period, all specimens were housed in plastic bags, to better contain the specimen and recurrent fungal growth.

DISCUSSION

We conclude that ethanol is an effective fungicide, appropriate for treating museum fish, amphibian, and reptile osteological specimens. We also report that it is possible to perform large-scale fungus mitigation in a natural history collection by contracting with professional fungus remediation firms and consultants. In the case of toxic fungal species, this method may be the only choice for fungus removal. We recommend storage in polyethylene bags and polystyrene or polypropylene boxes, with both interior and exterior labeling. This combination makes the specimens easy to use and examine, safeguards the specimens against

breakage, is inert to fungus infestation, and will contain fungal growth that initiates on the skeletal specimen.

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