MUCORMYCOSIS OF AMPHIBIANS

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WHAT IS MUCORMYCOSIS?

Amphibian mucormycosis is a systemic disease caused by the fungus, *Mucor amphibiorum*. Infected frogs and toads have fungi disseminated through their internal organs and skin. The fungi incite formation of granulomas that consist of inflammatory cells and fibrous tissue. At post mortem, the liver contains small pale nodules, up to about 5 mm in diameter, and usually in massive numbers (Fig. 1). These nodules can also be seen in other organs such as the kidney, lung, mesentery, urinary bladder, subcutaneous sinuses and skin. The microscopic fungi are found inside these nodules.

M. amphibiorum is a primary pathogen and can infect normal amphibians, but in the wild it appears to cause only sporadic infections. Possibly the usual inoculating dose in the wild is not high enough to cause epidemic disease. In captivity it can cause fatal outbreaks in collections.

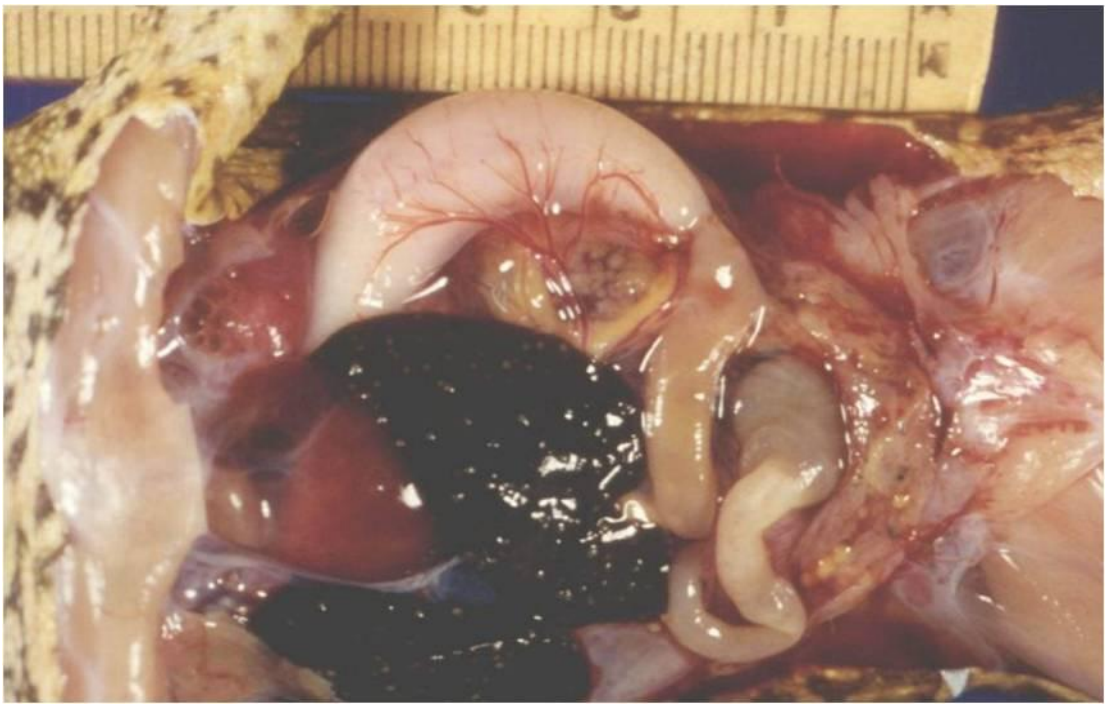


Figure 1: Mucormycosis due to *Mucor amphibiorum* in a cane toad, *Bufo marinus*, Townsville, Australia.

CAUSATIVE AGENT

The causative or aetiological agent, *Mucor amphibiorum*, is a zygomycete fungus not related to *Batrachochytrium dendrobatidis*, the amphibian chytrid. *M. amphibiorum* is a dimorphic fungus. In the amphibian host it is a yeast-like spherical structure, called a sphaerule. *M. amphibiorum* has the unusual characteristic of forming daughter spherules inside the mother spherule, and these can be seen in histological sections of organs or on direct microscopic examination of infected tissue (Fig. 2). The spherical shape and the daughter spherules is a key diagnostic feature in tissue sections. Sphaerules range in size from 37 to 4.9 microns. When *M. amphibiorum* is growing outside the amphibian host, it becomes thread like, forming hyphae that form a mat, or

mycelium (Fig. 3). Two mating types of *M. amphibiorum* exist, and when these meet they form resistant structures called zygospores (Fig. 4). Spores are eventually formed, and these are infectious to amphibians when ingested. *M. amphibiorum* grows on soil and will sporulate on the soil. Hence, we assume that amphibians can become infected when they catch prey along with soil containing spores.

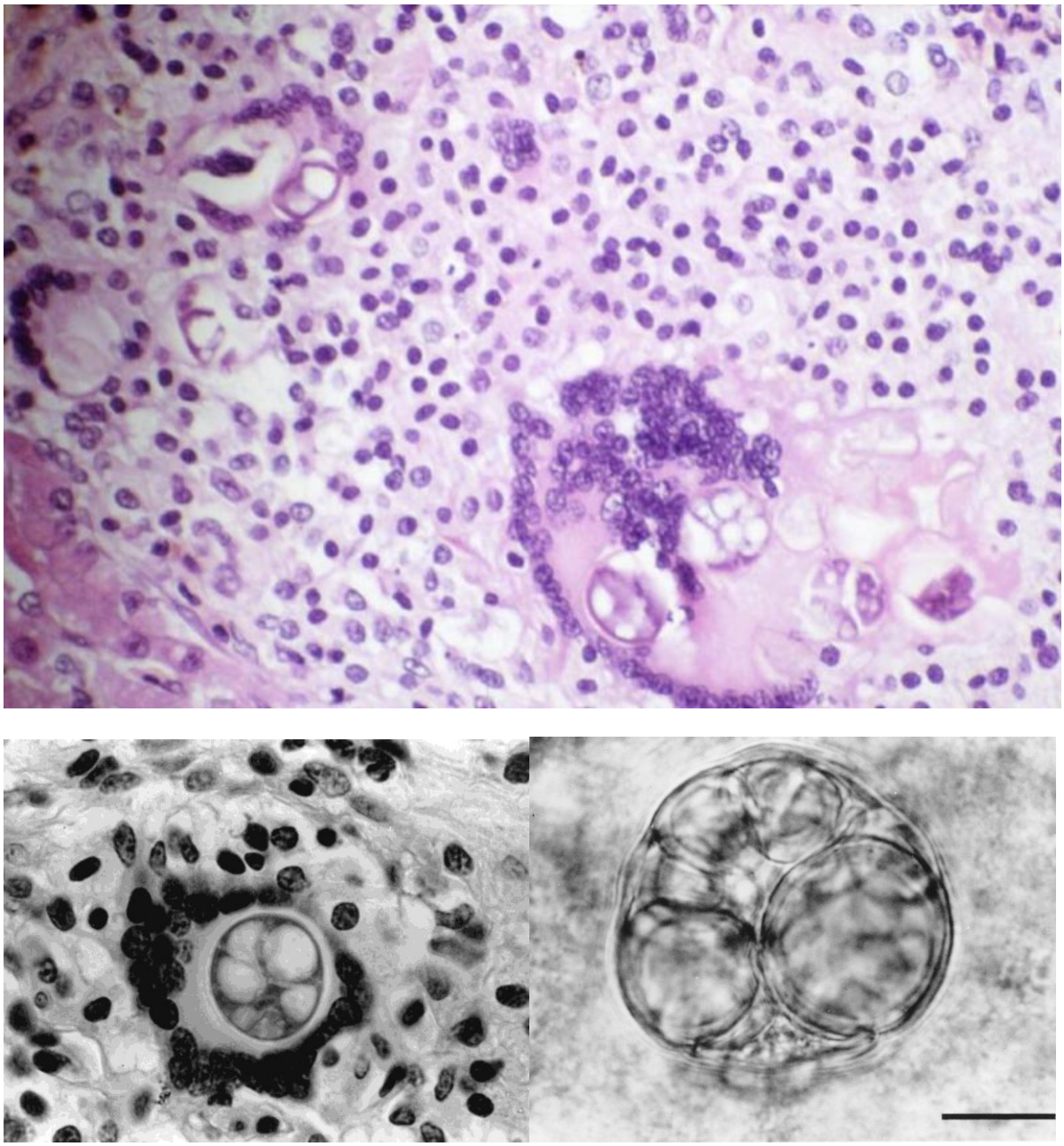


Figure 2a: Section of nodule in the liver of a *Bufo marinus* showing histiocytes and lymphocytes in a chronic inflammatory response with several foreign body giant cells. The sphaerules of *Mucor amphibiorum* appear as round bodies with a think, eosinophilic wall. The large giant cell at the bottom right of the image has phagocytosed at least two sphaerules, both of which contain daughter sphaerules. Several other sphareules, some degenerating, occur outside this giant cell to its right. Normal hepatocytes are present in the bottom left corner outlining the edge of the nodule. H&E.

Figure 2b: Sphaerule containing daughter sphaerules in a giant cell in the spleen of a *B. marinus*. H&E.

Figure 2c: Sphaerule of *M. amphibiorum* containing daughter sphaerules in a squash preparation from an infected organ of a *B. marinus*. Unstained. Sphaerules measure 14 to 5 microns in diameter when single and 37 to 15 microns when they contain daughter sphaerules (Speare et al 1994). The daughter sphaerules are released by dissolution of the wall of the mother sphaerule.

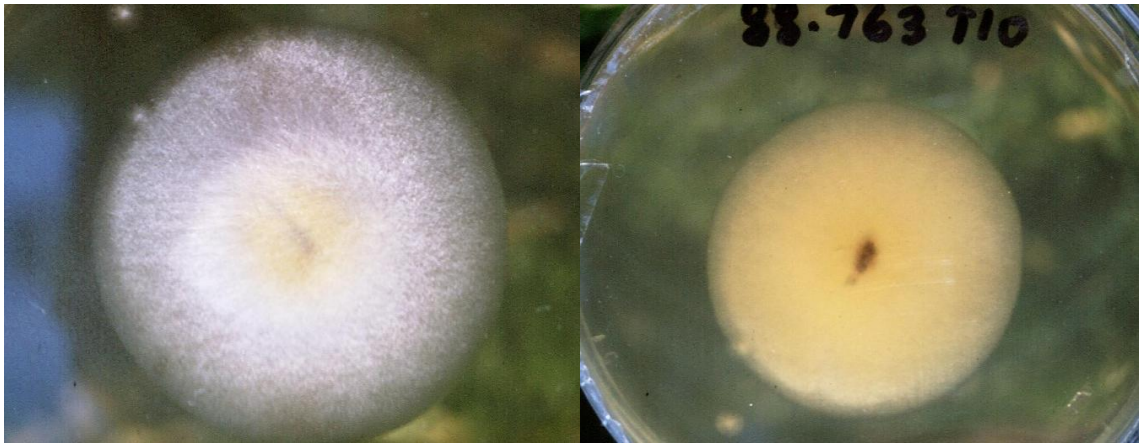


Figure 3a: *Mucor amphibiorum* growing on Saubaurad's agar as a typical filamentous fungus. *M. amphibiorum* is a dimorphic fungus being yeast like (spherical bodies) in the host and filamentous in the environment. Sporangiospores, the stage infective to amphibians, form on aerial structures produced by the mycelium. View from above and below.

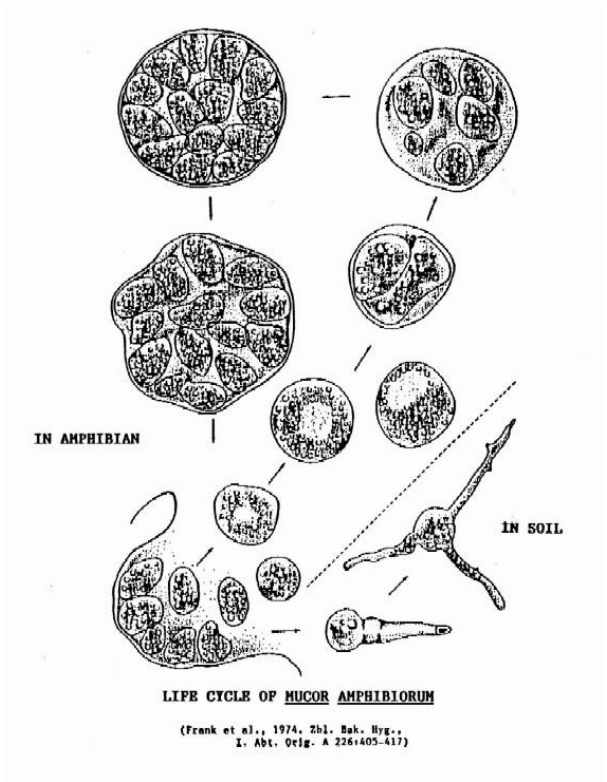


Figure 3b: Stylised *M. amphibiorum* life cycle showing dimorphic morphology. Only sphaerules occur in the amphibian host and hyphae in the external environment.

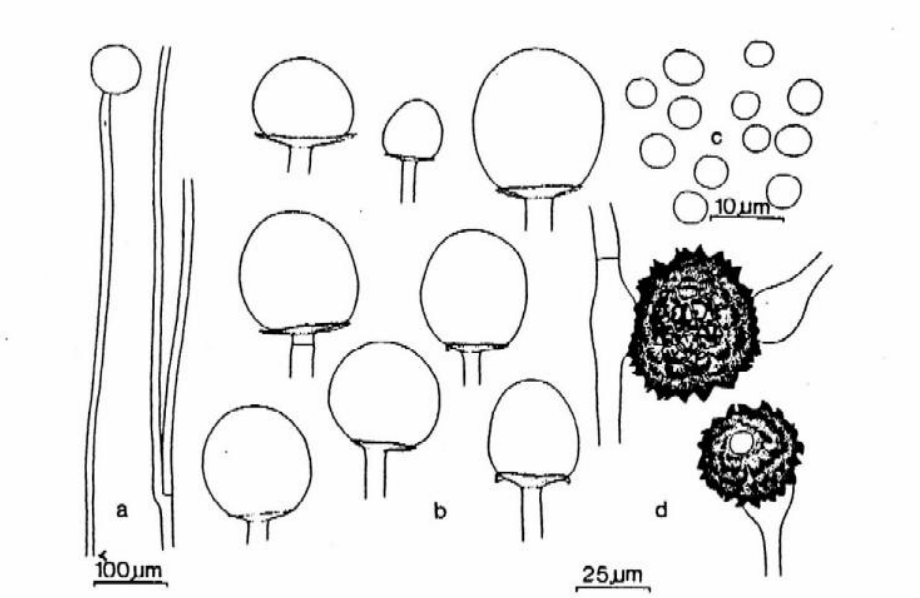


Fig. 9. *Mucor amphibiorum*, CBS 763.74. a. sporangiophore; b. columellae; c. sporangiospores; d. zygospores between suspensors (CBS 763.74 x 185.77).

(Schipper, 1978. Studies in Mycology 17:1-52)

Figure 3c: Drawings of reproductive stages of *M. amphibiorum* (Schipper 1978). The sporangiospores are infective orally to amphibians. Note the zygospores formed by the mating of positive and negative mating types (see Fig. 3d).

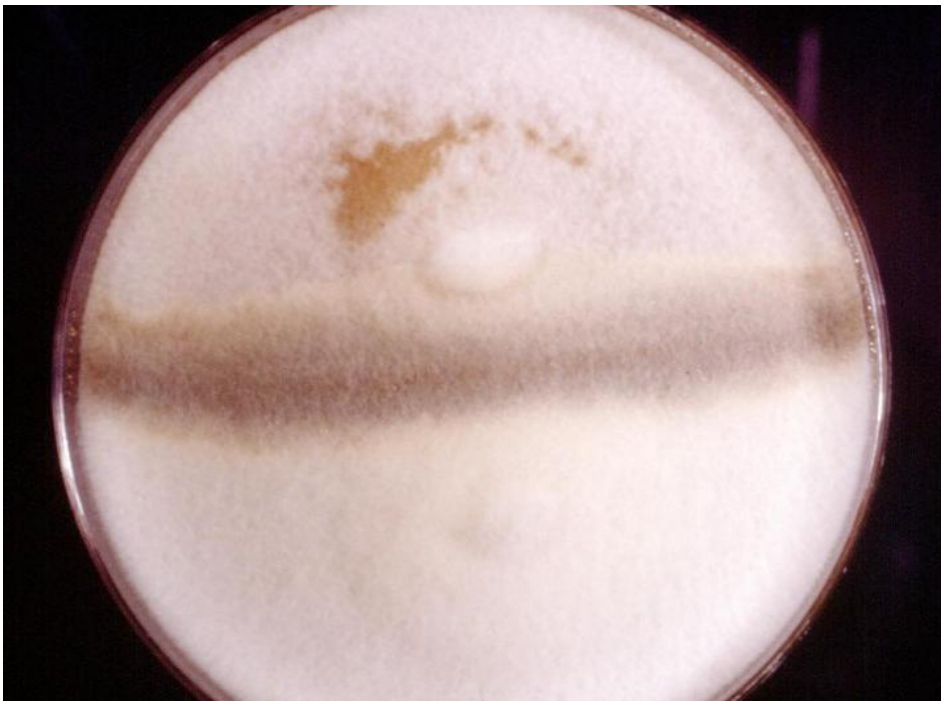
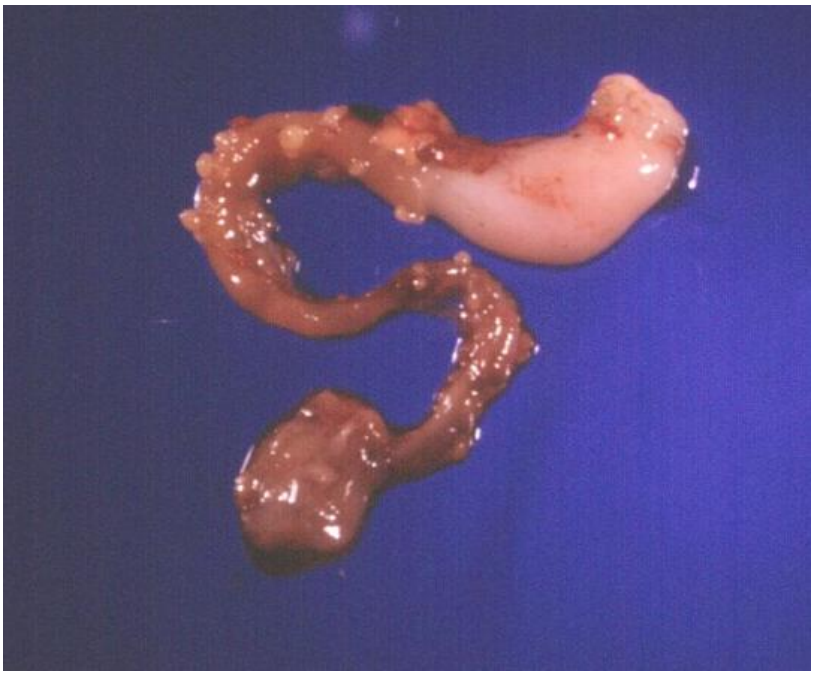


Figure 3d: *Mucor amphibiorum* has positive and negative mating types. When the mycelium of a positive mating type grows to meet the mycelium of a negative mating

type, they form reproductive bodies called zygospores. In this image the zygospores are seen as a brown line where the two types have met.



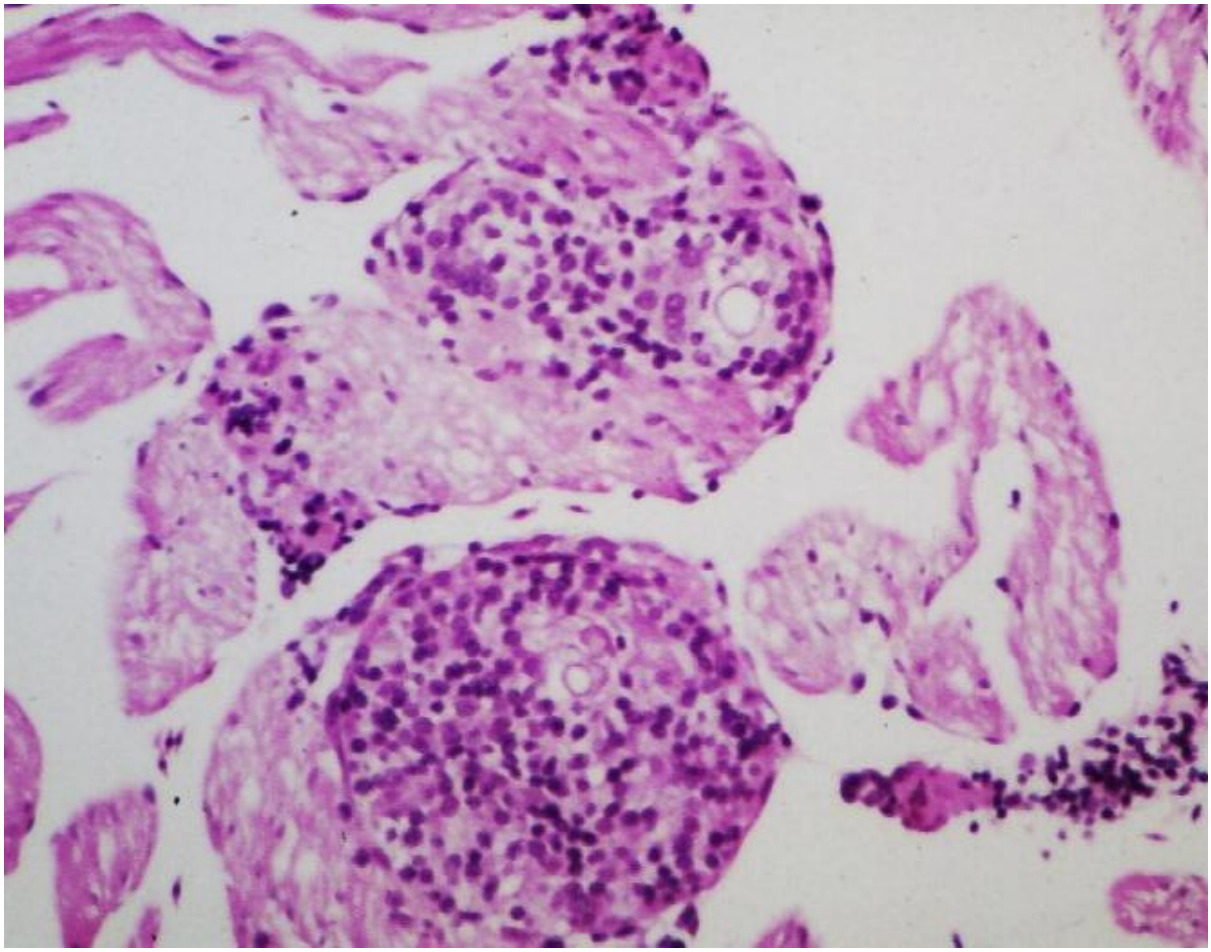


Figure 4a: When cane toads (*Bufo marinus*) were experimentally given sporangiospores of *M. amphibiorum* orally, the intestine was the initial organ to become infected. In this image the stomach is uppermost. Nodules containing sphaerules of *M. amphibiorum* are visible along the serosa of the small intestine. Infection spreads from there into the spleen and liver and is then disseminated via the blood and body cavity. The oral route is therefore regarded as the most likely route of infection for amphibians. *M. amphibiorum* may also be excreted in faeces of infected amphibians and can contaminate the environment.

Figure 4b: *M. amphibiorum* enters the systemic circulation and is widely disseminated via the bloodstream to many organs. This image confirms this hypothesis by showing inflammatory lesions in the endocardium and myocardium of *B. marinus*. Two sphaerule can be seen as spherical structures with a pink wall and clear contents in two lesions.

WHERE DOES *M. AMPHIBIORUM* OCCUR?

M. amphibiorum appears to be an endemic Australian fungus. Cases of mucormycosis have been found in amphibians in Queensland, Northern Territory, Western Australia, and New South Wales (see table below for details). *M. amphibiorum* also has been found causing disease in platypuses in Tasmania. However, it has not been found in amphibians in that state.

The quirky thing is that mucormycosis due to *M. amphibiorum* first became known to science after it was discovered in a captive collection in Germany (Frank et al 1974). In this collection it caused death in the Australian green tree frog (*Litoria caerulea*) and in *Dendrobates* from South America. The source of the fungus was not identified, but it may have been imported from Australia with the *L. caerulea*. Another notable Australian export!

M. amphibiorum can be found in the soil, and grows well on soil when infected tissues from frogs are placed on soil. In one epidemiological study *M. amphibiorum* was isolated from soil in a greenhouse where a series of cane toads (*Bufo marinus*) had died from mucormycosis over 2 years (Speare et al 1994). The available evidence suggests that *M. amphibiorum* is an environmental fungus that occasionally infects amphibians.

WHAT AMPHIBIANS HAVE BEEN FOUND TO BE INFECTED?

Mucormycosis has been found in cane toads (*B. marinus*), green tree frogs (*L. caerulea*), white lipped frogs (*Litoria infrafrenata*) and striped marsh frog (*Limnodynastes peronii*) in the wild in Australia and in slender tree frogs (*Litoria adelensis*), green tree frogs, white lipped tree frogs and dendrobatid frogs in captivity in Australia and Germany (see table). 0.7% of cane toads in a survey we did in Queensland, NSW and Northern Territory were infected (Speare et al 1994).

Species infected	Wild / captive	Deaths	Location	Reference
Litoria caerulea	captive	yes	Germany Australia (Adelaide)	Frank et al 1974; Frank 1976; Berger 2001
Dendrobates	captive	yes	Germany	Frank et al 1974; Frank 1976
Bufo marinus	wild	yes	Australia (Qld, NT, NSW)	Speare et al 1994; Speare et al 1997; Berger 2001
Limnodynastes peronii	wild	yes no	Australia (Rockhampton, Qld)	Berger 2001
L. caerulea	wild	yes no	Australia (Biloela, Qld) Australia (Brassall & Rockhampton, Qld)	Berger et al 1997 Berger 2001
Litoria infrafrenata	wild	yes	Australia (Cairns)	Speare & Mendez (pers obs)
Litoria adelensis	captive	yes	Australia (Perth)	Creeper et al 1998
L. infrafrenata	captive	yes	Australia (Perth)	Creeper et al 1998

HOW IS *M. AMPHIBIORUM* TRANSMITTED?

Experimentally we were able to infect cane toads by feeding them sporangiospores from cultures (Fig 4). The fungus appeared to establish first in the intestinal wall and

then to enter the bloodstream and become widely disseminated. After this the fungus appeared to spread further via the subcutaneous lymph sinuses and in the peritoneal cavity. Infections in the skin appeared to be initiated mainly from the subcutaneous lymph sinuses and to enter the epidermis from below in the dermis (Fig. 5). The initial intestinal route of infection was also indicated by severe infections with fungus in the intestines of captive frogs in the Perth Zoo (Creeper et al 1998). Slender tree frogs had started dying within 7 days of entering quarantine. In this outbreak transmission to a quarantined group of white lipped tree frogs may have occurred via contaminated feed bowls (Creeper et al 1998).

In a study on wild cane toads *M. amphibiorum* was isolated from the faeces of two toads. Ulcerative lesions in the skin may also allow *Mucor* to escape into the environment.

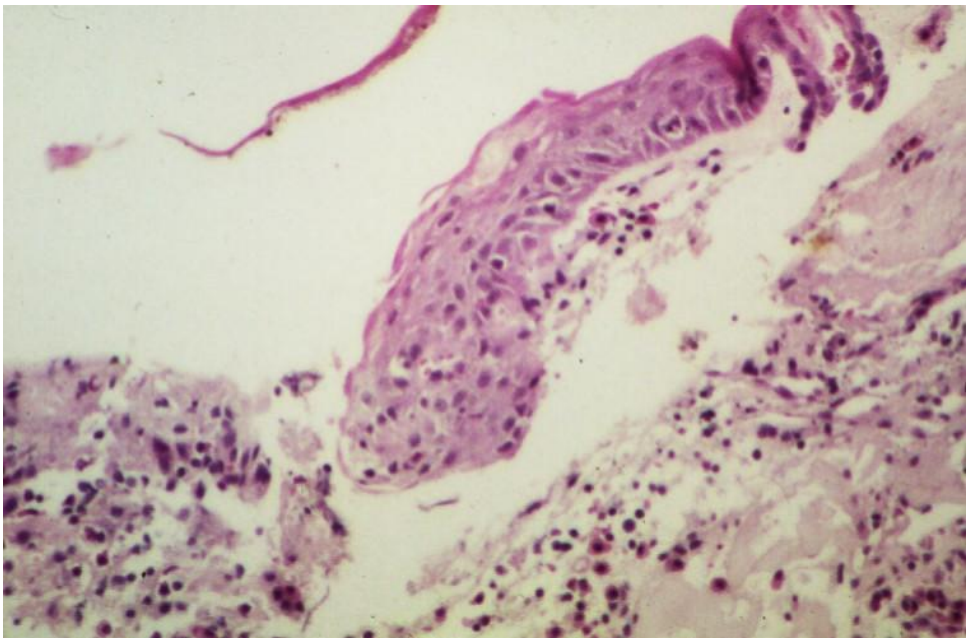


Figure 5a: Skin ulceration in a moribund *B. marinus* from Westmoreland Station, Northern Territory, Australia, showing ulcerating nodules on the skin due to mucormycosis.

Figure 5b: Histological section shows that the ulcer is associated with a necrosis of dermis and epidermis and an acute inflammatory response. Sphaerules of *M. amphibiorum* were found in the inflammatory reaction. H&E.

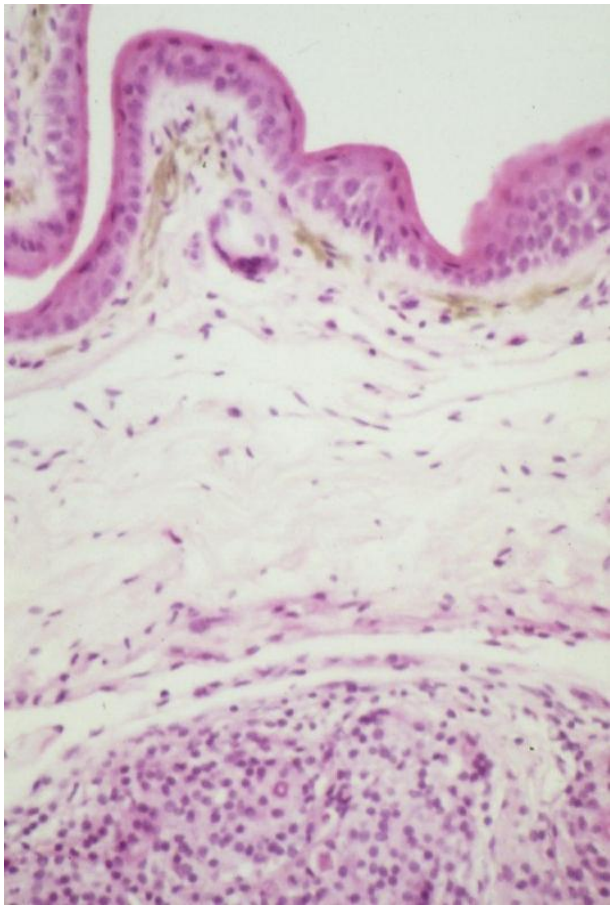


Figure 5c: Infection of the skin with *M. amphibiorum* appears to begin when sphaerules lodge in the subcutaneous lymphatic sinuses. This image shows an intact epidermis and dermis overlying a chronic granulomatous lesion in the lymph sinus. Involvement of the skin (dermis, epidermis and dermal lymphatic sinuses) was found in 42% of *B. marinus* surveyed in Australia (Speare et al 1999), but skin ulcerations were the exception. *M. amphibiorum* could gain access to the external environment through skin ulcerations, in faeces when the intestine is infected and in urine with bladder infection.

PREVENTING SPREAD IN CAPTIVITY

In the wild the fungus has yet to be shown to have a significant impact on amphibian populations. However, in captivity mortality rates in outbreaks have been high. No control strategies have been trialled. From what we know of the biology and

transmission of *M. amphibiorum*, we can devise theoretical control strategies for captive colonies.

STOPPING TRANSMISSION

INFECTIVE EVENT:

Sporangiospore is ingested with food.

STRATEGIES:

- Prevent infection by feeding food not contaminated with sporangiospores of *M. amphibiorum*. Since the sporangiospores can't be tested for, the next line of strategies are to avoid feeding insects contaminated with soil. Use only live food raised in captivity in a defined non-natural environment.
- Use the same feeding bowl in the same tank, and don't use in different tanks.
- Clean feedings bowls before use by using dimethyl didecyl ammonium chloride (DDAC).
- Keep handling equipment confined to a single tank. Don't cross contaminate tanks by hand or equipment.
- Use separate gloves to handle each tank or disinfect gloves between tanks with DDAC.
- Prioritise handling of amphibians according to risk of carrying pathogens; e.g., handle quarantined or ill amphibians after amphibians that are well or normal.

CONTROLLING THE SOURCE

M. amphibiorum in the captive environment: Sporangiospores are generated from the mycelium after the fungus has been growing for at least a week. In captive husbandry, the fungus would have to become established on a substrate that is suitable for growth. Soil is such a substrate. We do not know if the fungus grows on plant substrate. By preventing growth of the fungus sources within collections should be eliminated.

STRATEGIES:

- Sterilise all soil used in housing frogs by autoclaving. If this is not possible, kill fungi using DDAC. This fungicide is used in agriculture and forestry.
- Use DDAC as a fungicide for washing tanks and inanimate objects.

DIMETHYL DIDECYL AMMONIUM CHLORIDE (DDAC)

DDAC is a highly active fungicide. It kills *Batrachochytrium* at a concentration of 0.1% (Johnson et al 2002). DDAC has not been evaluated against *M. amphibiorum*, but it appears to be active against a wide range of fungi, including many found in the natural environment. Use at 0.1% concentration for soaking equipment and tanks

and at 1% for spraying in complex environments. DDAC is available in Australia as Path-X from Nutri Tech Solutions, Eumundi, Qld.

However, although DDAC is said to be safe in the environment, we have yet to test it for toxicity on frogs. Use it as you would any other disinfectant and do not apply it directly to amphibians or allow them to come into contact with DDAC.

For additional details on quarantine procedures for amphibians in captivity see Lynch 2001.

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