CHYTRIDIOMYCOSIS IN AMPHIBIANS

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1. OVERVIEW OF CHYTRIDIOMYCOSIS

Catastrophic amphibian population crashes over the last 30 years have been caused by the spread of an emerging fungal disease, chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis*. The arrival of this pathogen in naïve populations has caused about 122 extinctions worldwide, and is the worst example of a disease impacting on biodiversity (Skerratt et al 2007- http://www.jcu.edu.au/school/phtm/PHTM/frogs/papers/skerratt-2007.pdf).

For reviews see:


THE BD SEQUENCING PROJECT

The *Batrachochytrium dendrobatidis* sequencing project is part of the Broad Institute Fungal Genome Initiative. Its goal is to release an annotated assembly from 10X genome sequence coverage for *Batrachochytrium dendrobatidis* diploid strain JEL423, which was provided by Dr. Joyce Longcore at The University of Maine. This strain was isolated from a sick *Phyllomedusa lemur* frog in Panama. Genomic DNA from this strain was prepared by Tim James at Duke University.

http://www.broadinstitute.org/annotation/genome/batrachochytrium_dendrobatidis/MultiHome.html

2. DISTRIBUTION AND SPECIES INFECTED

GLOBAL

The global Bd mapping project maintains interactive maps and collates updated information and news at: http://www.bd-maps.net/


AUSTRALIA

A database of over 10,000 Australian records was collated and published in 2010:

The database is available online at http://esapubs.org/archive/ecol/E091/108/metadata.htm

The database is also available via interactive maps maintained by Atlas of Living Australia: http://collections.ala.org.au/public/showDataResource/dr642

### SUMMARY OF SPREAD AND IMPACT WITHIN AUSTRALIA

*B. dendrobatidis* is not native to Australia. It probably arrived in southeast Queensland in the mid-1970s on imported specimens of the African clawed frog, *Xenopus laevis*. However, this is speculative. Our earliest record in Australia is December 1978 in the Conondale Ranges, west of Brisbane and our hypothesis is that the amphibian chytrid fungus escaped from captivity in Brisbane and spread centripetally. Once *B. dendrobatidis* invades an area, it stays. Epidemiological evidence indicates that the amphibian chytrid fungus spread north and south as an advancing front, colonising frogs and water bodies as the front passed. The rate of advance has been estimated as 100 km/year in coastal Queensland and 20-30 km/year in Central America.

From museum records the estimate of the earliest appearance of *B. dendrobatidis* in each zone has been obtained; east coast zone - December 1978, southwest zone - October 1985, and Adelaide zone - May 1996. With more extensive retrospective searching, we may be able to establish earlier arrival dates in each zone. We speculate that each zone was initiated by *B. dendrobatidis* escaping into the wild from an infected amphibian imported from an infected Australian zone or another country.

As the invasive front infected naive amphibian populations, the outcome was determined by interplay between pathogen, host and environment. Some species of amphibians were exquisitely sensitive to chytridiomycosis, occurred in habitats that favoured virulence and became extinct. Of these, *Taudactylus acutirostris*, was a species made extinct by *B. dendrobatidis*; the last population crashed as a result of chytridiomycosis in the upland rainforests of North Queensland in September 1993 and the last known specimen died from chytridiomycosis in captivity at the Melbourne Zoo in 1995.
The Southern Corroboree frog is currently Australia’s most endangered frog, relying on intensive captive breeding until a solution is found to enable released frogs to survive.


3. PROTOCOLS AND TECHNIQUES FOR WORKING WITH THE AMPHIBIAN CHYTRID FUNGUS

DIAGNOSTIC METHODS

DIAGNOSIS OF CHYTRIDIOMYCOSIS IN AMPHIBIANS BY HISTOLOGIC EXAMINATION


Please see Attachment 1.

MOLECULAR DIAGNOSTIC PROTOCOLS FOR CHYTRIDIOMYCOSIS


MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS (OIE 2013)

The OIE Manual of Diagnostic Tests for Aquatic Animals describes standards for laboratory diagnostic tests. See Chapter 2.1.1 Infection with *Batrachochytrium dendrobatidis*

http://www.oie.int/en/international-standard-setting/aquatic-manual/access-online

PROTOCOLS FOR SURVEYING POPULATIONS


PROTOCOLS USED IN CULTURING *BATRACHOCHYTRIUM DENDROBATIDIS*

CULTURE METHODS FOR BD

Written by Rebecca Web. See Attachment 2.

ISOLATION OF *BATRACHOCHYTRIUM DENDROBATIDIS* FROM FROG SKIN

Written by Joyce Longcore. See attachment 3.

4. TREATMENT OF CHYTRIDIOMYCOSIS

Treatments include the following:

- Itraconazole (50-100 mg/L) is commonly used as a 5-minute bath exposure daily for 10 days in frogs, but is toxic to some species. In tadpoles, 5-minute daily baths of 0.5–1.5 mg/L itraconazole were effective (Garner et al. 2009).
- Voriconazole spray (1.25 mg/L) daily for 7 days cured frogs and toads (Martel et al. 2011). Voriconazole appeared safe in tadpoles but was not tested in infected tadpoles.
- Terbinafine hydrochloride in alcohol (available over the counter as Lamisil AT [Novartis]) was used at 0.01% or 0.005% as a 5-minute soak for 5 days, and successfully treated six amphibian species (Bowerman et al. 2010).
- Chloramphenicol (20 mg/L) was used successfully as a shallow continuous bath for 28 days; frogs were removed to a dry tub for 1 hour per day for feeding (Young et al. 2012).
- Heating to 32 °C for 5 days was also effective in a heat-tolerant species (Retallick & Miera 2007).

Clinically infected animals may require supportive electrolytes to recover and be cured (Young et al. 2012). Amphibians under treatment should be held in simple tubs with minimal substrates that can be disinfected or changed at each treatment application.

PCR testing is necessary to confirm that treatment has cleared infection. The suggested protocol involves three PCR tests at least a week apart, starting the week after the last treatment (Berger et al. 2010).
To increase knowledge of the efficacy and safety of treatments, results should be reported; where possible, treatments should be conducted as experimental controlled trials (Berger et al. 2010).


5. CHYTRIDIOMYCOSIS: KEY THREATENING PROCESS AND THREAT ABATEMENT PLAN FOR AUSTRALIA

The Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) provides for the identification and listing of key threatening processes.

A threatening process is defined as a key threatening process if it threatens or may threaten the survival, abundance or evolutionary development of a native species or ecological community. For example, invasive species listed as key threatening processes are predation by the European red fox, feral rabbits or unmanaged goats.

A process can be listed as a key threatening process if it could:

- cause a native species or ecological community to become eligible for inclusion in a threatened list (other than the conservation dependent category); or
- cause an already listed threatened species or threatened ecological community to become more endangered; or
- adversely affect two or more listed threatened species or threatened ecological communities.

The assessment of a threatening process as a key threatening process is the first step to addressing the impact of a particular threat under Commonwealth law.


On 23 July 2002 the Minister for the Environment and Heritage accepted that infection with the amphibian chytrid resulting in chytridiomycosis was a key threatening process. The conclusions of the advice given to the Minister by the Threatened Species Scientific Committee were that:

"The threatening process meets s188(4)(a), s188(4)(b) and s188(4)(c) of the EPBC Act. The threatening process could cause native amphibian species to become listed as threatened, could cause listed threatened species to become listed in another category representing a high degree of endangerment, and adversely affects at least 5 listed threatened amphibian species."
"A Threat Abatement Plan is considered to be a feasible, effective and efficient way to abate the process."

The 2006 Chytrid TAP is currently under review but has not yet been replaced. Please see Attachments 4a and 4b for the 2006 Chytrid TAP.

6. OTHER MANAGEMENT PLANS FOR CHYTRIDIOMYCOSIS

Tasmanian Chytrid Management Plan –

A Country-level Strategic Plan to Mitigate the Threat of Chytridiomycosis in Panama 2010 – 2015:

Chytridiomycosis management plan for the lesser antilles region: minimising the risk of spread, and mitigating the effects, of amphibian chytridiomycosis.

The Disease Strategy Manual for Batrachochytrium dendrobatidis (emergency outbreak response plan) 2013

RACE: Risk Assessment of Chytridiomycosis to European Amphibian Biodiversity
http://www.bd-maps.eu/


7. CONFERENCES AND NEWS

Please see our events page for a list of upcoming meetings, conferences and events.
REFERENCES


