

ATTACHMENT 7: Nomination of amphibian chytridiomycosis as a key threatening process

A nomination for listing amphibian chytridiomycosis as a key threatening process in Australia was lodged with the Threatened Species Scientific Committee for its consideration and subsequent advice to the Minister for Environment and Heritage. Nomination was made by Rick Speare on behalf of the Core Working Group which met on the 30 August 2000, the day after the Workshop at Getting the Jump on Amphibian Disease. The Core Working Group decided unanimously that a nomination should be submitted. The nomination was lodged electronically on 20 December 2000 and in hard copy on 11 January 2001.

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Nomination for listing of amphibian chytridiomycosis as a key threatening process under the *Environment Protection and Biodiversity Conservation Act 1999*

Key Threatening Process Details

A description of the threatening process that distinguishes it from any other threatening process, by reference to [EPBC Regulation 7.07 2(a)]:

(i). Its biological and non-biological components.

The nominated process

The process nominated is a specific disease, chytridiomycosis, caused by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*. Chytridiomycosis was first identified as a major disease of wild amphibians by Berger et al (1998) in a report that described the disease, the novel chytrid fungus that caused it, listed 11 species of Australian frogs and 7 species of Panamanian frogs killed by the amphibian chytrid and proposed that chytridiomycosis was a proximate cause of amphibian declines in eastern Australia and Panama.

***Batrachochytrium dendrobatidis*, the cause of chytridiomycosis**

B. dendrobatidis is a fungus in the phylum Chytridiomycota Class Chytridiomycetes Order Chytridales having characteristics separating it from all other chytrid fungi (Longcore et al 1999). *B. dendrobatidis* appears in two main forms; a spherical sessile zoosporangium (Fig. 1), 10-40 µm in diameter, and a motile zoospore (Fig. 3), about 2 µm in diameter. The motile zoospore attaches to the substrate, develops rhizoids, and becomes a zoosporangium (Fig. 1). Zoospores form within the zoosporangium and are released into the external environment as motile zoospores via discharge tubes (Fig. 2).

Most species within this phylum occur as free-living saprophytic fungi in water and soil. *B. dendrobatidis* is unique in the Chytridales in that it invades the skin of amphibians. Zoosporangia grow in the superficial keratinised layers of the epithelium only. In frogs *B. dendrobatidis* can invade any of keratinised epidermis (Fig. 4), but appears to be most commonly found on the feet. Discharge tubes penetrate through to the surface of the skin and allow zoospores to escape (Fig. 5). In early tadpole stages *B. dendrobatidis* is found in the mouthparts as this is the only keratinised epithelium on the body (Fig. 6). In later tadpole stages as the tail and feet become keratinised and the mouthparts are lost, zoosporangia begin to invade these sites.

Molecular biological studies using sequencing of the ITS 2 region so far support a conclusion that the isolates belong to a single, closely related population (Daszak, pers com 2000). Work in USA and Australia showed little variation within this region. Initial conclusions about 7 strains from molecular studies in Australia (Morgan 2000) appear to be a result of multiple poorly supported trees generated by this low variation. Joyce Longcore's initial results using a different technique also show little variation in the genome of *B. dendrobatidis* (Longcore, pers com 2000). However, the

work is not yet complete, and these are preliminary conclusions only. The lack of variation in a conserved region of the genome adds support to the hypothesis that *B. dendrobatidis* is a newly emerged agent.

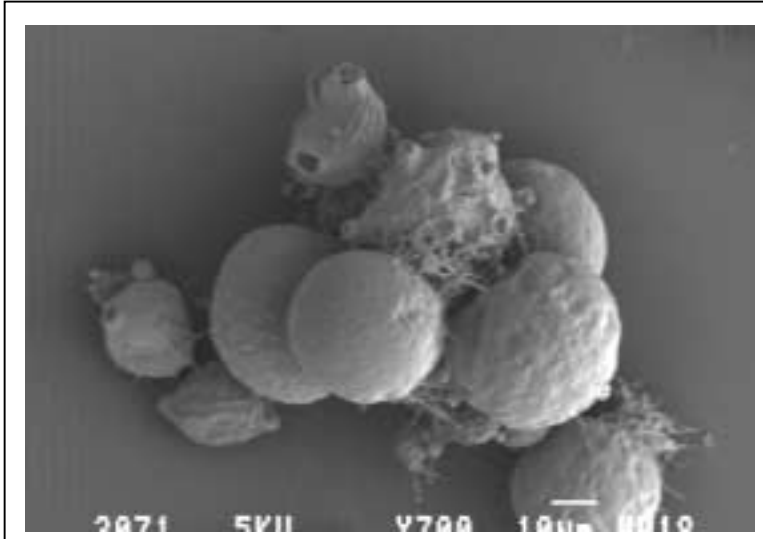


Figure 1: Zoosporangia of *Batrachochytrium dendrobatidis* growing in vitro. Note the roughly spherical shape and the discharge tubes through which the zoospores escape. In this picture most discharge tubes are open. The fine fibres are rhizoids growing from the zoosporangia. [High resolution of SEM (freeze dried) made by Lee Berger and Alex Hyatt]

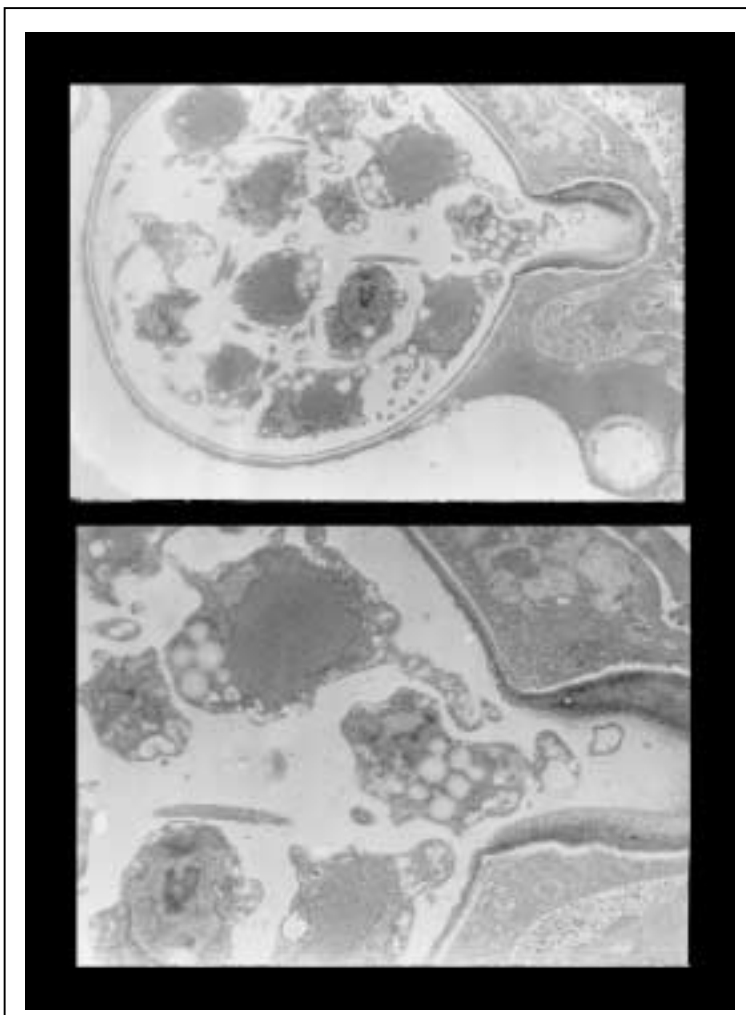


Figure 2: Zoosporangium of *Batrachochytrium dendrobatidis* showing early development of zoospores and prominent discharge tube with plug.

[Images from Lee Berger and Alex Hyatt]

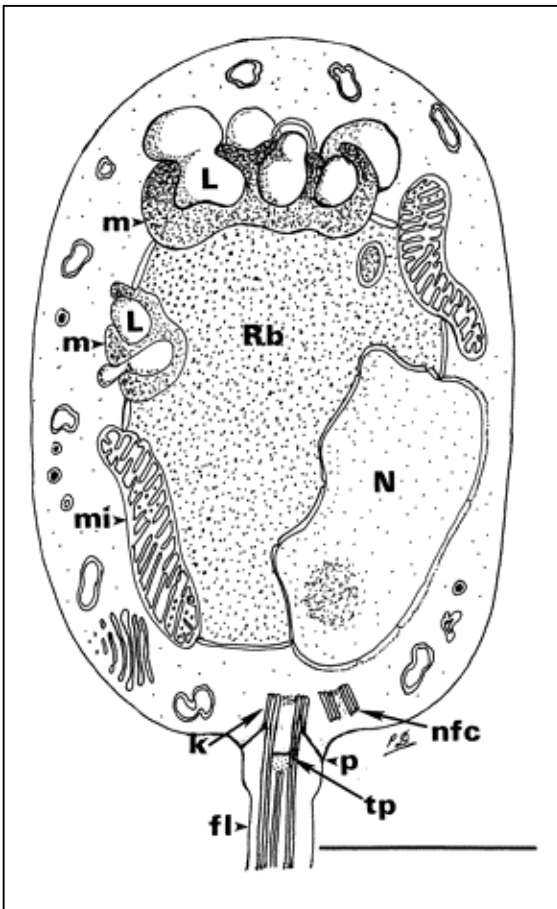


Figure 3: Drawing of the ultrastructure of a zoospore of *Batrachochytrium dendrobatidis*. The ultrastructural morphology of the zoospore is of major importance in the taxonomy of genera and species in the Chytridales. [Figure 4 from Berger et al (1998)]

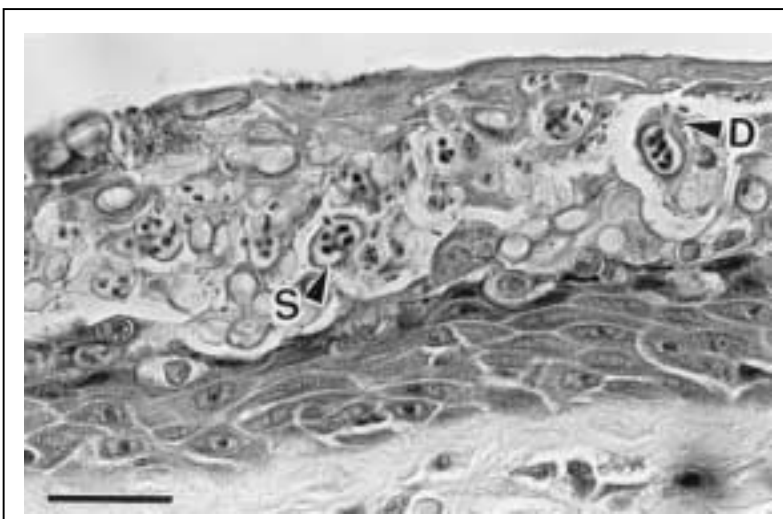


Figure 4: Histological section of the epidermis of *Litoria caerulea* infected with chytridiomycosis. The zoosporangia (S) invade the keratinised layer only, the most superficial layers. The dark bodies within the zoosporangia are mature zoospores. A discharge tube is visible on the right (D). [Fig 1 histological section from Berger et al (1998)].

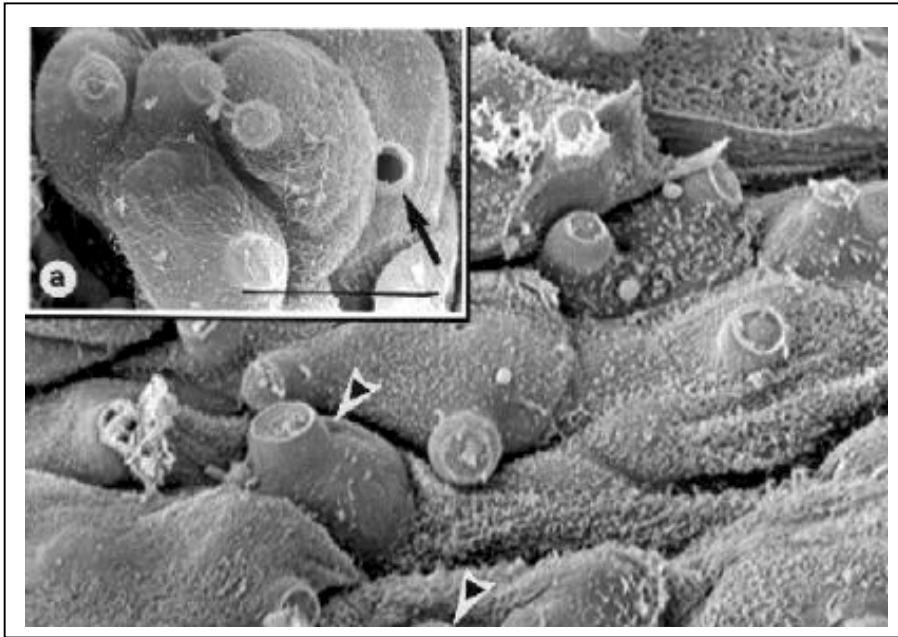


Figure 5: Surface of the epithelium of a *Litoria caerulea* with chytridiomycosis showing discharge tubes of zoosporangia of *B. dendrobatidis* emerging from the surface. Most plugs in the discharge tubes are still intact; in the inset one plug has disappeared (black arrow). [Fig 2 SEM from Berger et al (1998)].

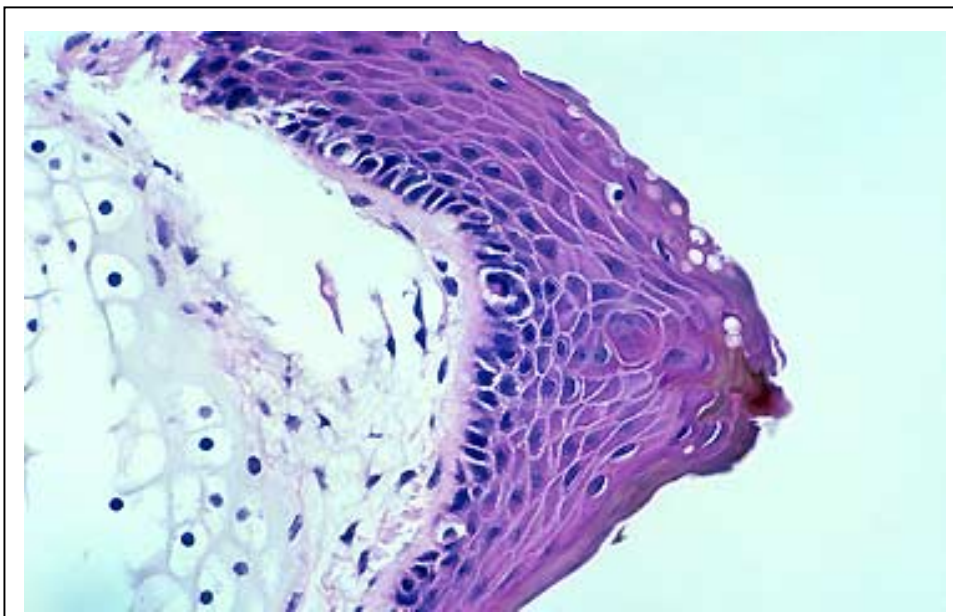


Figure 6: Histological section of mouthparts of tadpole infected with *B. dendrobatidis*. The zoosporangia are visible as clear circular spaces in the superficial keratinised epidermis on the right. [Fig. 6 from Berger et al (1999b)]

Chytridiomycosis: the disease

Typical clinical signs in Australian frogs with chytridiomycosis are related to the central nervous system and to the skin (Fig. 7). Neurological signs manifest as abnormal behaviour; typically lethargy, inappetance, sitting unprotected in the open often with limbs held slightly away from the body rather than tightly adducted against the body, and more obvious signs such as fitting with body rigid and hind limbs extended. Fitting is sometimes induced in an ill frog by handling. Skin signs include skin discolouration, and sloughing of the superficial layer of the skin. However, the degree of sloughing can vary markedly between specimens from obvious slough particularly on the feet to roughing of the skin barely detectable with the naked eye (Berger et al 1999a;). Death may take 12 or more hours in frogs with neurological signs, but can occasionally occur suddenly even in frogs showing minimal clinical signs.



Figure 7: *Mixophyes fasciolatus* in terminal phases of chytridiomycosis with depressed attitude, half closed eyes and accumulation of sloughed skin over the body (arrow head). [Fig. 2 in Berger et al (1999a)]

In the skin of frogs with chytridiomycosis the zoosporangia occur most commonly in the layer of epithelium just below the surface. The keratinised layer is thickened with an irregular surface. Typically, cells in the layer just below the epithelial surface disappear leaving empty spaces containing zoosporangia and debris of epithelial cells (Fig. 8).

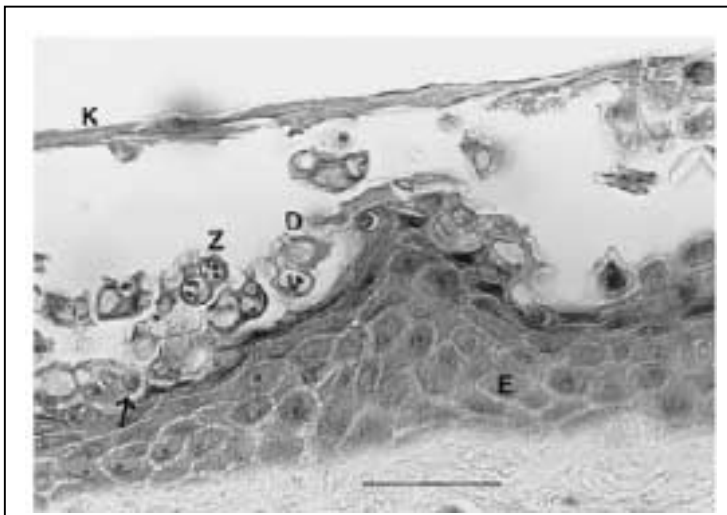


Figure 8: Histological section of heavily infected skin from a wild caught *Litoria caerulea*. The layer of epidermis just below the surface has disappeared and the surface layer is sloughing. [Fig. 4 in Berger et al (1999a)]

Changes in internal organs are non-specific. Neurological signs and death are probably due to absorption of a toxin secreted by *B. dendrobatidis*, but the mechanism has yet to be determined.

Transmission of chytridiomycosis

The infective stage of *B. dendrobatidis* is the zoospore and transmission requires water. The zoospore has no tolerance to dehydration (Berger et al 2000). Frogs can be experimentally infected by placing them in water containing zoospores. In one infection experiment an inoculating dose of 100 zoospores caused chytridiomycosis in all 3 *M. fasciolatus* while 10 zoospores did not cause disease in another 3 (Berger et al 2000). Zoospores released from an infected amphibian, enter the water and can potentially infect other amphibians in the same water. This has been demonstrated experimentally (Berger et al 1998), and it is assumed that the same process occurs in the natural situation. However, the dynamics of infection in the wild has not been studied.

Batrachochytridium dendrobatidis appears to persist in the environment independently of the size of amphibian populations. In stable populations once an amphibian has been found with chytridiomycosis subsequent infection with *B. dendrobatidis* is found in members of that population if the search effort is adequate (Kirkpatrick and Aplin 2000). The current hypothesis is that *B. dendrobatidis* does not depend on amphibians for its existence and either lives saprophytically or has alternative hosts.

The pattern of occurrence of *B. dendrobatidis* can be interpreted as indicating that it is an exotic pathogen that spreads from infected foci into adjacent areas both along and between waterbodies. In Queensland, Victoria and Western Australia chytridiomycosis may have spread at approximately 100 km per year (Kirkpatrick and Aplin 2000; Speare 2000a,b), although at least the Queensland data have alternative interpretations (Alford and Richards 1999). The mechanism of spread is unknown, but may involve normal movement of individual infected amphibians, waterbodies and surface water possibly during rain.

Impact of chytridiomycosis on wild amphibian populations

The evidence to support the significant negative impact of the amphibian chytrid fungus on wild amphibian populations has become more convincing with time and further research. The amphibian chytrid has now been associated with amphibian population declines in eastern Australia, Western Australia, New Zealand, USA, Panama, Ecuador, and Spain. At least one Australian species, *Taudactylus acutirostris*, appears to have been made extinct by the amphibian chytrid, even the last specimen dying in captivity from chytridiomycosis in Melbourne Zoo in 1995. Several other species may well have succumbed to the disease as it has been isolated from sick and dying animals in the field as well as in museum specimens (Berger et al 1998, Berger et al 1999a). A number of species currently listed as 'extinct' may well have been eliminated by the disease. However, the frogs had disappeared prior to any knowledge of this disease and specimen numbers in museums were too small to enable statistically useful samples to be assessed. Other species have undergone population declines with loss of upland populations; had reduction in population numbers (Richards et al 1993, McDonald and Alford 1999, Hines et al 1999, Gillespie and Hines 1999, Osborne et al 1999); and had sporadic deaths associated with chytridiomycosis (Speare 2000a).

Chytridiomycosis is the major disease threatening wild amphibians

In a conference / workshop, Getting the Jump on Amphibian Diseases, held in Cairns on 26-29

August, chytridiomycosis was identified as the major formidable infectious disease of amphibians globally and strategies developed to lessen its spread and impact. Amphibian chytridiomycosis is an emerging infectious disease of amphibians and has been recognised as such on a global scale (Daszak et al 1999; Daszak et al 2000).

The amphibian chytrid can probably infect all amphibian species

The amphibian chytrid appears capable of infecting any species of amphibian. To date in Australia 44 species of native frogs have been found infected, along with 2 introduced species of amphibian, the cane toad and the axolotl (Table 1). Globally, infections with the amphibian chytrid have been detected in 2 orders (Anura and Caudata), 15 families and 94 species of amphibians.

Table 1: Amphibians found infected with the amphibian chytrid globally by family and number of species. (Speare & Berger (2000b) is a collation of reports from many sources).

Country	Families	Number of species	Year of first record in country	Reference
Australia	Ambystomatidae Bufonidae Hylidae Myobatrachidae	1 1 22 24	1978	Speare & Berger (2000b)
New Zealand	Hylidae	2	1999	Waldman et al (2000)
Europe: Spain	Discoglossidae	1	1997	Bosh et al (2000)
Europe: Germany	Amphiumidae Dendrobatidae Hylidae Proteidae Ranidae Salamandridae Sirenidae	1 10 3 1 1 1 1		Speare & Berger (2000b)

North America: USA	Ambystomatidae	1	1974	Speare & Berger (2000b)
	Bufoidea	3		
	Cryptobranchidae	1		
	Dendrobatidae	3		
	Hylidae	2		
	Microhylidae	1		
	Ranidae	6		
Central America: Panama	Bufoidea	3	1994	Berger et al (1999)
	Centrolenidae	2		
	Leptodactylidae	2		
Central America: Costa Rica	Bufoidea	1	1992	Speare & Berger (2000b)
South America: Ecuador	Bufoidea	1	1989	Berger et al (1999)
	Hylidae	1		
	Leptodactylidae	1		
South America: Uruguay	Ranidae	1	1999	Mazzoni (2000)
Africa: South Africa	Pipidae	1	2000	Speare & Berger (2000b)
Africa: Kenya	Ranidae	1	1999	Speare & Berger (2000b)
Africa: west Africa	Pipidae	1	1998	Speare & Berger (2000b)

The amphibian chytrid is a virulent pathogen of amphibians

B. dendrobatidis is a highly virulent pathogen of amphibians capable at the minimum of causing sporadic deaths in some populations and 100% mortality in other populations. The inoculating dose is low, 100 zoospores able to cause clinical chytridiomycosis within 4 weeks (Berger et al 2000). Some species of amphibians appear highly susceptible to develop disease progressing to death, while other species appear less susceptible to disease manifestations (Berger et al 1999, McDonald and Alford 1999, Speare 2000a,b).

The amphibian chytrid appears to expand its geographic range

In Australia, Panama and New Zealand *B. dendrobatidis* has appeared suddenly and then expanded its geographic range. The word "appeared" is used here since the initial prospective and retrospective searching has been concentrated on areas where amphibian declines have occurred and

without a similar intensity of searching in areas where no amphibians have died from chytridiomycosis, a sampling bias is possible (Alford 2000). However, in the countries listed above as the intensity of retrospective searching has increased, the evidence in support of the hypothesis of sudden arrival of *B. dendrobatidis* and expansion into adjacent zones has increased not decreased.

In Australia chytridiomycosis has been found in amphibians from 4 geographic areas: a large eastern coast zone extending from Big Tableland near Cooktown in the north to Melbourne in the south, an Adelaide zone, a southwest zone which includes the whole of south west Western Australia to just north of Perth, and a central Kimberley zone. Based on current records (Speare and Berger 2000a), the Australian epidemic appears to have commenced in southeast Queensland (SEQ) in the late 1970s (December 1978 is the earliest record) and extended north and south along the east coast. However, the appearance of commencing in SEQ may be an artifact of searching since intensive monitoring and collecting of amphibians was occurring in this region; declines in northern NSW where monitoring was less intense may not have been detected. Chytridiomycosis in Western Australia appears to have commenced south of Perth in mid-1985 and subsequently spread in all directions, currently occurring over a wide area of southwest Western Australia (Kirkpatrick and Aplin 2000). The sudden appearance of *B. dendrobatidis* has been demonstrated in both locations of SEQ and southwest WA by retrospective examination of museum specimens, with 700 amphibians negative prior to the 1985 appearance in WA and 110 negative prior to the 1978 appearance in SEQ. Two other Australian foci of chytridiomycosis have been shown: Adelaide and environs - 1996 and Kimberley Region of WA - 1999 (Speare and Berger 2000a), but retrospective testing at these locations has not been done. One hundred and twenty amphibians tested from Northern Territory in 1999 were free of chytridiomycosis (Speare pers com 2000) and prompted a prohibition of imports of amphibians into NT by the Northern Territory Parks and Wildlife Commission (Freeland 2000).

Since no resting stage of *B. dendrobatidis* has been found, the hypothesis is that new zones are initiated by the escape of the amphibian chytrid from infected amphibians bought into that area. In the case of amphibians deliberately moved infection could be initiated by release of the infected amphibian or by release of water containing zoospores. This hypothesis applies to new infection in countries and new infections in regions within countries. The later is the situation with respect to apparently chytrid-free areas in Australia. The earliest record of the amphibian chytrid globally is in USA in *Rana pipiens* in Colorado in 1974 (Carey et al 1999). The initial origin of *B. dendrobatidis* has yet to be determined. The most likely explanation for the appearance of *B. dendrobatidis* as an emerging infectious disease of global significance is the escape of the pathogen from a host and locality with which it has evolved into host species and environments that are novel to the pathogen. The current likely source is *Xenopus* spp. in Africa. *B. dendrobatidis* is present at a high prevalence in *Xenopus laevis* in South Africa, but appears to cause no obvious morbidity (Speare and Berger 2000b).

Amphibians with chytridiomycosis are not uncommon in the amphibian pet trade. In Australia, 3 of 6 axolotyls were infected when purchased in Townsville and Perth (Speare 2000a). In Germany over 200 amphibians in the pet trade have been found to have chytridiomycosis over a number of years. However, chytridiomycosis in wild amphibians was reported in Germany only in 1999 in a localised die off of *Rana arvalis* outside Berlin (Mutschmann 2000). Infected amphibians moved deliberately in the pet trade between or within countries are a risk for bringing chytridiomycosis to new areas. Similarly, amphibians sold and moved for scientific studies can be a potential infection risk. *Xenopus laevis* and *X. tropicalis* caught in the wild in Africa and moved into scientific institutions in South Africa (Speare and Berger 2000b) and USA (Reed et al 2000) respectively have been shown to be infected with chytridiomycosis. Amphibians accidentally moved in agricultural produce

also have the potential to move *B. dendrobatidis* long distances, either within or between countries. Native Australian frogs rescued from produce in Melbourne have been found to be infected with *B. dendrobatidis* (Marantelli and Hobbs 2000).

(ii). **The processes by which those components interact (if known)**

Interaction between host, environment and pathogen is important

In Australia and Panama the most severely affected populations have been upland populations. Only populations at higher altitude (>400 m) have suffered 100% mortality. This has been seen in the wet tropics (Richards et al 1993, Laurance et al 1996, McDonald and Alford 1999), NSW uplands (Hines et al 1999, Gillespie and Hines 1999) and in the Victorian alps (Gillespie and Marantelli 2000). This effect appears to be due to low temperature. In an experimental study the mortality rate of chytridiomycosis was 100% at temperatures of 17°C and 22°C while at 27°C 50% survived and remained asymptomatic carriers (Berger 2000). The effect of low temperature is also supported by data from translocation studies in the wet tropics which showed increased mortality rates with low temperature at both upland and lowland sites (Retallick and Dwyer 2000). Increases in prevalence of infection with *B. dendrobatidis* in winter have also been shown in samples of apparently healthy amphibians examined from Western Australia and the wet tropics (Kirkpatrick and Aplin 2000, Retallick and Dwyer 2000).

For many host populations of poikilotherms exposed to virulent pathogens increases in morbidity and mortality of infectious diseases often occur with increases in stressful environmental conditions. Amphibian examples of this phenomenon are less well documented, but some data are available. In an experimental infection disease was more severe in a group of *Bufo marinus* that was artificially stressed and exposed to a fungus that caused chromomycosis (Cicmanec et al 1973). Similarly, susceptibility to bacterial infection increased with various physical and chemical environmental stresses (Colt et al 1984a,b; Carey et al 1996), including exposure to sublethal doses of pesticides (Taylor et al 1999). Amphibian populations living in regions where *B. dendrobatidis* is endemic are liable to be much more vulnerable if environmental stressors are present than if they are not. When other stressors impact on populations where *B. dendrobatidis* is present, the mortality rate due to chytridiomycosis will be expected to rise. However, this has not been proven by experimental studies. If this does prove to be the case, reducing both physical and chemical environmental stressors to a minimum becomes a major tool in decreasing the impact of chytridiomycosis on populations where *B. dendrobatidis* is present.

A name for the threatening process [EPBC Regulation 7.07 2(b)]:

Chytridiomycosis due to the amphibian chytrid fungus.

Name any species or ecological communities listed as threatened under the Environment Protection and Biodiversity Conservation Act 1999 that are considered

to be adversely affected by the threatening process [EPBC Regulation 7.07 2(c)]:

The epidemiological and pathological evidence relevant to threatened amphibians is:

1. *B. dendrobatidis* appears capable of infecting any species of amphibian
2. *B. dendrobatidis* appears capable of causing death of individuals of most amphibian species
3. Some species of amphibian appear to be highly susceptible to death once infected with *B. dendrobatidis* owing to innate characteristics of the species or to environmental factors operating at their geographic location or a combination of the two.

Hence, all amphibian species listed as threatened and vulnerable species could be infected by *B. dendrobatidis* and some individuals would be expected to die as a direct result of this infection. The appearance of *B. dendrobatidis* in any amphibian population will at the minimum make it much more susceptible to adverse effects of other factors, including environmental ones, acting to reduce the population size. To date 44 species of native frogs, including 14 species listed as threatened, have been found to be infected with *B. dendrobatidis*.

Some amphibian species listed as threatened are known to have been severely impacted by *B. dendrobatidis*, including one species made extinct by chytridiomycosis. The populations of *Taudactylus acutirostris* (now listed in the category "Species that are extinct") appear to have suddenly declined as a result of epidemic chytridiomycosis. Two animals were seen in the field in late 1995 and November 1996, but none have been detected since. Three threatened species in Queensland (*Litoria nannotis*, *Litoria rheocola* and *Nyctimystes dayi*) had upland populations killed by chytridiomycosis (Berger et al, 1998). Chytridiomycosis is a known cause of death to several other species listed as threatened (*Litoria aurea*, *Litoria spenceri*, *Mixophyes fleayi*, *Pseudophryne corroboree*, *Pseudophryne pengilleyi*, *Taudactylus eungellensis*).

Table 2: Species of amphibians that are threatened and their known status re chytridiomycosis. (Species reported as infected are bolded)**Amphibian species that are endangered**

Scientific name	Common name	Status re amphibian chytrid
<i>Geocrinia alba</i>	White-bellied Frog	No reports
<i>Litoria castanea</i>	Yellow-spotted Tree Frog	No reports
<i>Litoria lorica</i>	Armoured Mistfrog	No reports
<i>Litoria nannotis</i>	Waterfall Frog	Upland populations disappeared in Qld; <i>B. dendrobatidis</i> now endemic in lowland populations (Berger et al 1998)
<i>Litoria nyakalensis</i>	Mountain Mistfrog	Upland populations disappeared in Qld
<i>Litoria rheocola</i>	Common Mistfrog	Upland populations disappeared in Qld; <i>B. dendrobatidis</i> now endemic in lowland populations (Berger et al 1998)
<i>Litoria spenceri</i>	Spotted Tree Frog	<i>B. dendrobatidis</i> associated with disappearance of some populations (Gillespie and Marantelli 2000)
<i>Mixophyes fleayi</i>	Fleay's Frog	<i>B. dendrobatidis</i> endemic in SEQ populations (Speare & Berger 2000a)
<i>Mixophyes iteratus</i>	Southern Barred Frog	Endemic in eastern NSW (Mahony 2000)
<i>Nyctimystes dayi</i>	Lace-eyed Tree Frog	Upland populations disappeared in Qld; <i>B. dendrobatidis</i> now endemic in lowland populations (Berger et al 1998)
<i>Philoria frosti</i>	Baw Baw Frog	No reports
<i>Pseudophryne corroboree</i>	Southern Corroboree Frog	<i>B. dendrobatidis</i> endemic (Speare & Berger 2000a)
<i>Spicospina flammocaerulea</i>	Sunset Frog	No reports
<i>Taudactylus eungellensis</i>	Eungella Day Frog	<i>B. dendrobatidis</i> associated with declines ; now endemic (Speare & Berger 2000a)
<i>Taudactylus rheophilus</i>	Tinkling Frog	No reports

Amphibian species that are vulnerable

Scientific name	Common name	Status re <i>B. dendrobatidis</i>
<i>Geocrinia vitellina</i>	Orange-bellied Frog	Endemic in WA population (Speare & Berger 2000a)
<i>Heleioporus australiacus</i>	Giant Burrowing Frog	Endemic in NSW population (Speare & Berger 2000a)
<i>Litoria aurea</i>	Green and Golden Bell Frog	Endemic in NSW populations (Mahony 2000; Speare & Berger 2000a)
<i>Litoria littlejohni</i>	Littlejohn's Tree Frog	No reports
<i>Litoria olongburensis</i>	Wallum Sedge Frog	No reports
<i>Litoria piperata</i>	Peppered Tree Frog	No reports
<i>Litoria raniformis</i>	Southern Bell Frog	Endemic in South Australia population (Speare & Berger 2000a)
<i>Litoria verreauxii alpina</i>	Alpine Tree Frog	No reports
<i>Mixophyes balbus</i>	Stuttering Frog	No reports
<i>Pseudophryne covacevichae</i>	Magnificent Brood Frog	No reports
<i>Pseudophryne pengilleyi</i>	Northern Corroboree Frog	Endemic in population (Speare & Berger 2000a)
<i>Taudactylus pleione</i>	Kroombit Tinker Frog	No reports

Amphibian species that are extinct

Scientific name	Common name	Status re <i>B. dendrobatidis</i>
<i>Rheobatrachus silus</i>	Gastric-brooding Frog	Not found in museum specimens
<i>Rheobatrachus vitellinus</i>	Eungella Gastric-brooding Frog	Not looked for
<i>Taudactylus acutirostris</i>	Sharp-snouted Day Frog	Extinction due to <i>B. dendrobatidis</i> (Berger et al 1998; Speare & Berger 2000a)
<i>Taudactylus diurnus</i>	Southern Day Frog	Not found in Museum specimens

Name any species or ecological community, other than those that are listed under the *Environment Protection and Biodiversity Conservation Act 1999*, that could become eligible for listing in one of those categories because of the threatening process [EPBC Regulation 7.07 2(d)]:

Predicting which species are liable to be severely impacted by chytridiomycosis is difficult. Based on past data species that are stream associated in high elevations appear to be the most susceptible to population declines (McDonald and Alford 1999, Hines et al 1999, Gillespie and Hines 1999, Hero et al 2000). Chytridiomycosis, however, does occur in arid zone amphibians (Kirkpatrick and Aplin 2000) and across a wide range of other habitats especially those in high elevations. The species currently known to be infected with chytridiomycosis are listed in Table 3. *Adelotus brevis*, one of these, has recently declined over much of its range with upland populations particularly decreased. The New England Tableland population has recently been listed as threatened by New South Wales.

Table 3: Species of native Australian amphibians that are not listed, but have populations with chytridiomycosis. All records are from the data collated by Speare and Berger (2000a).

Species	State
Hylidae	
<i>Litoria adelaidensis</i>	Western Australia
<i>Litoria sp. Kroombit Tops</i>	Queensland
<i>Litoria caerulea</i>	New South Wales, Queensland
<i>Litoria chloris</i>	New South Wales, Queensland
<i>Litoria citropa</i>	New South Wales
<i>Litoria ewingi</i>	South Australia
<i>Litoria genimaculata</i>	Queensland
<i>Litoria gracilentia</i>	Queensland
<i>Litoria infrafrenata</i>	(Captive) Adelaide
<i>Litoria lesueuri</i>	New South Wales, Queensland, Victoria
<i>Litoria moorei</i>	Western Australia
<i>Litoria nasuta</i>	Queensland
<i>Litoria pearsoniana</i>	New South Wales, Queensland

<i>Litoria tyleri</i>	Queensland
<i>Uperoleia laevigata</i>	Queensland
<i>Adelotus brevis</i>	New South Wales, Queensland
<i>Crinia georgiana</i>	Western Australia
<i>Crinia glauerti</i>	Western Australia
<i>Crinia insignifera</i>	Western Australia
<i>Crinia pseudinsignifera</i>	Western Australia
<i>Geocrinia rosea</i>	Western Australia
<i>Heleioporus eyrei</i>	Western Australia
<i>Lechriodus fletcheri</i>	Queensland
<i>Limnodynastes dorsalis</i>	Western Australia
<i>Limnodynastes dumerilii</i>	Queensland, South Australia, (captive) Melbourne
<i>Limnodynastes tasmaniensis</i>	South Australia
<i>Limnodynastes terraereginae</i>	Queensland
<i>Mixophyes fasciolatus</i>	New South Wales, Queensland, (captive) Victoria
<i>Neobatrachus kunapalari</i>	(captive) Melbourne
<i>Neobatrachus pelobatoides</i>	Western Australia

Nominee Information

The following information is subject to the provision of the Privacy Act and will not be divulged to third parties if advice regarding the nomination is sought from such parties [EPBC Regulation 7.04].

Full Name

Rick Speare

Address

School of Public Health & Tropical Medicine

James Cook University

Townsville 4811

Daytime Telephone

07-47225777 or 0419993031

E-mail

richard.speare@jcu.edu.au

Facsimile

07-47225788

Body, Organisation or Company Name (if nominee acts for an organisation):

**Core Working Group, Getting the Jump! on Amphibian
Disease Conference and Workshop, Cairns 26-30 August
2000**

Address

Business Telephone (daytime)

E-mail

Facsimile

Declaration (Please read carefully and sign the declaration)

I declare that the information in this nomination and its attachments is true and correct to the best of my knowledge.

Signed _____ Rick Speare_____

DATE __20__/_12__/_2000__

Justification for this nomination

Provide justification for listing the threatening process as a key threatening process under the *Environment Protection and Biodiversity Conservation Act 1999* (section 188), with particular reference to [EPBC Regulation 7.07 2(e)]:

- (i) the likelihood of the threatening process causing a species or ecological community to become eligible for listing in any category, other than conservation dependent;**

Since the amphibian chytrid is a highly virulent pathogen it has the potential to cause amphibian species to change threatened species status. Four patterns of response to the appearance of the amphibian chytrid have been seen; 1) species extinction, 2) extinction of local populations, but survival of the species, 3) population decline and recovery, 4) sporadic deaths. All populations in which *B. dendrobatidis* has been found and which have been intensively studied appear to show some pathological effect. No intensively studied population appears to be unaffected by the amphibian chytrid; i.e., no deaths.

Species extinction

Extinction is illustrated by the response of the sharp snouted day frog, *Taudactylus acutirostris*. The status of this frog changed from not listed, to endangered in 1992 to extinct in 1999. This frog occurred in Queensland only in a range that extended 310 km in upland wet tropics from Mt Graham to Big Tableland, just south of Cooktown (McDonald 1992). Populations were abundant in all locations, with 50-100 frogs per 100 metre transect being common (Richards et al 1993). The first populations to disappear were in 1990. Populations then disappeared in a progressively northern direction, the final population at Big Tableland disappearing by early 1994. The decline of this population was precipitous, most frogs disappearing over 3 months and survivors dying over the next 6 months. Chytridiomycosis was found in wild frogs as well as being responsible for the death of adult frogs and metamorphs bought into captivity in late 1993. The last surviving member of *T. acutirostris* was a male that died from chytridiomycosis at Melbourne Zoo in 1995. Two adults were observed in the field in late 1995 and November 1996. Since the causative agent had not been identified at that time, the therapeutic measures adopted in captivity were ineffective.

Local extinction, but survival of species

Several examples are known where chytridiomycosis has caused local extinctions of populations. Good examples of this pattern are provided by the upland wet tropics species, *Litoria nannotis*, *Litoria rheocola* and *Nyctimystes dayi*. These species followed the same pattern of population decline as *T. acutirostris*, with extinction of all upland populations. However, lowland populations on the same watercourses persisted. In the lowlands sporadic deaths occur, but population numbers appear to be stable. Experimental studies in upland wet tropics rainforest confirmed high rates of mortality in winter from chytridiomycosis in *L. rheocola* translocated from lowland sites. Frogs translocated in summer did not show a pattern of sudden high mortality and frogs at lowland sites

showed some no mortality due to chytridiomycosis in summer and a slight mortality in winter (Retallick and Dwyer 2000). The experimental studies mimicked the natural events with local extinctions at upland sites and sporadic deaths at lowland sites.

The spotted tree frog, *L. spenceri*, also showed a similar pattern when a stable population at Bogong Creek suddenly declined in 1996 and the last frog was seen in 1999 (Gillespie and Marantelli 2000). Chytridiomycosis was a cause of death in frogs autopsied. The first appearance of the fungus in a retrospective survey of toe clips was in March 1996, the last day the frogs were seen in high numbers. In lowland populations of *L. spenceri* chytridiomycosis was present, but the population did not decline.

Population declines, but recovers

At Big Tableland, *Litoria genimaculata*, a species sympatric with *T. acutirostris*, *L. nannotis* and *L. rheocola* suffered a sudden decline in numbers at the same time that *T. acutirostris* disappeared (Laurance et al 1996, McDonald and Alford 1999, McDonald unpublished data). However, the population of *L. genimaculata* recovered to close to former numbers after 2.5 years. Occasional individuals of *L. genimaculata* are now found ill or dying from chytridiomycosis, demonstrating that *B. dendrobatidis* occurs in the environment, but apparently a balance between *L. genimaculata* and the microorganism has been arrived at. The incidence of infected and dead frogs increases during the colder months.

Sporadic deaths

A pattern of sporadic deaths due to chytridiomycosis is seen with many of the species infected by *B. dendrobatidis* in Australia (Speare and Berger 2000a). Often the mortality rate rises in the colder months. The seasonal pattern has been well documented in southwest Western Australia for the motorbike frog, *Litoria moorei* (Kirkpatrick and Aplin 2000). On the east coast the common green tree frog, *L. caerulea*, in southeast Queensland shows a similar pattern. Once *B. dendrobatidis* has become established in an area and its amphibian species, sporadic death is to be expected as the minimum impact.

The amphibian chytrid is a key impact on any amphibian population that is infected. Depending on a complex interaction between the innate characteristics of individual species, the pathogen and the environment including how the species is distributed across a range of environments, the outcome for a species can range from extinction of the species as the worst case scenario, to a stable population with sporadic deaths due to chytridiomycosis. However, the vulnerability of the apparently stable population to the effect of "normal" environmental variations and to abnormal environmental factors is probably increased.

Chytridiomycosis is probably the cause of the disappearance of *Adelotus brevis* from upland areas of southeastern Queensland and northern New South Wales, and has resulted in listing of the New England Tableland population by New South Wales could result eventually in national listing of this species.

Species most likely to become newly listed as a result of chytridiomycosis are those that occupy habitat in currently chytrid-free zones. In particular frog communities in Tasmania, Cape York Peninsula and the Gulf Country in Queensland, northwest Western Australia, the Northern Territory and most of central Australia are potentially at risk as *B. dendrobatidis* expands from its current zones. The susceptibility of individual species is difficult to predict since we do not know what innate characteristics correlate with high mortality rates.

(ii) evidence that the threatening process could cause a listed threatened species or ecological community to become eligible for listing in another category representing a higher degree of endangerment;

From the summary given in section (i) chytridiomycosis in general would be expected to have a negative impact on any amphibian population. The severity of the impact due to chytridiomycosis will depend on the individual species (particularly on innate characteristics of the species), characteristics of the location that the particular populations occupy and other general environmental factors particularly sudden lowering of ambient temperature.

Chytridiomycosis by causing sudden population declines has already resulted in several species being listed as endangered; notably, *T. acutirostris*, *L. nannotis*, *L. rheocola*, *L. nyakalensis*, *L. lorica*, *M. fleayi* and *Nyctimystes dayi*. By implication chytridiomycosis is the likely cause of the population declines that led to the listing of *R. silus*, *R. vitellinus*, *T. diurnus*, *T. eungellensis*, *T. rheophilus* and *L. aurea*. Chytridiomycosis is known to be the proximate cause of the change in listing of *T. acutirostris* from threatened to extinct. The ability of any threatened species to recover is made less likely by the presence of chytridiomycosis.

Remnant populations of endangered species with low numbers could be made extinct by the arrival of chytridiomycosis in a previously chytrid free population, or by environmental stressors upsetting the balance between amphibian chytrid and frog host in populations where chytridiomycosis is present.

(iii) evidence that the threatening process adversely affects two or more listed threatened species (other than conservation dependent species) or two or more listed threatened ecological communities.

Of the 44 native species of amphibians in Australia infected by *B. dendrobatidis* 15 species are listed as threatened; chytridiomycosis has been detected in roughly 48% of the listed species. Chytridiomycosis has been found in 60% of endangered species, 42% of threatened species, 25% of extinct species, and in 15% of non-listed species. Only one of the 4 species listed as extinct has been adequately examined, and this one, *T. acutirostris*, was made extinct by chytridiomycosis. Since the disappearance of the other 3 extinct species had the same epidemiological pattern as *T. acutirostris*, the likelihood that these 3 species disappeared because of chytridiomycosis is high. Although the higher prevalences of chytridiomycosis in the listed species may be an artifact of searching due to

more intense searching of threatened species, it does suggest there may be a positive relationship between the occurrence of chytridiomycosis in amphibian populations and the vulnerability of that population.

As more species of amphibians in Australia become infected with *B. dendrobatidis* the number of listed species would expect to increase as a result.

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CORE WORKING GROUP FOR "GETTING THE JUMP ON AMPHIBIAN DISEASE" CONFERENCE AND WORKSHOP

The Steering Committee and Core Working Group were the driving force behind the Conference and Workshop. The Core Working Group met on 30 August to consider whether amphibian chytridiomycosis should be nominated as a key threatening process. The decision for nomination was unanimous at that meeting.

Members of Steering Committee and Core Working Group:

Ross Alford, C/O Tropical Biology, James Cook University, Townsville, QLD 4811 Australia.

Ken Aplin, W.A. Museum, Francis Street, Perth, Western Australia 6000.

Lee Berger, CSIRO Australian Animal Health Laboratory, PO Bag 24, Geelong, Victoria 3220

Bradford Cullen, School of Biomedical and Molecular Biology, JCU, Townsville, Queensland 4811

Liz Dovey, Environment Australia, Canberra

Tim Halliday, International Director, Declining Amphibian Task Force, Department of Biological Sciences, The Open University, Milton Keynes, MK7 6AA, UK

Harry Hines, Queensland Parks and Wildlife Service, PO Box 42, Kenmore, Queensland 4069

Alex Hyatt, CSIRO Australian Animal Health Laboratory, PO Bag 24, Geelong, Victoria 3220

Michael Lynch, Melbourne Zoo Veterinary Department, PO Box 74, Parkville, Victoria 3052

Gerry Marantelli, Amphibian Research Centre, PO Box 424 Brunswick, Victoria 3056

Keith McDonald, Queensland Parks and Wildlife Service, PO Box 834, Atherton, Queensland 4883

Stan Orchard, Frogs Australia, Worldwide Fund for Nature Australia, GPO Box 528, Sydney, New South Wales, 2001

Leigh Owens, Microbiology and Immunology, School of Biomedical and Molecular Sciences, James Cook University, Townsville Queensland 4811

Rick Speare, School of Public Health and Tropical Medicine, James Cook University, Townsville Queensland 4811 (Chair of Steering Committee and of Core Working Group)