

## Amphibian disease detectives: The History of Chytridiomycosis (Bd) discovery in Australia (1993 - 1999)

In 1993, Keith McDonald of the Queensland Department of Environment and Heritage asked Rick Speare of James Cook University to help investigate the role of disease in the disappearance of upland frogs in Queensland. Keith, a renowned frog ecologist and taxonomist, had been monitoring frog populations since the late 1970s. He had followed the disappearance of frog populations in upland sites from the original crashes in the D'Aguilar and Conondale ranges through central Queensland into the wet tropics. In 1993 one upland population of frogs remained unaffected in the wet tropics on Big Tableland, near Cooktown. This was the last known habitat of the sharp snouted day frog, *Taudactylus acutirostris*, a frog only found above 400 m altitude.

Keith realised that monitoring frog numbers allowed declines to be detected, but had not provided answers about the cause. Monitoring pesticides, heavy metals, and water quality had also shown that pollution was not involved in these pristine sites.

Rick Speare, although now working in human public health, had spent several years (1988-1990) investigating disease in the introduced cane toad, *Bufo marinus*. With funding from the Wet Tropics Management Authority, Rick joined Keith and his team in monitoring the frogs on O'Keefe Creek, Big Tableland. Soon after, mortality occurred and adults along the study site were found ill or dead. During a trip in November 1993 *T. acutirostris* numbers appeared to still be adequate, but ill waterfall frogs (*Litoria nannotis*) and common mistfrogs (*Litoria rheocola*) were found.



*Litoria rheocola* (Common mistfrog) declined due to Bd. Photo: Lee Skerratt (left)  
*Litoria genimaculata* (Green-eyed treefrog) declined due to Bd. Photo: Lee Skerratt (right)

A group of *T. acutirostris* were collected to be maintained in captivity at James Cook University, Melbourne Zoo, and Taronga Zoo. Within 3 months of this initial episode all adults of *T. acutirostris*, *L. nannotis*, and *L. rheocola* were gone from O'Keefe Creek above 400 m. Tadpoles of some species survived, but no establishment by

metamorphs were detected. Numbers of green-eyed treefrogs (*Litoria genimaculata*) declined at the same time, but the population started recovering after 2 years.

Although this was a disaster for frog populations, it was a wonderful pathological opportunity as it was the first time ill and non-autolysed dead frogs were available for examination. A disease team headed by Rick Speare, and including Kelly Field and Joy Koehler, investigated the specimens and were quickly able to rule out bacterial septicaemia as a cause; aeromonads had been postulated as agents in ecological work overseas. However, the pathology was unspectacular and indicative of a toxic or peracute viral cause. Viral isolations were unsuccessful. Early immunoperoxidase tests on histological sections for ranavirus were positive, but could not be confirmed.

Rick's epidemiological analysis of the spreading pattern of declines and the mortality event led to the breakthrough conclusion that declines in Queensland rainforest were most likely due to an introduced infectious disease. This theory was published in *Conservation Biology* in 1996 and was highly controversial.

<http://www.jcu.edu.au/school/phtm/PHTM/frogs/papers/laurance-1996.pdf>

Ron Slocombe, a veterinary pathologist working for the Melbourne Zoo, made an interesting observation early in 1994. All the *T. acutirostris* sent to the zoo had died in spite of intensive and highly skilled husbandry. Even metamorphs emerging from tadpoles died within a month of emergence. The only consistent lesions were heavy infections of the epidermis with a microscopic organism that looked like a protozoa or microspora. Undescribed, of course!

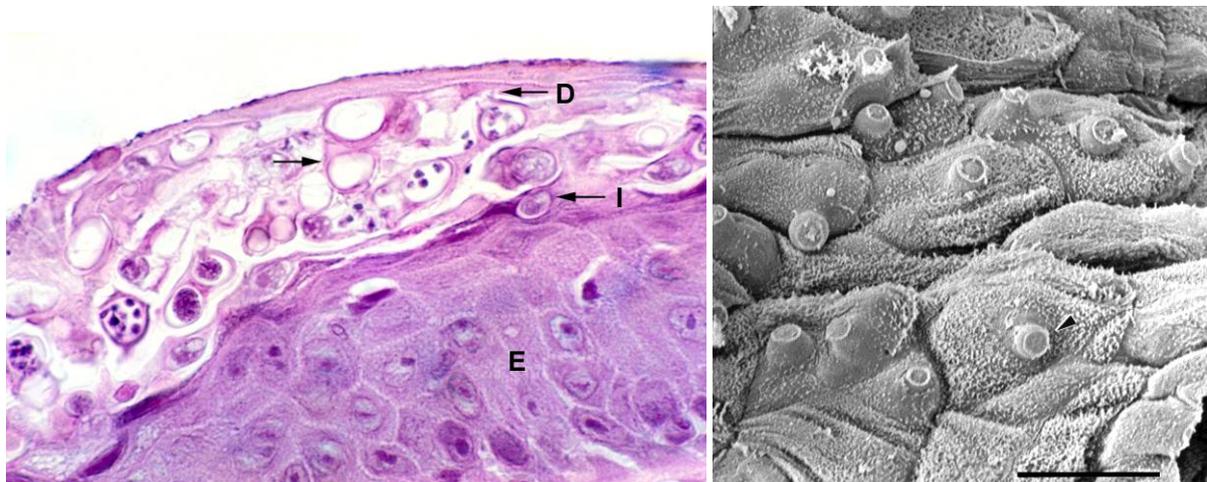
Rick Speare had also noted these organisms occasionally in the skin of the Big Tableland frogs, and more commonly in the *T. acutirostris* that had died in husbandry at JCU. At this stage, however, he dismissed them as being minor parasites unrelated to the deaths in the wild.

Rick and the Queensland Frog Recovery team decided a full time vet pathologist was needed to continue investigations into the cause of death of these frogs, and so funding was obtained from the Australian Nature Conservation Agency for a PhD stipend. Lee Berger, a veterinarian with an interest in wildlife, began work in 1995 at the CSIRO Australian Animal Health Laboratory (AAHL) to study disease in Australian frogs under the supervision of Rick Speare and Alex Hyatt. Alex and Rick had worked together previously on the Bohle ranavirus from north Queensland. Alex was also conducting experimental work on ranaviruses and *Bufo marinus*, and had experience in amphibian diseases. The combination was remarkable; AAHL with its world class expertise in the laboratory investigation of unknown viral agents, Rick Speare with expertise in field and lab investigation of infectious diseases of unknown causes in many species of animals, Keith McDonald with his extensive knowledge of Queensland frog populations, and Lee Berger with her tenacity and persistence.

As little was known about diseases in any Australian frogs, the group decided to investigate disease in both declining and non-declining populations. A

multidisciplinary network was set up involving herpetologists in universities and government departments. Sick frogs were collected from all over Australia and forwarded to the amphibian disease group for diagnostic tests. The team also recruited people with expertise in specialty areas, e.g., parasitology and molecular biology, who were keen to donate their time, and contacted groups around the world working on amphibian disease in order to share information. The approach adopted was that collaboration and open information exchange would allow us to progress much more rapidly.

After much detailed work looking for the mythical virus in the Big Tableland and other frogs, Lee Berger decided that the only possible agent was the "minor" skin parasite detected in both wild and captive frogs. In an experiment, Lee demonstrated that this agent, the amphibian chytrid, was indeed a primary pathogen. From the ultrastructure of the zoospore Peter Daszak identified the agent as a new species in a new genus. Further weight was given to this by Louise Goggin's molecular biological work which placed the organism most closely to *Chytridium confervae*.



Histopathology and SEM image of *Batrachochytrium dendrobatidis* (Bd) within the epidermal layer of an infected frog. Note the fungal tubes poking through the skin surface on the Scanning EM picture. Images: Lee Berger

The Australian frog recovery effort has been well coordinated for a number of years. As amphibian pathologists have been involved in the recovery effort since 1993, Australia has led the world in disease investigations into declining populations. We had been investigating the role of chytrid fungi in mass mortalities, and had determined it caused 100% mortality in experimental trials when it was discovered in a mass mortality event in Panama. We published the first evidence for a chytrid as a threatening force in collaboration with the US researchers, David Green and Karen Lips, working in Panama - "Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Panama" by L. Berger, R. Speare, P. Daszak, DE Green et al, in Proceedings of The National Academy of Sciences 1998; 95: 9031-9036." (<http://www.pnas.org/content/95/15/9031.full>) This

is a remarkable publication from the collaborative perspective - 14 authors from 3 continents and 11 institutions!

Independent of the Australian group, chytrid was identified as a cause of mortality in frogs at the Washington Zoo by a group consisting of Don Nichols, Alan Pessier and Joyce Longcore. Don had seen the chytrid in the skin of frogs at the zoo since 1991, and had established its identity working with Joyce Longcore, an expert on the Chytridales. To have two groups independently arrive at the same conclusion namely that the agent caused death of amphibians, and that the agent was an undescribed member of the Chytridales, increased the confidence in the conclusion.

Frog disease research is an excellent example of the benefits of global collaboration. Each person contributes in their own field, and information is shared freely for the benefit of all.

\*The Amphibian Diseases Home Page was originally funded under the Endangered Species Program of Department of the Environment and Heritage by funds from the National Heritage Trust with the assistance of Queensland Parks and Wildlife Service.

\*\* For more information on the sharp snouted day frog please go to [http://www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon\\_id=1911](http://www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=1911).

