Histological evidence of chytridiomycete fungal infection in a free-ranging amphibian, *Afrana fuscigula* (Anura: Ranidae), in South Africa

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ABSTRACT

The 1st recorded histological evidence of chytridiomycete fungal infection in a free-ranging ranid amphibian in South Africa is presented. Literature on causes of a worldwide decline in amphibian populations is briefly reviewed.

Key words: amphibian, Chytridiomycosis, dermatitis, fungus.

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Declining amphibian populations are a worldwide concern. Proposed aetiological factors, either singly or in combination, include habitat destruction; changes in weather patterns; pollution of water supplies with urea, nitrates, carbaryl, paraquat and petroleum; increased road traffic; increased exposure to ultraviolet radiation; and infectious diseases such as chytridiomycosis, saprolegniasis and infection with *Aeromonas hydrophila* or teratogenic trematodes[1,3,7,9–11,13,14,17,18,21–23].

One of the most commonly identified infectious causes of mortality is cutaneous or oral chytridiomycosis caused by the chytridiomycete fungus, *Batrachochytrium dendrobatidis*[3,8]. Dermatitis associated with a chytridiomycete was described from a number of captive anurans before the original discovery of the amphibian chytrid[15]. Clinical symptoms associated with chytridiomycosis include abnormal behaviour and body posture, as well as excessive sloughing of the skin[4].

Chytridiomycosis has been associated with population declines in Australia, New Zealand, North, Central and South America and, most recently Spain[4,6,16,19]. Amphibian cutaneous chytridiomycosis has been reported in wild *Xenopus laevis* frogs from the Western Cape Province[24]. Retrospective studies of archived material in South African museums have also identified the amphibian chytrid in South African frogs (Weldon, unpubl. data). *B. dendrobatidis* may either have been recently introduced into South Africa or it might have evolved within an African host species such as *Xenopus* spp., and been disseminated around the world in *Xenopus* used as laboratory animals[19]. This communication reports the presence of *B. dendrobatidis* zoosporangia in the superficial epidermis of a Cape river frog (*Afrana fuscigula*) collected in the Eastern Cape province of South Africa; and is the 1st confirmation of the disease in a ranid frog from South Africa.

As part of a small initial survey of free-ranging amphibians in South Africa, a freshly dead amphibian, identified as *Afrana fuscigula,* was collected from Kologha Forest, near Stutterheim, in the Eastern Cape (32°32′29″S, 27°21′57″E). No macroscopic lesions were noted, and the whole frog was preserved in 10 % formalin.

Routine histological examination of strips of the abdominal skin and whole severed feet was performed on paraffin-embedded sections (6 μm), which were stained with haematoxylin and eosin, as well as with periodic acid Schiff, and Gomori methamine silver stain[2]. Near the ends of the toes, and in the crevices between the toes, the superficial epidermis contained rare fungal structures consistent with the zoosporangia of *B. dendrobatidis* (Fig. 1). These were often empty spherical structures (up to 8 μm in diameter) whose walls stained strongly with silver stains, but weakly with periodic acid Schiff stain. A few contained 4–6 basophilic zoospores, but discharge papillae were rarely seen. Associated changes in the epidermis included moderate diffuse epidermal hyperplasia and oedema. Sloughed empty zoosporangia and necrotic epithelial cells lay on the surface of the skin. Scattered degenerative

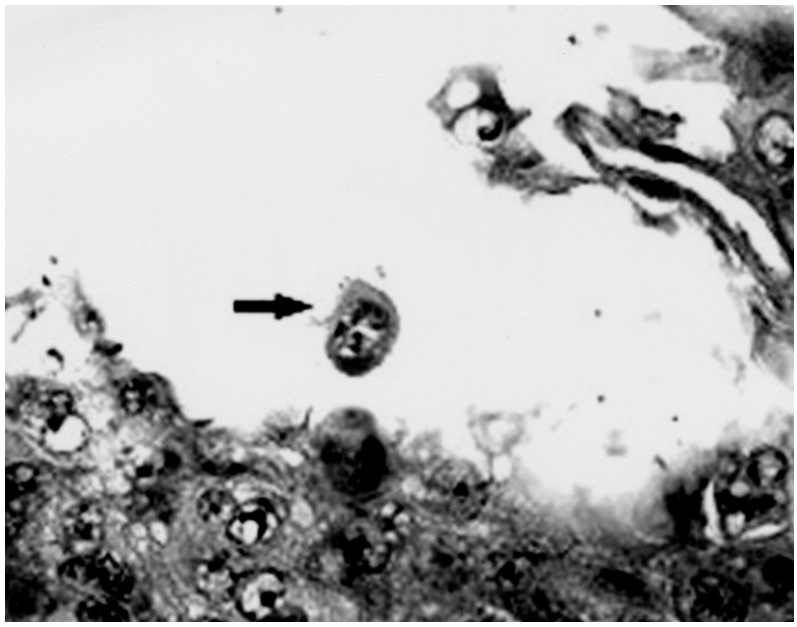

Fig. 1: **A zoosporangium, containing 4 zoospores** (arrow)**, in a sloughed epithelial cell** (H&E, ×100).

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and necrotic epithelial cells and clefts filled with granular amphophilic cellular debris in the superficial epidermal layers were present. The epidermis and superficial dermis contained small numbers of heterophils. In addition, multifocal rare foci of acute mild necrotising purulent dermatitis were present, not usually in proximity to zoosporangia. These were characterised by large numbers of heterophils in the dermis admixed with small numbers of large pigment-laden cells and fibrin, and moderate heterophilic dermal leucostasis. No infectious agents were seen in or adjacent to these lesions. Remaining tissues showed no other lesions. A mild infestation of intestinal helminths was noted, which was not associated with enteritis.

The detection of cutaneous chytridiomycosis in *A. fuscigula* adds yet another amphibian species to the extensive list of free-ranging and captive hosts. It is not clear whether or not the chytrid infection caused the death of this frog, since the separate necrotising dermatitis may also have played a role. No other infectious agents or life-threatening lesions were noted in the frog. No infectious agent, including *B. dendrobatidis,* was seen in association with the necrotising dermatitis. *B. dendrobatidis* is often associated with other pathogens and has been considered the primary pathogen in a number of epizootics of amphibians[3,12,16].

This report highlights the importance of the value of *post-mortem* samples in the study of the epidemiology and distribution of chytridiomycosis in the subcontinent and the rest of Africa. Chytridiomycosis can be diagnosed by routine histology of skin specimens preserved in 10 % neutral buffered formalin, as well as by examination of unstained skin scrapings, although the latter requires some expertise in identifying the organisms[5,12].

Risk assessments, to determine which amphibians are infected with *B. dendrobatidis*, should be carried out in all countries, including South Africa[19,20]. Amphibian disease research is currently under way at Potchefstroom University, in conjunction with James Cook University, Australia. The programme is a multi-disciplinary investigation that includes a survey of the distribution of *B. dendrobatidis* in Africa by examining the genus *Xenopus*; risk assessment of the dissemination of chytridiomycosis locally and globally via the *X. laevis* trade; quantifying the factors that modify the interaction between *X. laevis* and *B. dendrobatidis*; and determining the level of genetic variation in *B. dendrobatidis* from Africa. Information on the epidemiology, pathogenesis and phylogeny of chytridiomycosis will help to elucidate its role as a pathogen of African amphibians.

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