A Conservation Strategy for the Amphibians of Madagascar	Monografie del Museo Regionale di Scienze Naturali di Torino, XLV (2008): pp. 95-106

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Lack of detection of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in Madagascar

ABSTRACT

The global fungal disease chytridiomycosis can have catastrophic effects on amphibian populations leading to declines and even extinctions. Madagascar with its highly endemic and diverse amphibians is particularly vulnerable to emerging infectious diseases. In this study we report on a histological survey of chytridiomycosis at multiple localities in eastern Madagascar. The amphibian chytrid fungus was not detected in 527 frogs that altogether were examined. A more comprehensive survey involving all biogeographic zones on the island is urgently needed before a conclusion can be made about the chytridiomycosis classification of Madagascar. Suggestions on future research aimed at managing the disease are also made.

Key words: Amphibians, Chytrid, Histopathology, Madagascar.

INTRODUCTION

The amphibians of the world are currently facing an extinction crisis that calls for major conservation efforts at all levels of research, law and policy (Mendelson et al., 2006). The magnitude of the proportion of taxa that are either declining or presumed extinct make amphibians the most threatened vertebrate class (Stuart et al., 2004). The greatest contributors to amphibian declines include habitat loss, environmental contamination, exotic predators, climate change and disease. One of the most virulent diseases of amphibians is

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chytridiomycosis caused by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Berger et al. 1998; Collins & Storfer 2003). Two factors in particular contribute to the potency of chytridiomycosis; the ability to affect entire amphibian assemblages and a rapid rate of spread (e.g., Berger et al., 1998; Lips et al., 2006). Chytridiomycosis has been associated with amphibian population declines and extirpations from the Australian and American tropics, western North America, Europe and East Africa (Berger et al., 1998; Bosch et al., 2001; Weldon & Du Preez, 2003; Rachowicz et al., 2006). In contrast, evidence from southern Africa indicates that *B. dendrobatidis* is an endemic infection with no apparent adverse effects on amphibian communities at large (Weldon et al., 2004).

A highly diverse and almost exclusively endemic amphibian assemblage is found on the island of Madagascar, one of the world's biodiversity hotspots (Glaw & Vences, 2000; Myers et al., 2000). The Madagascan batrachofauna includes approximately 23% threatened taxa (including all vulnerable, endangered and critically endangered taxa) mainly because of a small distribution area, habitat destruction, and exploitation for the pet trade (IUCN, 2004; Andreone et al., 2005). Forest ecosystems are disappearing at an alarming rate (Ganzhorn et al., 2001), which could be potentially devastating, because they sustain the highest frog diversity. Furthermore, the high numbers of frogs being exported for the pet trade locally threaten some species such as *Dyscophus, Mantella* and *Scaphiophryne* (Behra & Raxworthy, 1991; Jenkins & Rakotomanampison, 1994). Chytridiomycosis has not been included as a threat to Madagascan batrachofauna, because the likelihood was not investigated until now (see an appraisal of threats by Andreone & Luiselli, 2003). Taxa particularly prone to extinction include those with limited geographical distribution and low reproductive rate (McKinney, 1997: Purvis et al., 2000; Cardillo, 2003) of which Madagascar has many. The moment that the live animal trade is involved in an infectious disease system, the risks of pathogen dispersal within the system (Madagascan frogs) and beyond (export countries) increases considerably (Daszak et al., 2000, Hanselmann et al., 2004). Dealing in the international pet trade implies that Madagascar is a high-risk country for disseminating associated disease agents. Because the frog trade via Madagascar is unidirectional (exports only; e.g., Rabemananjara et al., 2008) the biggest risk for frog-to-frog transmission between countries is exporting potential pathogens from Madagascar.

Given the high conservation profile of Madagascar's frogs and augmented by the trade in live frogs, classification of *B. dendrobatidis* at regional, species and population level is both warranted and timely. Here we present the findings from a preliminary survey in eastern Madagascar as part of a larger initiative to survey the whole of Madagascar's frog diversity, and suggest future research directions. The results from here and the survey, once completed, will provide researchers and policy makers with information that can aid the management of *B. dendrobatidis* either pro-actively or responsively. Chytridiomycosis in this paper is the designation for infection of frogs by *B. dendrobatidis*.

MATERIAL AND METHODS

Month-long surveys of Madagascan frogs were conducted in January 2005 and February 2006 to determine if *B. dendrobatidis* was present on the island. The regions surveyed included Maroantsetra in the north-east, Andasibe, An'Ala and Ranomafana in the central east, and Ambohitantely, Antananarivo, Ambatolampy and Ankaratra in the central highlands (Fig. 1).

There were no prior reports of enigmatic declines that could help direct survey effort. Therefore our study sites were selected either for their high biodiversity and unique frog assemblages (Andasibe, An'Ala, Ranomafana, Ambohitantely), because they have been in the center of previous studies on threats of fragmentation and deforestation on Madagascan amphibians (Ambohitantely, An'Ala), because they are in areas of major tourist destinations (Andasibe, Ranomafana) or close to laboratories where in the past clawed frogs may have been kept (gardens of Institut Pasteur and of Parc de Tsimbazaza in Antananarivo) and where chytrid introduction would therefore be most likely. Clawed frogs have been identified as a high-risk species for disseminating the amphibian chytrid fungus through the trade in this species from South Africa (Weldon et al., 2004). The selected locations as summarized in Tab. I span a wide range of biogeographic regions and elevations, including some specimens from high elevations above 2000 m in the Ankaratra Massif, various mid-altitude to high-altitude sites (900-1700 m) and one coastal site, virtually at sea level.

Post-metamorphic and adult frogs were collected by hand at night and placed in separate, clean plastic bags to minimize the risk of potential disease transmission. All footwear and equipment were thoroughly cleaned and air dried between locations. Frogs were examined for any clinical symptoms associated with chytridiomycosis including loss of fear, abnormal body posture, excessive sloughing, loss of righting reflex and fitting when handled. All frogs were anaesthetized with chlorobutanol and a representative subset was sampled for chytridiomycosis. Two phalanges from the fifth toe of the left hind foot were clipped and preserved in vials of 70% alcohol for histopathology. Dissecting instruments were wiped clean and alcohol flamed between animals. Collected specimens were fixed in 70% ethanol and will be deposited in the herpetological collections of the Université d'Antananarivo, Département de Biologie Animale, Madagascar, and the Zoologische Staatssammlung München, Germany.

Toe clips for histological sectioning were dehydrated in an alcohol series (70, 96 and 2 x 100% alcohol), elucidated with xylene, decalcified with Perreni's fixative and impregnated with paraffin wax at 60°C. Following the wax impregnation the tissue samples were embedded in paraffin wax blocks using a SLEE MPS/P2 embedding center and sectioned at 6 µm with a Reickert-Jung 2050 automated microtome. Slides were stained with Erlich's haematoxylin, counter stained with eosin and examined using a Nikon Eclipse E800 compound microscope for the presence of *B. dendrobatidis* using the criteria described in Berger et al. (1999).

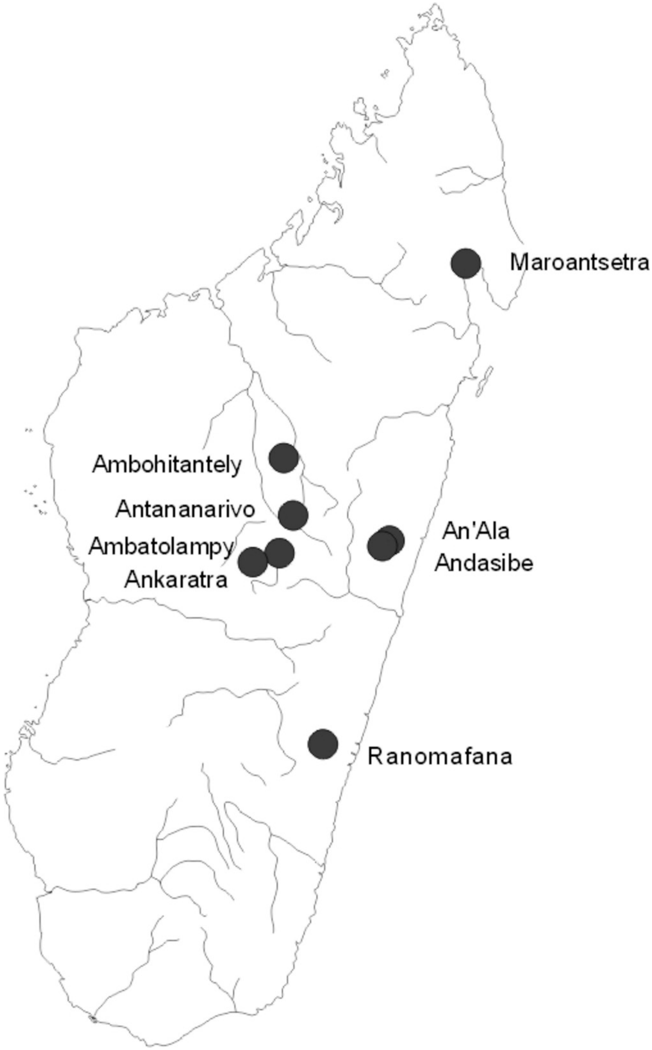

Fig. 1. Map showing study sites that were surveyed for chytrid infection using histological methods, based on samples collected in 2005 and 2006.

RESULTS

Collectively 527 frogs representing a minimum of 79 species were collected and screened during the two surveys (Tab. I). Among these were a number of new species that await description. The sample represented all four families known from Madagascar: Mantaellidae, Microhylidae, Ranidae and Hyperoliidae (Vences & Glaw, 2001). Sample sizes broken up between the two surveys varied between 20 and 178 per locality (Tab. II) and are a reflection of the time spent at different localities and of species richness.

We did not encounter any frogs that demonstrated any clinical symptoms usually associated with chytrid fungus infections. All sections were carefully examined, but no indication of chytridiomycosis was detected in any of the specimens that were screened through histopathology.

DISCUSSION

Our results demonstrate that histologically detectable chytridiomycosis was not present in any of the 74 species screened from Maroantsetra, Andasibe, Ranomafana, An'ala, Ambohitantely, Antananarivo, Ambatolampy and Ankaratra. Although molecular confirmation is still missing, we see this as a very strong indication that in fact *B. dendrobatidis* was absent from the

Family	**Genus**	**N species**	**% Infected**
Hyperoliidae	*Heterixalus*	3	0
Mantellidae	*Aglyptodactylus*	1	0
	Blommersia		0
	Boophis	22	0
	Gephyromantis	6	0
	Guibemantis	7	0
	Mantella	7	0
	Mantidactylus	21	0
	Spinomantis	1	0
Microhylidae	*Dyscophus*	1	0
	Platypelis	2	0
	Plethodontohyla	5	0
Ptychadenidae	*Ptychadena*	1	0

Tab. I. Summary of families and genera sampled.

Locality	Altitude	Collection date	N	% infected
Maroantsetra	10	2006, Feb	34	0
Andasibe	910	2005, Jan	28	0
Andasibe	920	2006, Feb	42	0
An'Ala	840	2006, Feb	178	0
Ranomafana	600-1100	2006, Feb	115	0
Ambohitantely	1580	2005, Jan	27	0
Antananarivo	1290	2006, Feb	20	0
Ambatolampy	1650	2006, Feb	32	0
Ankaratra	1700-2500	2005, Jan	29	0
Ankaratra	1700-2500	2006, Feb	22	0

Tab. II. Localities and outcome of chytrid survey of frog samples included in this study.

specimens sampled. This outcome is encouraging for several reasons: (1) the survey took place in three major biogeographic zones (Glaw & Vences, 1994), (2) these zones include regions known for their high amphibian species richness (Andreone, 1994; Vences et al., 2002), (3) these are the zones from which most of the pet trade collecting takes place (Behra & Raxworthy, 1991; Jenkins & Rakotomanampison, 1994). In addition the sample included species which conform to eight of nine parameter ranges selected by Andreone & Luiselli (2003) as indicators of population survival among Madagascan frogs namely: environmental adaptability, habitat breadth, arboreality, reproductive mode, activity type, altitudinal distribution, number of findings and extent of occurrence. The only parameter that was not completely represented by our sample was geographic distribution, because our survey did not include western and northern Madagascar, both of which are areas of considerable endemic and threatened amphibian diversity (Andreone et al., 2005). However, this study is by no means a conclusive indication of the chytridiomycosis classification of Madagascar nor does it suggest that chytridiomycosis is not a threat. Evidence is accumulating that climate change could under particular conditions foster the outbreak of chytridiomycosis (Pounds et al., 2006). Moreover, compelling evidence from Panama indicated how *B. dendrobatidis* arrived and spread like an epidemic wave through the country (Lips et al., 2006). As a consequence, even regions so far not affected by the disease could become so in the near future. Ron (2005) indicated that large parts of Madagascar have a favorable climate for *B. dendrobatidis* based on a prediction model developed on distribution data from the New World. Besides obvious advantages for conservation, Leung et al. (2002) determined that an approach at preventing biodiversity loss could be less costly and more effective than future control and eradication measures.

A limitation of the data from this study can be expressed as an order of magnitude in terms of sample size and diagnostic sensitivity. Sample sizes for the 2005 surveys of Andasibe and Ambohitantely, as well as the 2006 surveys of Antananarivo and Ankaratra were too small to detect disease at an estimated prevalence of 10%. However when we combine the number of specimens during the two surveys, only Ambohitantely and Antananarivo did not have large enough sample sizes for this estimated prevalence. Although histology has been used to great effect in other surveys as primary technique for detecting *B. dendrobatidis* (Lips et al., 2003; Carnaval et al., 2006), detection through quantitative polymerase chain reaction (qPCR) has been demonstrated as the most sensitive assay for chytridiomycosis (Boyle et al., 2004; Kriger et al., 2006). The results presented from this survey should therefore be considered reliable yet preliminary until sufficient samples from all regions of Madagascar have been surveyed for chytridiomycosis through assays of higher sensitivity.

The speed with which chytridiomycosis has spread to naïve populations causing extinctions or declines may be forewarning of the problems to be caused by this disease in the future in countries that are currently chytrid free. A rapid assessment of the vectors and mechanisms contributing to the global spread of amphibian chytrid is therefore essential, and should be conducted in especially countries involved in the amphibian trade. A call for pro-active conservation action towards protecting Madagascar's frogs from habitat loss and amphibian chytrid was recently voiced (Andreone et al. 2008). A more detailed and comprehensive study in Madagascar is of the utmost importance. We propose that a dichotomous approach should be followed to address the research and policy issues relating to *B. dendrobatidis* in Madagascar depending on the final outcome of the survey (Fig. 2). If *B. dendrobatidis* is detected the first objective should be to determine the extent of the infection in Madagascar to classify areas as *B. dendrobatidis* infected or *B. dendrobatidis* free. This can be done by continuing to survey frogs across taxonomic and geographic ranges. Detailed information about the occurrence of *B. dendrobatidis* can then aid in conducting a risk assessment involving threats and spread of the disease. It will also be important to establish long-term monitoring sites as part of a management strategy. Concurrently with conducting the survey, attempts should be made to isolate and culture the fungus using described criteria from Longcore (2001). The cultured Madagascan strains can then be used to determine their relatedness to other strains in an attempt to find a genetic basis for the region of endemism of *B. dendrobatidis* (see Morehouse et al., 2003). Knowing whether *B. dendrobatidis* is endemic or introduced in a region determines how biosecurity strategies should be designed. If *B. dendrobatidis* is not detected a risk assessment that focuses on the likelihood of introduction and subsequent spread of the amphibian chytrid should be conducted. Part of this risk assessment should involve exposing Madagascan frogs to *B. dendrobatidis* strains outside of Madagascar to determine susceptibility to infection and disease. Such challenge experiments, although replicated, have to consider dose and ecological parameters that most accurately simulate exposure under natural conditions.

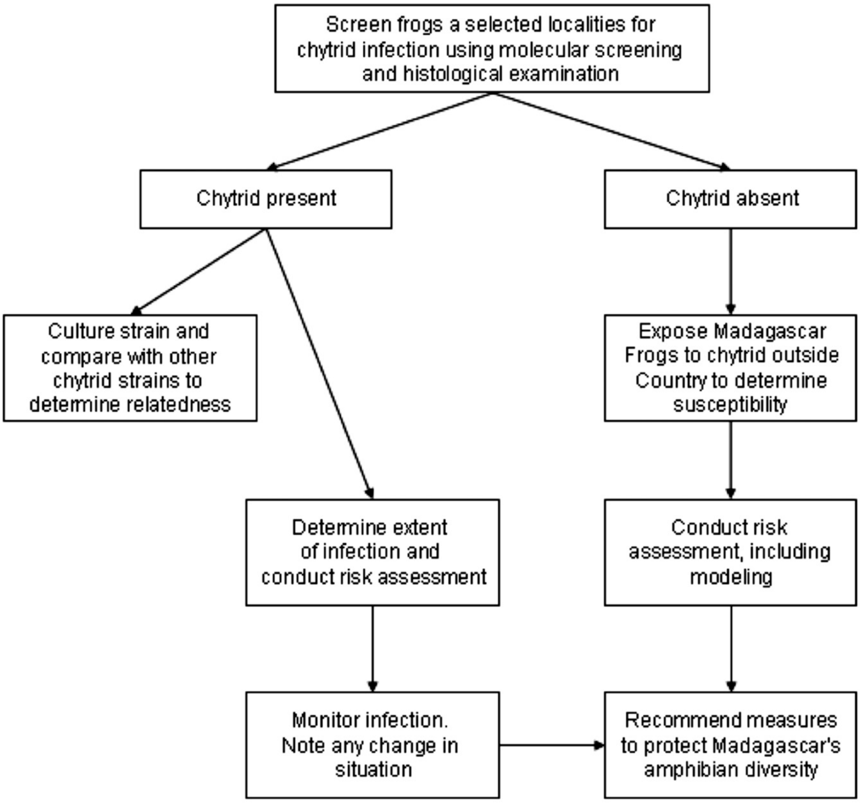

Fig. 2. Diagrammatic outline of proposed research activities aimed at curtailing chytridiomycosis in Madagascar.

Integration of the knowledge gained from the disease survey and risk assessment can aid in developing recommendations to protect Madagascar's amphibian diversity. The management plan must be made available to Madagascan conservation authorities, research facilities and other stakeholders to ensure concerted implementation. Effective functioning of a management plan would have to involve continued communication through the interactive exchange of information between risk assessors, research community, conservation authorities and all parties involved in the management process (stake holders, amphibian trade etc.).

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RÉSUMÉ

Lac de détection du chytride (Batrachochytrium dendrobatidis) *des amphibiens à Madagascar.*

La mycose du chytridiomycosis peut avoir des effets catastrophiques sur les populations des amphibiens en entraînant leurs déclins, voire leurs extinctions. Madagascar, de part sa grande diversité d'amphibiens hautement endémiques est particulièrement vulnérable à l'émergence de maladies infectieuses. Dans cette étude, nous enquêtons sur le recensement histologique du chytridiomycosis dans de multiples localités de l'est de Madagascar. Le champignon chytride n'a pas été détecté chez les 527 grenouilles que nous avons examinées. Un recensement plus global qui prend en compte toutes les zones bio géographiques de l'île constitue une nécessité urgente avant de pouvoir donner des conclusions sur la catégorisation de la chytridiomycosis de Madagascar. Des indications à propos d'une future recherche qui vise à contrôler la maladie sont également effectuées.

Mots clés: Amphibiens, Chytride, Histopathologie, Madagascar.

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