Chytrid distribution and pathogenicity among frogs of the Udzungwa Mountains, Tanzania

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1. INTRODUCTION

One of the causal factors in global amphibian declines is thought to be infection by a chytrid fungus, *Batrachochytrium dendrobatidis*. It remains unclear whether this chytrid represents an emerging disease, or an organism that commonly co-occurs benignly with amphibians and becomes pathogenic when environmental perturbations increase susceptibility of individuals. However, Chytridiomycosis is, at minimum, proximately responsible for rapid extirpations of entire populations (Pounds et al. 2006). Furthermore, pathogenic infection of frogs by chytrids are leading to massive die-offs in relatively pristine habitats, suggesting that Chytridiomycosis may indeed be an emerging disease epidemic ultimately responsible for species extinctions and extirpations in several areas of the world. Thus, there is a pressing need to expedite investigations into Chytridiomycosis outbreaks when and where they occur. Epidemiological data from such rapid response studies are crucial to identifying species in imminent risk of extinction, and developing appropriate countermeasures to stave off further amphibian losses.

This project was conceived following reports of a Chytridiomycosis outbreak in several species of amphibians in the Kihansi Gorge, Udzungwa Mountains, Tanzania, (Weldon & du Preez, 2004). Particularly alarming, were reports that the only know population of the critically endangered Kihansi Spray Zone Toad, *Nectophrynoides asperginus*, had virtually disappeared from the gorge. The possibility of a similar outcome for other populations of endemic Eastern Arc amphibians is very grim.

The aim of this project was to sample amphibian populations from localities across the Udzungwa Mountains to determine if Chytrid infection was present, and to assess levels of risk to endemic species. There were no previous reports of Chytridiomycosis in Tanzania prior to the discovery of *B. dendrobatidis* infection in amphibians of the Kihansi Gorge. Therefore, it is likely that little resistance to this fungus has built up in local populations.

Amphibians were collected from localities across the Udzungwa Mountains, by David Moyer and Elia Mulungu of the Wildlife Conservation Society (WCS), and Dr. Charles Msuya, University of Dar es Salaam (Figure 1, Appendix I). Fieldwork took place from May 2004 to May 2005. A total of 696 specimens were collected, of which 554 have been screened for Chytrid fungus infection by Dr. Ché Weldon at North-West University, Potchefstroom, South Africa.

Twenty-three skin scrapes were collected by Dr. Don Church of Conservation International at Kihanga stream in Uzungwa Scarp Forest Reserve. These were sent to the Pisces laboratory for PCR analysis to determine if *B. dendrobatidis* DNA was present (Appendix II).

Field sites

Field sites were situated so as to give the widest geographic and habitat type coverage possible. These spanned an altitudinal gradient of 1930 m at Luala in the West Kilombero Scarp Forest Reserve down to 260 m near Ifakara in the Kilombero Valley. The highland sites were in a variety of habitat types and situations.

Maganga is on an active coffee and dairy farm in the Mufindi Highlands. The area was formerly montane forest / grassland mosaic but has been extensively modified by human activities. All of the small forest streams on this farm have been dammed and the reservoirs have been stocked with Salmonids (Rainbow Trout).

At Uhafiwa, two sites were sampled; both were within 10 km of the Kihansi Gorge where *B. dendrobatidis* was first found in the Udzungwa Mountains. The first site, Uhafiwa, was in reed beds along the Unyazungwa Stream that runs through the Village. The second, Ihambwi, was in montane forest in the Udzungwa Scarp Forest Reserve a few km east of Uhafiwa. At Ihambwi, there are extensive reed beds in the valley bottom and forest on ridges and valleys and along streams running out of the site and into the Ruaha River (which joins the Kihansi River about 4 km down stream).

Two sites were sampled in the central Udzungwa Scarp Forest Reserve, Kihanga and Mapimbili. These are both named after streams, and Kihanga is the type locality of Kihanga Reed Frog, *Hyperolius kihangensis*. This strict endemic was not found during the sampling work there as it was too late in the season and many species were no longer calling.

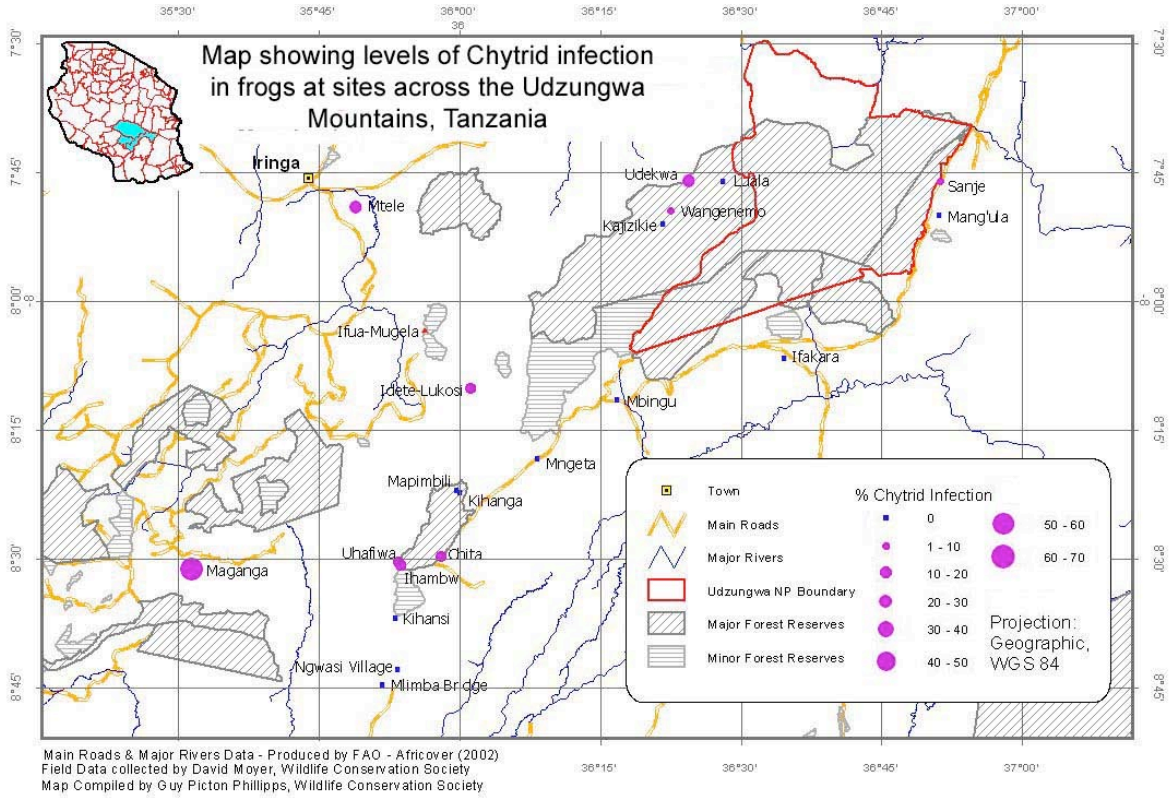

Figure 1
Collecting localities and percent of the amphibian populations that were infected with Chytrid, *Batrachochytrium dendrobatidis*, in the Udzungwa Mountains, Tanzania.

Two sites further sites were samples outside of the forest at Idete-Lukosi and Ifua-Mugela, between the main northern and southern forest blocks in the Udzungwas. Both of these were close to villages and in reed beds along rivers and streams flowing out of forested areas. A number of localities in southern Tanzania share the same place names. Thus, the village of Idete on the Udzungwa highlands is referred to as Idete-Lukosi in this report to distinguish it from Idete Village in the Kilombero Valley along the main Ifakara to Mlimba road. The Ifua-Mugela site was along the Mugela Stream just north of Kidabaga Village and west of the Dabaga-Ulang'mbi Forest Reserve. This area has been visited by many herpetologists in the past and is the type locality of Keith's Wot-wot, *Phlyctimantis keithae*, another Udzungwa endemic (Loveridge 1933, 1944, Schiøtz 1975).

The Mtele site is quite close to Iringa. This is a seasonally flooded wetland with short grass standing in water that varies from 5 – 50 cm deep. The wetland is surrounded by miombo woodland and cultivation mosaic. This is a typical high savanna site with a relatively high diversity of widespread species.

Several sites were sampled from Udekwa Village. Researchers have visited all of these in the past. The first site, 'Udekwa', was in the valley to the east of the village and within the West Kilombero Forest Reserve. Sampling was done near the Tanzania National Parks ranger post in the extensive reed beds along streams flowing from the Ndundulu Ridge and Nyumbanitu Mountain. Further sampling was done at Luala, a grassy valley on the Ndundulu Ridge surrounded by wetlands, montane forest, montane bamboo and honeydew thickets. Two further sites were sampled in the Nyumbanitu Mountains south of Udekwa – Wangenemo and Kajizikie. Both of these were along streams running through forest but with some smaller areas of wetlands in valley bottoms.

The lowland sites were broadly similar and were spread along the ecotone between the Udzungwa Mountains and the Kilombero Valley between Mlimba and Sanje. All of these areas were around 300 m above sea level and were a variety of flooded grassland, stream banks and wetlands along the main road. A majority of the species collected here were widespread savanna forms. One species of *Ptychadena* collected near Ifakara was probably an undescribed form and may well be a new Kilombero endemic.

2. METHODS

Field methods:

Frogs were collected by hand at night in the field. Specimens were generally located by listening for advertisement calls of males after dark. Whenever possible, calls were recorded to aid in identification to species level. Other specimens were collected opportunistically by searching along riverbanks, in overhanging vegetation along streams, on rocks in torrents, in reed beds and forest leaf litter.

Specimens were euthanized with MS 222 (3-Aminobenzoic Acid Ethyl Ester or Methanesulfonate Salt) and fixed in 10% formaldehyde. After fixation the specimens were cleared with water then transferred to 70% ethanol for long-term storage and preservation.

Lab methods:

Histology

- One whole foot of small specimens or 1-2 toes of larger specimens were surgically removed and decalcified in Perreni's fixative for 18 h.
- Skin tissue was dehydrated further, elucidated with xylene, infiltrated with paraffin wax at 60°C (under vacuum) and embedded in paraffin wax blocks.
- Specimens were sectioned at 6 µm. Two slides containing two ribbons of 4-10 sections each were prepared for every specimen.
- Mayer's Haematoxylin was used as staining solution and eosin as counter stain.
- Slides were examined under a Nikon Eclipse E800 compound microscope for the presence of *Batrachochytrium dendrobatidis* using criteria described in Berger et al. (1999). Approximately 5 min of screening were spent per specimen.

PCR Assay

A small sample of 23 skin scrapes was collected by Dr. Don Church at Kihanga Stream, in the central Uzungwa Scarp Forest Reserve in May 2005. These were sent to the Pisces lab for a PCR analysis testing for the presence of *B. dendrobatidis* DNA.

Sample Preparation

Each skin scrape sample was mixed by pipetting the liquid up and down, then the entire volume, including any visible skin/tissue pieces, was transferred to a microfuge tube. After spinning at maximum speed in a microcentrifuge (~16,000 x G) for 3 minutes, the supernatant was drawn off and discarded, tissue lysis buffer added, and any pellet re-suspended by vortexing. 10 µg of carrier DNA was added to the lysis buffer. Total DNA was extracted from all samples using a spin-column DNA purification procedure.

PCR assay

All sample DNA preparations were assayed for the presence of the *Batrachochytrium dendrobatidis* ribosomal RNA Intervening Transcribed Sequence (ITS) region by 45 cycle single-round PCR amplification using an assay developed by Seanna Annis and modified for greater specificity and sensitivity at Pisces.

Each PCR run included the following controls:

Positive DNA: DNA prepared from a laboratory culture of B*. dendrobatidis,* Strain JEL 270, kindly provided by Joyce Longcore. This sample was previously demonstrated to be positive by PCR. The signal from this sample is the standard for a strong positive (++) signal.

Negative DNA: DNA prepared from a laboratory culture of a non-batracho chytrid fungus*,* Strain JEL 151, kindly provided by Joyce Longcore. This sample was previously demonstrated to be negative (-) by PCR.

No DNA: H_2O in place of template DNA. This reaction remains uncapped during addition of sample DNA to the test reactions, and serves as a control to detect contaminating DNA in the PCR reagents or carryover of positive DNA during reaction set-up.

3. RESULTS

One of the major obstacles in this project was to confirm species-level identifications of specimens. The inventory of amphibians is not yet complete in the Udzungwa Mountains and there are still many undescribed species. There were at least four species new to science among the samples collected during this project – an Arthroleptid, a Ranid, a Hyperoliid, and a Bufonid. Descriptions of these will be published when further specimens and data on their calls and ecology can be collected.

A minimum of 48 amphibian species were collected during this fieldwork (Appendix III). This represents 84% of the 57 species of amphibians known from the Udzungwas at the start of the survey (Menegon pers com. 2004). Unusual weather patterns during the field periods caused many delays in project implementation. Late rains in the 2004 and 2005 rainy season, and a mid-season drought, resulted in many species staying out of site and not calling during some of field visits. Also, it was difficult to collect samples of any one species that were large enough to determine the true chytrid infection level in that species at a given site. As a result, data were pooled across species at a site to arrive at a meaningful estimate of total infection levels in an amphibian community.

Table 1
Batrachochytrium dendrobatidis infection data of frogs from the Udzungwa Mountains, by genus.

Genus	No. screened	No. infected	Prevalence
Afrana	26		
Afrixalus	75	6	8%
Arthroleptides	1		
Arthroleptis	107		
Bufo	13		
Chiromantis	11		
Hemisus	6		
Hyperolius	124	22	17.74%
Kassina	7	5	71.43%
Leptopelis	31		
Nectophrynoides	24		
Phrynobatrachus	37		
Phrynomantis	8	2	25%
Probreviceps	2		
Ptychadena	48		
Schoutedenella	1		
Strongylopus	1		
Xenopus	32		
Total	554	35	6.32%

3.1 Chytridiomycosis levels in screened samples

Chytridiomycosis was detected in 35 of the 554 (6.3%) specimens and 10 of 48 (8.7%) species screened in the test sample (Appendix IV). Geographically, *B. dendrobatidis* is spread across the Udzungwa Mountains at both high and low altitude sites. The fungus is present at more of the highland sites (64%) than the lowland sites (22%). It was not found in some of the more remote sites in the central Udzungwa Scarp and West Kilombero Forest reserves.

Table 2
Prevalence of *Batrachochytrium dendrobatidis* across species at 20 localities in the Udzungwa Mountains.

Locality	Altitude	Prevalence
Chita	300	14.29%
Idete-Lukosi	1560	11.54%
Ifakara NW	260	
Ihambwi	1400	12.96%
Kajizikie	1500	
Kihanga	1760	
Kihansi	300	
Luala	1930	
Maganga	1760	63.64%
Mang'ula	300	
Mapimbili	1760	
Mbingu	280	
Mlimba Bridge	300	
Mngeta	300	
Mtele	1620	22.22%
Ngwasi Village	320	
Sanje	300	3.70%
Udekwa	1480	22.22%
Uhafiwa Village	1500	20.00%
Wangenemo	1540	1.10%

3.2 Pathology

All developmental stages of *B. dendrobatidis* were observed in histological sections of infected animals. Usually, between one and three cell layers contained sporangia, and infected regions developed hyperkeratosis and hyperplasia (Figure 2). Most infected animals showed evidence of sloughing. Animals that had recently undergone sloughing had only a single layer of *B. dendrobatidis* thalli embedded in the skin, because most of the fungus thalli were sloughed with the superficial epidermis (Figure 3). Infection was not restricted to any particular region on the toe, however, ventral surfaces, especially tubercles and toe discs, were frequently infected (Figure 4).

3.3 Taxonomic distribution of Chytridiomycosis

3.3.1 *Family Level*

Two of the eight anuran families included in the test sample, *Microhylidae* and *Hyperoliidae*, had species infected with *Batrachochytrium dendrobatidis*. The other six families - *Arthroleptidae*, *Bufonidae, Hemisotidae, Pipidae, Ranidae,* and *Rhacophoridae* – were chytrid free. Prevalence of the number of infected individuals per family was 13.9% for *Hyperoliidae* and 20% in *Microhylidae*. The number of specimens collected for each family differed considerably, with *Hyperoliidae* being the most abundant group followed by *Arthroleptidae* and *Ranidae*.

3.3.2 *Genus Level*

Chytridiomycosis was detected in 4/18 genera (22.2%) of the test sample (Table 1). Three quarters of the infected genera were *Hyperoliidae*. The highest prevalence per genus was detected for *Kassina*, *Phrynomantis* and *Hyperolius*. Sample sizes in some genera, such as *Arthroleptides, Schoutedenella,* and *Strongylopus* were too small to be able to say with confidence that these are chytrid-free.

3.4 Geographic & population level distribution of *Chytridiomycosis*

Specimens were collected from 20 localities in the Udzungwas (Appendix I, Figure 1). Chytridiomycosis was detected at 9 (45%) of these (Table 2). The average prevalence of infection across all anuran species from infected localities was 19.1%, and 8.6% overall for all localities. A maximum of 63.4% prevalence was recorded for Maganga (Fox) Farm in the Mufindi District. Apart from this locality, infection levels were never higher than 23%. Combined *B. dendrobatidis* infection levels for lowland localities in Kilombero District was only 2%, whereas, combined levels for highland localities was 14%.

Table 3
Infection levels of frogs by *Batrachochytrium dendrobatidis* within anuran communities in the Udzungwa Mountains.

Locality	No. species screened	No. species infected	% species infected
Chita	3	1	33.3%
Idete-Lukosi	6	2	33.3%
Ifakara NW	5	0	-
Ihambwi	7	2	28.6%
Kajizikie	6	0	-
Kihanga	3	0	-
Kihansi	3	0	-
Luala	4	0	-
Maganga	5	3	60.0%
Mang'ula	8	0	-
Mapimbili	2	0	-
Mbingu	3	0	-
Mlimba Bridge	2	0	-
Mngeta	10	0	-
Mtele	9	2	22.2%
Ngwasi	3	0	-
Sanje	10	1	10.0%
Udekwa	3	1	33.3%
Uhafiwa	3	1	33.3%
Wangenemo	7	1	14.3%

The number of species screened per site ranged from 2–10. Percent of species with Chytridiomycosis at infected sites ranged from a low of 10% at Sanje to a high of 60% at Maganga (Table 3).

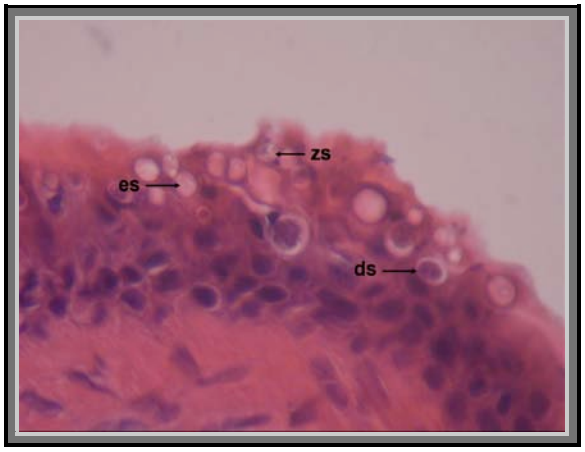

Figure 2: Haematoxylin and eosin stained section of *Hyperolius acuticeps* skin showing a cluster of *Batrachochytrium dendrobatidis* thalli in various stages of development. The developing sporangia (ds) are embedded in the outer layer of the *stratum corneum*, whereas mature sporangia with zoospores (zs) are present in the superficial *stratum corneum*. Sporangia that have already released their contents (empty sporangia) are visible as light colored vesicles (es).

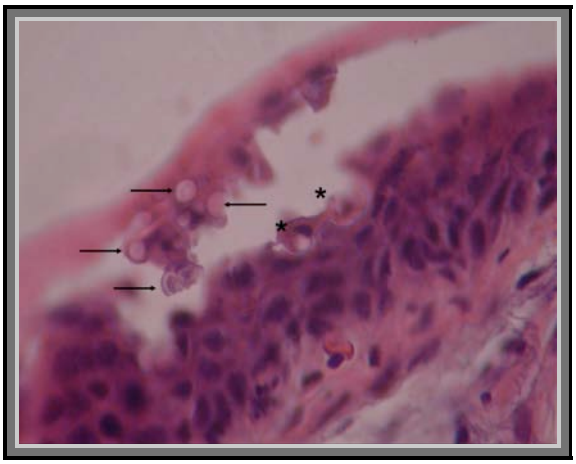

Figure 3: Haematoxylin and eosin stained section of *Hyperolius pictus* skin. The *stratum corneum* containing numerous *Batrachochytrium dendrobatidis* thalli (arrows) is in the process of being sloughed. A few developing sporangia (asterisk) only remain on the skin.

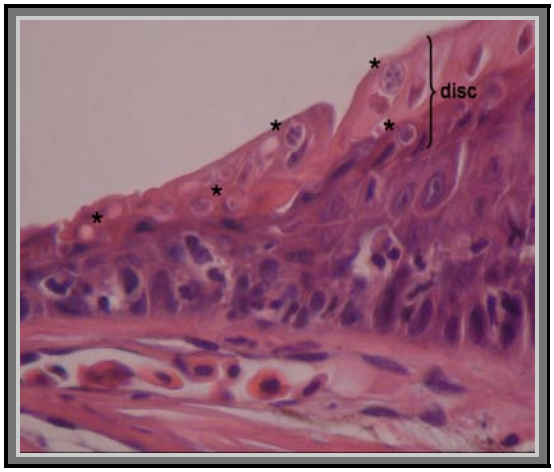

Figure 4: Haematoxylin and eosin stained section of *Hyperolius acuticeps* skin. Toe discs are common regions of infection for *Batrachochytrium dendrobatidis* as illustrated by this micrograph.

4. DISCUSSION AND RECOMMENDATIONS

Apart from the fatal infection in the Kihansi Spray Zone Toad, *Nectophrynoides asperginus,* and concurrent infection in Anchieta's Ridged Frog, *Ptychadena anchietae,* and Southern Torrent Frog, *Arthroleptides yakusini,* from Kihansi Gorge in 2003, Chytridiomycosis has not been reported in Tanzania (Weldon and du Preez 2004). These records from the Udzungwas are the first documents infections of *B. dendrobatidis* in amphibians outside of the Kihansi Gorge. This is also the first time that the disease has been detected in 9 of the 10 infected species. Only *Kassina senegalensis* from the Eastern Cape, South Africa has previously been found with Chytridiomycosis (Weldon unpublished data).

The odds for detecting infected individuals are better with larger samples, and an absence of Chytridiomycosis in large samples is an indication that the infection is not present in the taxon. However, when infection levels are high, detecting Chytridiomycosis in a taxon is possible even with small sample sizes. Good examples from this study are *Kassina* and *Phrynomantis*. Where large sample sizes were collected and no evidence of *B. dendrobatidis* found, such as at Luala, Kajizikie and Kihanga, it can be safely concluded that these localities were free of amphibian chytrid.

Given that *B. dendrobatidis* was found to be widespread in the Udzungwas, it may be that this fungus will be found elsewhere in amphibian populations throughout the Eastern Arc Mountains. Researchers have been implicated in the past for inadvertently facilitating the spread of this fungus through contamination of their field clothes and equipment. Scientists have visited all of the high altitude localities where B. dendrobatidis was found in the past. Sampling sites that were found to be chytrid-free but have been visited by field researchers in the past were Luala, Kajizikie, Mapimbili and Kihanga. If Chytridiomycosis is an emerging disease, and *B. dendrobatidis* is now spreading out from Kihansi to other localities, past fieldwork at these sites may have predated the arrival of this fungus in the Udzungwas.

There are several scenarios to consider when speculating about the source of B. dendrobatidis infection in the Udzungwas. It may be that this is an endemic fungus and that local amphibian populations have acquired some level of immunity to its harmful effects. This would be the 'best case" situation. Other sources to consider are the contamination of researcher's equipment from fieldwork in other areas where the chytrid fungus is found and the introduction of Salmonids in the dams and streams of the southern highlands. Stocking Salmonids has been implicated in the spread of *B. dendrobatidis* in the USA. In fact, both of these are likely as potential sources of *B. dendrobatidis* introduction if Chytridiomycosis is found to be a new and emerging disease in this area.

The present data are inconclusive and conflicting as to the source and nature of Chytridiomycosis in the Udzungwas. The site with the highest level of infection was Maganga in the Mufindi area. All the streams and dams at Maganga were stocked with trout within the past 10 years. Other rivers and streams in the southern highlands have been stocked with trout since the 1930's and most of the streams and dams in the Mufindi area were stocked in the 1950s (Fraser 1937). So a high level of infection at Maganga is consistent with trout stocking being a source of chytrid fungus. However, frog populations at Maganga are particularly numerous and diversity is high. All specimens handled there in the past have appeared to be healthy and normal and no pathological symptoms of Chytridiomycosis were observed.

The fact that *B. dendrobatidis* was not found at several remote sites in the Udzungwas supports the idea that Chytridiomycosis is a new and emerging disease rather than an endemic infection. However, data are not sufficient to draw a conclusion at this time. Further fieldwork and re-sampling of sites investigated during this survey will be necessary before any conclusion can be reached about the nature and origin of Chytridiomycosis in the Udzungwa Mountains. There is no doubt that Chytridiomycosis can be pathological in amphibians in the Udzungwas that are under environmental stress as evidenced by the disappearance of the Kihansi Spray Toad. Therefore, mapping the extent and trend of Chytridiomycosis, and taking steps to stop its spread are imperative if the endemic species of this area are to be saved from extinction.

It is critically important to carry out further sampling and monitoring of infected amphibian populations to determine whether the geographical distribution of *B. dendrobatidis* is limited to certain sites within the Udzungwas, or if the fungus is widespread throughout the Eastern Arc and Tanzania. This will

also help to determine whether Chytridiomycosis is new and spreading through the Udzungwas or is stabilized and fits the profile of an endemic infection.

Although Chytridiomycosis was present in only one species in five of the nine infected sites (55%), the infection of more than one species at the other four sites demonstrates that any species of frog is vulnerable to infection once the disease is present within a population. Further data on the effects of Chytridiomycosis on amphibian population dynamics – sporadic death in metamorphs, population declines and extirpations – are urgently needed. These data will supplement epidemiological data so that sound conclusions may be made about threats posed by Chytridiomycosis amphibians in this biodiversity hotspot, and realistic mitigation measures implemented.

Recommendations

Further field and laboratory research will be necessary before any of the questions about the origin and distribution of *B. dendrobatidis* and the nature of the Chytridiomycosis outbreak in the Udzungwas can be answered. The highest priority for future investigation is to determine if the fungal infection is spreading, both within and between populations and species. This can be done by (1) re-sampling populations sampled during this survey with the aim of getting larger sample sizes to determine infection levels and species affected more accurately.

Furthermore, (2) a number of sites where researchers have not visited in the past should be chosen to use as a control to help determine whether *B. dendrobatidis* is an endemic species or a newly introduced fungus. These sites should be in similar habitats to those already sampled during this survey.

On the wider level, if *researchers spread B. dendrobatidis* then it is likely to be found at other sites in the Eastern Arc such as Amani in the East Usambaras, and the Ulugurus. This would be comparatively easy to test, and (3) sites where Frontier expeditions and other researchers have visited in recent years should be sampled, along with controls from places where no researchers have been.

Finally, if introduction of Salmonids was a factor in the introduction of *B. dendrobatidis*, then Chytridiomycosis will be found in frogs at other localities where trout have been stocked in the past. The main places for further investigation will be (4) streams around Njombe and Tukuyu in the southern highlands, the Kirawira River on Mt. Rungwe, the Kigogo and Luisenga rivers at Mufindi, streams on Mt. Kilimanjaro and the southeastern sides of Mt. Meru (above Usa River) and the Ngorongoro Highlands. It would be useful to sample areas with similar streams that have never been stocked with trout to use as a control for this study.

The most important short-term precaution that must be taken is to advise all researchers working in Tanzania, and the Eastern Arc in particular, of the need to thoroughly disinfect all of their clothing and field equipment before traveling to other field research sites. This should be done through the Tanzania Commission for Science and Technology, the Tanzania Wildlife Research Institute, the Wildlife Division and the Forestry and Beekeeping Division. All researchers and tourists visiting forests in the Eastern Arc should be given information on how to disinfect their clothing and equipment and required to sign a commitment to do so before being issued research or entry permits to vulnerable areas.

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Appendix I. Sampling localities across the Udzungwa Mountains from which frogs were screened for Chytridiomycosis.

Locality	District	Latitude	Longitude	Elevation (m)	Description
Chita	Kilombero	8°29'46.41"	35°56'32.60"	300	ca. 3 km North of Chita
Idete-Lukosi	Kilolo	8°10'13.20"	36°00'06.00"	1560	Idete Village along the Lukosi River
Ifakara NW	Kilombero	8°06'43.18"	36°36'39.09"	260	ca. 8 km northwest Ifakara on Mlimba Road
Ifua-Mugela	Kilolo	8°03'18.26"	35°54'44.29"	1900	3 km north northwest of Kidabaga Village
Ihambwi	Mufindi	8°30'54.00"	35°51'53.99"	1400	ca. 2 km southeast of Uhafiwa
Kajizikie	Kilolo	7°50'59.99"	36°22'29.99"	1500	West Kilombero Forest Reserve, ca. 10 Km South of Udekwa
Kihanga	Mufindi	8°22'18.02"	35°58'54.01"	1760	11 km southeast Masisiwe Village
Kihansi	Kilombero	8°36'54.00"	35°51'24.00"	300	Between TANESCO office & tailrace
Luala	Kilolo	7°46'06.01"	36°29'36.00"	1930	Ndundulu Mtns., Luala Valley, 13 km East of Udekwa Village
Maganga	Mufindi	8°31'10.57"	35°27'39.77"	1760	Small dams on the Fox's Farm
Mang'ula	Kilombero	7°50'00.01"	36°54'41.99"	300	Along stream 500 m SE Udzungwa Mtns. National Park offices
Mapimbili	Mufindi	8°21'59.98"	35°58'30.00"	1760	10 km southeast Masisiwe Village
Mbingu	Kilombero	8°11'31.99"	36°17'14.07"	280	ca. 1.5 km northwest of the bridge over the Ruipa River
Mlimba Bridge	Kilombero	8°44'45.37"	35°49'53.23"	300	Road bridge over Ngwasi River, 6 km northeast of Mlimba
Mngeta	Kilombero	8°18'25.11"	36°07'53.88"	300	ca. 1.5 km northeast of Mngeta
Mtele	Kilolo	7°49'08.99"	35°46'43.99"	1620	10 km East southeast of Iringa
Ngwasi Village	Kilombero	8°42'52.80"	35°51'37.37"	320	ca. 11 km northwest of Mlimba
Sanje	Kilombero	7°46'6.88"	36°54'52.6"	300	ca. 500 m southwest of Sanje Village
Udekwa	Kilolo	7°46'00.02"	36°25'29.99"	1480	Udekwa, TANAPA Ranger Post
Uhafiwa Village	Mufindi	8°30'27.60"	35°51'38.99"	1500	Uhafiwa Village, Unyazungwa Stream
Wangenemo	Kilolo	7°49'29.99"	36°23'29.99"	1540	West Kilombero Forest Reserve, ca. 8.5 km South of Udekwa

Appendix II. Results of skin scrape samples from Amphibians in the Udzungwas Mountains tested for presence of *B. dendrobatidis*, DNA.

Source	Source ID #	Species	Date	Locality	Specimen No.	Pisces #	sample	tested for	*results
CI/DC	AFAN-1	Afrana angolensis	13-May-04	Kihanga	no specimen	54285	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	AFAN-2	Afrana angolensis	13-May-04	Kihanga	no specimen	54286	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	AFAN-3	Afrana angolensis	13-May-04	Kihanga	no specimen	54287	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	AFAN-4	Afrana angolensis	13-May-04	Kihanga	no specimen	54288	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	AFAN-5	Afrana angolensis	13-May-04	Kihanga	no specimen	54289	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	AFAN-6	Afrana angolensis	13-May-04	Kihanga	no specimen	54290	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	AFAN-7	Afrana angolensis	13-May-04	Kihanga	no specimen	54291	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	ARTHRO-1	Arthroleptis sp.	14-May-04	Kihanga	MO263	54292	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	ARTHRO-2	Arthroleptis sp.	13-May-04	Kihanga	MO163	54293	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	ARTHRO-3	Arthroleptis sp.	14-May-04	Kihanga	MO265	54294	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	ARTHRO-4	Arthroleptis sp.	13-May-04	Kihanga	MO160	54295	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	ARTHRO-5	Arthroleptis sp.	13-May-04	Kihanga	MO167	54296	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	ARTHRO-6	Arthroleptis sp.	13-May-04	Kihanga	MO162	54297	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	ARTHRO-7	Arthroleptis sp.	13-May-04	Kihanga	MO165	54298	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	LEPA-1	Leptopelis parkeri	13-May-04	Kihanga	MO164	54299	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	LEPA-2	Leptopelis parkeri	14-May-04	Kihanga	MO262	54300	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	LEPA-3	Leptopelis parkeri	14-May-04	Kihanga	MO261	54301	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	NECTO-1	Nectophrynoides sp.	14-May-04	Kihanga	MO260	54302	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	NECTO-2	Nectophrynoides sp.	14-May-04	Kihanga	MO259	54303	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	NECTO-3	Nectophrynoides sp.	13-May-04	Kihanga	MO161	54304	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	NEVI-1	Nectophrynoides viviparus	14-May-04	Kihanga	MO258	54305	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	NEVI-2	Nectophrynoides viviparus	13-May-04	Kihanga	no specimen	54306	skin scrape/EtOH	*Batrachochytrium d.*	-
CI/DC	NEVI-3	Nectophrynoides viviparus	13-May-04	Kihanga	no specimen	54307	skin scrape/EtOH	*Batrachochytrium d.*	-

***Scoring:** + = positive signal
w+ = weak positive signal
- = no signal/below limit of detection

Appendix III. Amphibian species screened for Chytridiomycosis from sample sites across the Udzungwa Mountains, Tanzania.

Locality	Genus	Species	No. Screened	No. Infected	% Prevalence
Chita	Phrynobatrachus	acridoides	1		0
Chita	Phrynomantis	bifasciatus	6	2	33.3
Chita	Xenopus	muelleri	7		0
Idete-Lukosi	Afrixalus	sp.	5	1	20
Idete-Lukosi	Hyperolius	pictus	1		0
Idete-Lukosi	Hyperolius	pseudargus	2		0
Idete-Lukosi	Hyperolius	puncticulatus	14	2	14.3
Idete-Lukosi	Strongylopus	fasciatus	1		0
Idete-Lukosi	Xenopus	laevis	3		0
Ifakara NW	Afrixalus	fornasinii	1		0
Ifakara NW	Bufo	gutturalis	2		0
Ifakara NW	Hyperolius	reesi	3		0
Ifakara NW	Phrynobatrachus	acridoides	2		0
Ifakara NW	Ptychadena	mascareniensis	16		0
Ihambwi	Afrixalus	sp. nov.	19	5	26.3
Ihambwi	Hyperolius	puncticulatus	28	2	7.1
Ihambwi	Leptopelis	(barbouri?)	2		0
Ihambwi	Phrynobatrachus	sp.	3		0
Ihambwi	Probreviceps	rungwensis	1		0
Ihambwi	Schoutedenella	sp.	1		0
Kajizikie	Afrixalus	sp.	1		0
Kajizikie	Arthroleptis	sp.	32		0
Kajizikie	Hyperolius	puncticulatus	5		0
Kajizikie	Hyperolius	spinigularis	1		0
Kajizikie	Leptopelis	parkeri	8		0
Kajizikie	Nectophrynoides	sp.	3		0
Kajizikie	Nectophrynoides	viviparus	1		0
Kihanga	Afrana	angolensis	23		0
Kihanga	Arthroleptis	sp.	20		0
Kihanga	Leptopelis	parkeri	4		0
Kihansi	Chiromantis	xerampelina	1		0
Kihansi	Ptychadena	anchietae	2		0
Kihansi	Xenopus	sp.	9		0
Luala	Afrixalus	uluguruensis	35		0
Luala	Arthroleptis	sp.	1		0
Luala	Schoutedenella	sp.	2		0
Maganga	Hyperolius	(spinigularis)	1	1	100
Maganga	Hyperolius	pictus	5	5	100
Maganga	Hyperolius	pseudargus	3	1	33.3
Maganga	Xenopus	sp.	2		0
Mang'ula	Afrana	angolensis	1		0
Mang'ula	Arthroleptides	sp.	1		0
Mang'ula	Chiromantis	sp.	5		0
Mang'ula	Hyperolius	sp.	2		0
Mang'ula	Phrynobatrachus	acridoides	3		0
Mang'ula	Phrynobatrachus	sp.	3		0
Mang'ula	Ptychadena	sp.	15		0
Mang'ula	Xenopus	sp.	1		0
Mapimbili	Arthroleptides	yakusini	1		0

Locality	Genus	Species	No. Screened	No. Infected	% Prevalence
Mapimbili	Arthroleptis	sp.	2		0
Mbingu	Bufo	gutturalis	1		0
Mbingu	Phrynobatrachus	acridoides	7		0
Mbingu	Xenopus	muelleri	1		0
Mlimba Bridge	Ptychadena	anchietae	3		0
Mlimba Bridge	Ptychadena	mascareniensis	3		0
Mngeta	Bufo	gutturalis	1		0
Mngeta	Bufo	maculata	2		0
Mngeta	Hyperolius	mitchelli	1		0
Mngeta	Hyperolius	tuberilinguis	1		0
Mngeta	Leptopelis	argentatus	1		0
Mngeta	Phrynobatrachus	acridoides	10		0
Mngeta	Phrynobatrachus	natalensis	6		0
Mngeta	Ptychadena	anchietae	2		0
Mngeta	Ptychadena	mascareniensis	1		0
Mngeta	Xenopus	muelleri	8		0
Mtele	Afrixalus	sp.	3		0
Mtele	Bufo	taitanus	5		0
Mtele	Hemisus	marmoratus	7		0
Mtele	Hyperolius	acuticeps	13	6	46.2
Mtele	Hyperolius	argentovittis	7		0
Mtele	Kassina	senegalensis	6	4	66.7
Mtele	Ptychadena	grandisonae	3		0
Mtele	Ptychadena	taneoscelis	1		0
Ngwasi Village	Bufo	maculata	1		0
Ngwasi Village	Hyperolius	reesi	3		0
Ngwasi Village	Hyperolius	sp.	6		0
Ngwasi Village	Phrynomantis	bifasciatus	2		0
Sanje	Afrixalus	fornasinii	6		0
Sanje	Bufo	gutturalis	1		0
Sanje	Chiromantis	xerampelina	6		0
Sanje	Hyperolius	mitchelli	3		0
Sanje	Hyperolius	puncticulatus	1		0
Sanje	Hyperolius	tuberilinguis	5		0
Sanje	Kassina	senegalensis	1	1	100
Sanje	Phrynobatrachus	acridoides	1		0
Sanje	Ptychadena	anchietae	1		0
Sanje	Ptychadena	oxyrhynchus	1		0
Sanje	Xenopus	muelleri	1		0
Udekwa	Afrixalus	sp.	3		0
Udekwa	Hyperolius	pictus	1		0
Udekwa	Hyperolius	puncticulatus	5	2	40
Uhafiwa Village	Afrixalus	sp.	1		0
Uhafiwa Village	Hyperolius	pictus	6	2	33.3
Uhafiwa Village	Hyperolius	puncticulatus	3		0
Wangenemo	Afrana	angolensis	2		0
Wangenemo	Afrixalus	sp.	3		0

Locality	Genus	Species	No. Screened	No. Infected	% Prevalence
Wangenemo	Arthroleptis	sp.	48		0
Wangenemo	Hyperolius	puncticulatus	5		0
Wangenemo	Leptopelis	parkeri	11		0
Wangenemo	Nectophrynoides	sp.	13		0
Wangenemo	Nectophrynoides	viviparus	2		0
Wangenemo	Nectophrynoides	sp.	2	1	50
Wangenemo	Nectophrynoides	viviparus	4		0
Wangenemo	Probreviceps	rungwensis	1		0

Appendix IV: Collection data for frogs of the Udzungwa Mountains that tested positive for *Batrachochytrium dendrobatidis.*

Colectors No.	Date	Family	Genus	Species	Locality
Moyer 1272	4-Apr-2005	Hyperoliidae	*Afrixalus*	*sp. nov.*	*Ihambwi*
Moyer 1275	4-Apr-2005	Hyperoliidae	*Afrixalus*	*sp. nov.*	*Ihambwi*
Moyer 1284	5-Apr-2005	Hyperoliidae	*Afrixalus*	*sp. nov.*	*Ihambwi*
Moyer 1302	6-Apr-2005	Hyperoliidae	*Afrixalus*	*sp. nov.*	*Ihambwi*
Moyer 1304	6-Apr-2005	Hyperoliidae	*Afrixalus*	*sp. nov.*	*Ihambwi*
Moyer 1078	9-Feb-2005	Hyperoliidae	*Afrixalus*	*uluguruensis*	*Idete-Lukosi*
Moyer 1309	11-Apr-2005	Hyperoliidae	*Hyperolius*	*acuticeps*	*Mtele*
Moyer 1311	11-Apr-2005	Hyperoliidae	*Hyperolius*	*acuticeps*	*Mtele*
Moyer 1313	11-Apr-2005	Hyperoliidae	*Hyperolius*	*acuticeps*	*Mtele*
Moyer 1315	11-Apr-2005	Hyperoliidae	*Hyperolius*	*acuticeps*	*Mtele*
Moyer 1316	11-Apr-2005	Hyperoliidae	*Hyperolius*	*acuticeps*	*Mtele*
Moyer 1318	11-Apr-2005	Hyperoliidae	*Hyperolius*	*acuticeps*	*Mtele*
Moyer 1345	21-Apr-2005	Hyperoliidae	*Hyperolius*	*pictus*	*Maganga*
Moyer 1347	21-Apr-2005	Hyperoliidae	*Hyperolius*	*pictus*	*Maganga*
Moyer 1348	21-Apr-2005	Hyperoliidae	*Hyperolius*	*pictus*	*Maganga*
Moyer 1349	21-Apr-2005	Hyperoliidae	*Hyperolius*	*pictus*	*Maganga*
Moyer 1350	21-Apr-2005	Hyperoliidae	*Hyperolius*	*pictus*	*Maganga*
Moyer 1152	15-Feb-2005	Hyperoliidae	*Hyperolius*	*pictus*	*Uhafiwa*
Moyer 1159	15-Feb-2005	Hyperoliidae	*Hyperolius*	*pictus*	*Uhafiwa*
Moyer 1346	21-Apr-2005	Hyperoliidae	*Hyperolius*	*pseudargus*	*Maganga*
Moyer 1063	9-Feb-2005	Hyperoliidae	*Hyperolius*	*puncticulatus*	*Idete-Lukosi*
Moyer 1064	9-Feb-2005	Hyperoliidae	*Hyperolius*	*puncticulatus*	*Idete-Lukosi*
Moyer 1256	4-Apr-2005	Hyperoliidae	*Hyperolius*	*puncticulatus*	*Ihambwi*
Moyer 1257	4-Apr-2005	Hyperoliidae	*Hyperolius*	*puncticulatus*	*Ihambwi*
Moyer 1163	11-Mar-2005	Hyperoliidae	*Hyperolius*	*puncticulatus*	*Udekwa*
Moyer 1164	11-Mar-2005	Hyperoliidae	*Hyperolius*	*puncticulatus*	*Udekwa*
Moyer 1341	4-May-2005	Hyperoliidae	*Hyperolius*	*sp.*	*Wangenemo*
Moyer 1344	21-Apr-2005	Hyperoliidae	*Hyperolius*	*spinigularis*	*Maganga*
Moyer 1038	6-Feb-2005	Hyperoliidae	*Kassina*	*senegalensis*	*Mtele*
Moyer 1039	6-Feb-2005	Hyperoliidae	*Kassina*	*senegalensis*	*Mtele*
Moyer 1040	6-Feb-2005	Hyperoliidae	*Kassina*	*senegalensis*	*Mtele*
Moyer 1042	6-Feb-2005	Hyperoliidae	*Kassina*	*senegalensis*	*Mtele*
Howell 29018	25 April 2005	Hyperoliidae	*Kassina*	*senegalensis*	*Sanje*
Howell 28920	23 April 2005	Microhylidae	*Phrynomantis*	*bifasciatus*	*Chita*
Howell 28921	23 April 2005	Microhylidae	*Phrynomantis*	*bifasciatus*	*Chita*