

# Survey of the chytrid fungus *Batrachochytrium dendrobatidis* from montane and lowland frogs in eastern Nigeria

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**Abstract.** The chytrid fungus *Batrachochytrium dendrobatidis* (Bd) that causes the amphibian disease chytridiomycosis might have originated in sub-Saharan Africa. Based on historical museum collections, it has been detected in frog specimens from Central Africa (Cameroon) as early as 1933. Yet, to date, there are few surveys of Bd for recently collected specimens from Central Africa. We present the results of a study undertaken to evaluate the presence and prevalence of Bd in eastern Nigeria at lowland and montane sites in Gashaka-Gumti National Park and in or near Ngel Nyaki Forest Reserve, respectively. Using quantitative real-time PCR, we detected three low-intensity cases of *B. dendrobatidis* infection each in a different genus of frog (*Amietophrynus*, *Astylosternus*, and *Petropedetes*), including in an undescribed species, at the lowland site in Gashaka-Gumti National Park. While it is promising that Bd was detected at only low intensities and at only one site, the extent to which Bd is distributed in this region of high amphibian diversity in Africa requires further evaluation.

**Keywords.** Amphibian declines, Africa, chytridiomycosis, Gashaka-Gumti National Park, Mambilla Plateau, Ngel Nyaki Forest Reserve.

## Introduction

Many recent amphibian declines are associated with the emergence of a pathogenic fungus, *Batrachochytrium dendrobatidis* (Bd), that causes the disease chytridiomycosis in amphibians (Berger et al. 1998). This deadly disease has been linked to the decline of over 200 amphibian species worldwide (Skerratt et al. 2007), with the majority of these declines observed in Australia and the New World (e.g., Fisher et al. 2009). Recent studies have documented the presence of Bd in a variety of genera in at least 13 countries in sub-Saharan Africa, mostly in southern and eastern Africa (Blackburn et al. 2010). However, aside from one possible Bd-caused decline in Tanzania (for the toad *Nectophrynoides asperginis*: Weldon & du Preez 2004; Channing et al. 2006), there have been no reported declines in African amphibians associated with Bd infection. Recent surveys of museum specimens reveal that Bd has a long history of presence in sub-Saharan Africa, beginning in at least the early 1930s

(Soto-Azat et al. 2010), bolstering previous suggestions that this pathogen may be endemic to Africa (Weldon et al. 2004). Weldon et al. (2004) further proposed that the global trade of *Xenopus laevis* from South Africa may have caused the spread of Bd from Africa to other regions (Weldon et al. 2004).

The earliest potential record of Bd in Africa (1933) derives from preserved museum specimens collected in Cameroon (Soto-Azat et al. 2010), which hosts one of Africa's richest amphibian faunas. However, there are few previously published surveys of Bd in western and central Africa, including Cameroon and neighboring Nigeria. Investigation of five individuals of *Hoplobatrachus occipitalis* collected in Nigeria during 1971 did not detect Bd (Oullett et al. 2005). Surveys in Okomu National Park in Nigeria between 2007 and 2008 found one individual of *Chiromantis rufescens* with a confirmed Bd infection (Imasuen et al. 2009). Bd has also been detected recently much farther east in Central Africa (Greenbaum et al. 2008). However, rather than view this record as suggestive of Bd infection elsewhere in Central Africa, this record of Bd from eastern Democratic Republic of Congo might be best interpreted as indicative of the margins of the Bd-positive region encompassing East African countries such as Kenya (Kielgast et al. 2009), Tanzania (Weldon and du Preez 2004; Channing et al. 2006), and Uganda (Goldberg et al. 2007; Soto-Azat et al. 2010). Additional information is sorely needed to determine the prevalence and potential impact of Bd in central and

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**Table 1.** Species screened for Bd in this study.

Locality	Coordinates	Elevation	Species (Family)	No. Tested	No. Positive
Kwano Camp, Gashaka Gumti N.P.	07 19' 47.9" N 011 35' 06.0" E	~540 m	<i>Amietophrynus</i> sp. (Bufonidae)	3	1
			<i>Astylosternus</i> sp. (Arthroleptidae)	1	1
			<i>Cardioglossa leucomystax</i> (Arthroleptidae)	2	0
			<i>Hylarana galamensis</i> (Ranidae)	2	0
			<i>Hyperolius</i> sp. (Hyperoliidae)	1	0
			<i>Petropedetes</i> sp. nov. (Petropedetidae)	2	1
			<i>Phrynobatrachus cornutus</i> (Ranidae)	4	0
			<i>Phrynobatrachus natalensis</i> (Ranidae)	1	0
Ngel Nyaki F.R.	07 05' 53.3" N 011 03' 19.6" E	~1460 m	<i>Phrynobatrachus steindachneri</i> (Ranidae)	2	0
Kurmi Danko F.R.	07 06' 54.6" N 011 01' 35.8" E	~1500 m	<i>Phrynobatrachus danko</i> (Ranidae)	3	0
Ngel Nyaki F.R.	07 05' 10.0" N 011 03' 59.9" E	~1550 m	<i>Arthroleptis palava</i> (Arthroleptidae)	1	0
			<i>Astylosternus</i> sp. (Arthroleptidae)	1	0
			<i>Leptodactylodon</i> cf. <i>bicolor</i> (Arthroleptidae)	2	0
Yelwa	07 05' 17.0" N 011 04' 41.1" E	~1550 m	<i>Amietophrynus</i> cf. <i>villiersi</i> (Bufonidae)	4	0
			<i>Leptopelis nordequatorialis</i> (Arthroleptidae)	1	0
Yelwa	07 05' 18.1" N 011 05' 06.6" E	~1580 m	<i>Arthroleptis palava</i> (Arthroleptidae)	1	0
Riparian forest, near Ngel Nyaki F.R.	07 05' 00.5" N 011 03' 51.0" E	~1630 m	<i>Leptopelis nordequatorialis</i> (Arthroleptidae)	3	0

western Africa as well as to help resolve whether Bd may have originated in Africa. We conducted a survey of the distribution and prevalence of Bd in eastern Nigeria at lowland and montane sites on or near the Mambilla Plateau.

## Materials and Methods

During the course of short opportunistic surveys in April 2009, chytrid screening was carried out (by DCB) at lowland and montane sites near or on, respectively, the Mambilla Plateau (Fig. 1; Table 1). The lowland field site is based near the Kwano field camp of the Gashaka Primate Project within Gashaka-Gumti National Park (Adamawa State). At this site, most frogs were collected in close proximity to a stream, including in the leaf litter, in the water of the streams, on sandy banks, or on rock faces within the stream. Frogs were collected from a handful of montane localities all near the field station of the Nigerian Montane Forest Project adjacent to the Ngel-Nyaki Forest Reserve near Yelwa village (Taraba State). These montane sites included forested streams in the forest reserve as well as tree-lined riparian corridors, grassland streams, eucalyptus plantations, and farms outside of the reserve. Nearly all frogs were collected active in the grass and leaf litter, though some, especially male *Leptopelis nordequatorialis*, were collected calling from trees at night. All specimens screened for chytrid are deposited in the herpetology collections of the Museum of Comparative Zoology (Harvard University).

Infection status was determined through a standardized swabbing protocol (Hyatt *et al.* 2007). Using a synthetic cotton swab, each individual was swabbed with 30 strokes (five times between the toes on each hindfoot, five times on each thigh, and five times on each side of the ventral abdomen). Swabs were extracted using a PrepMan extraction and run in duplicate by quantitative real-time PCR (qPCR) as described by Boyle *et al.* (2004) and Hyatt *et al.* (2007). Each plate contained negative controls. Standard samples of known Bd quantity served as positive controls on each plate;

standards were obtained from the Australian Animal Health Laboratory (courtesy of A. Hyatt). Samples were considered positive for Bd if qPCR revealed a measurable quantity (Genomic Equivalent, GE > 0) of Bd in either of the two replicates during the 40 cycle thresholds (CT) of the run. False positives were eliminated by ensuring that amplification curves followed standard exponential growth patterns (Hyatt *et al.* 2007). Infection levels on individual amphibians were quantified as zoospore equivalents obtained by multiplying genomic equivalents by 80 to correct for dilution during extraction and PCR.

## Results and Discussion

We tested 34 individuals from five currently recognized families and ten genera from the lowland and montane sites (Table 1). The overall prevalence of Bd for our survey was 8.8% (3/34), with 0% (0/18) prevalence at the montane site, and 18.8% (3/16) prevalence at the lowland site (Table 1). Positive samples all produced low-level infections (less than 1 zoospore equivalents), and were detected in three lowland species: *Astylosternus* sp., *Amietophrynus* sp., *Petropedetes* sp. nov. Specimens from these species were all collected in or adjacent to a large stream running past the Kwano field camp within Gashaka Gumti National Park.

Specimens of two of the species tested for Bd comprise type specimens of species described only recently (*Arthroleptis palava*: Blackburn *et al.* 2010; *Phrynobatrachus danko*: Blackburn 2010) Other specimens represents an undescribed species of *Petropedetes* (Blackburn unpublished data). Bd was not detected in *Arthroleptis palava* and *Phrynobatrachus*

*danko*, both from montane sites, but was detected in one specimen of the undescribed species of *Petropedetes*.

The data presented here expand our knowledge of the geographic and taxonomic distribution of Bd in Nigeria and Africa. Though Bd was present in three frog specimens, we did not observe any sick or dying frogs associated with Bd infection during our survey. In addition, the animals that were positive have very low zoospore equivalents or low infection intensities. Previous field studies showed that high infection intensities lead to disease and eventually population extinctions whereas frogs with low infection intensities (at the levels we found in this study) show no signs of disease and populations persist with Bd (Vredenburg et al. 2010; Briggs et al. 2010). Our results showing low levels of Bd infection intensities corresponds with the observation that there are no reports of mortality events associated with Bd infection anywhere in central or western Africa. Unfortunately, it is impossible to know the dynamics of this host-pathogen system in the areas surveyed in this study because of the opportunistic nature of the survey and the lack of previous Bd

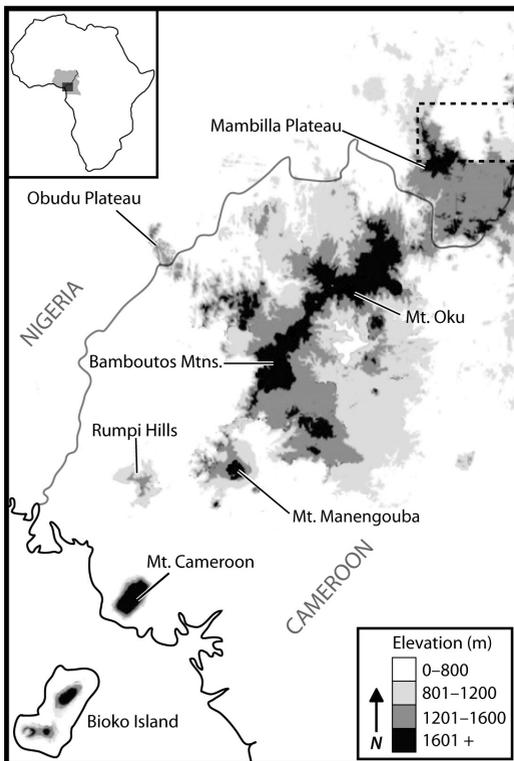
surveys at these sites.

Further studies are needed to test the susceptibility or resistance of Nigerian amphibians to Bd. At present, it remains difficult to evaluate the potential threat that Bd represents to these amphibian populations. Future studies could also potentially benefit from a better understanding of the natural immune responses of these species to Bd infection. In addition, the testing of additional historical museum specimens from Nigeria should be conducted to determine the prevalence of Bd in Nigeria, at least over the past century. Finally, due to our limited sample sizes and geographic sampling, our understanding of the distribution and threat of Bd in Nigeria remains incomplete. With our discovery of several low-level Bd-positive individuals, we hope that this will lead to more thorough, repeated, and widespread surveys of Bd in Nigeria.

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**Figure 1.** Location of region of sampling (within box with dotted line) on or near Mambilla Plateau with inset showing location within Africa.

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