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Short Communication

High Prevalence of the Amphibian Chytrid Pathogen in Gabon

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Abstract: Amphibian chytridiomycosis is an infectious disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) that is implicated in the worldwide decline and extinction of amphibians. Africa has been proposed as a potential source for the global expansion of *Bd*, yet the distribution of *Bd* across the continent remains largely unexplored. Using quantitative polymerase chain reaction (qPCR), we screened for the presence of *Bd* in 166 adult anurans from two national parks in Gabon (Monts de Cristal and Ivindo). *Bd* was detected in 20 of the 42 species and was present at all three sites surveyed (two in Monts de Cristal, and one in Ivindo) with high prevalence (19.6%–36.0%). Both national parks were *Bd*-positive at all elevations and across habitat types, though no dead or dying frogs were encountered. To our knowledge, this study presents the first evidence of *Bd* in Gabon and the first record of infection for 19 of the 20 species that were *Bd*-positive. Documenting the distribution and virulence of *Bd* across Africa will be essential for understanding the dynamics of amphibian chytridiomycosis across the globe.

Keywords: amphibian pathogen, *Batrachochytrium dendrobatidis*, chytridiomycosis, Gabon

Amphibian chytridiomycosis is an infectious disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) that is implicated in the worldwide decline and extinction of numerous amphibian species (Berger et al., 2005; Lips et al., 2006). Recent studies detected *Bd* in museum specimens of African pipid frogs from the 1930s, providing evidence of a widespread historical presence of *Bd* across the continent, and resulting in the hypothesis that Africa might be the source from whence *Bd* spread globally (Weldon et al., 2004; Soto-Azat et al., 2010). Although Africa may be a potential source, the verified distribution of *Bd* across the continent remains largely unexplored. The contemporary presence of *Bd* has been documented in South Africa (Hopkins and Channing, 2003), Uganda (Goldberg et al., 2007), the eastern Democratic Republic of Congo (Greenbaum et al., 2008), Nigeria (Imasuen et al., 2009), and Kenya (Kielgast et al., 2009). A recent field survey of anurans in the Cameroonian highlands failed to detect *Bd* (Doherty-Bone et al., 2008), though a museum specimen of *Xenopus fraseri* collected in Cameroon in the 1930s is the earliest confirmed case of *Bd* infection (Soto-Azat et al., 2010). Mapping the contemporary and historic distribution of *Bd* in Africa will help clarify whether amphibian populations are naive to the pathogen or if it is endemic, which will be essential for understanding the dynamics of chytridiomycosis. Here we report the first records, to our knowledge, of *Bd* from Gabon using quantitative

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polymerase chain reaction (qPCR) methods and community-level sampling in three wild populations of anurans.

Amphibian surveys were conducted October 8–14, 2009 in northwest Gabon at Monts de Cristal National Park (at the Kinguélé and Tchimbélé rivers) and October 17–22, 2009 in northeast Gabon at Ivindo National Park (in the vicinity of Ipassa Station). Surveyed habitats included pristine forest, natural forest clearings (*bais*), and disturbed forest, ranging in elevation from approximately 75–550 m, and 480–530 m at Monts de Cristal and Ivindo, respectively. Both Monts de Cristal and Ivindo National Parks harbor extraordinary amphibian biodiversity and endemic species (Lötters et al., 2005; Pauwels and Rödel, 2007).

Amphibians were captured by hand and placed in individual plastic bags until they were processed the next morning. We collected samples from a total of 166 adult individuals (64 from Monts de Cristal and 102 from Ivindo) with sterile fine-tip swabs (MW113; Medical Wire & Equipment Co., Wiltshire, England) following the methods of Hyatt et al. (2007). Swabs were stored in 95% EtOH and kept as cool as possible in the field, and then stored at −80°C until processing. The swabbed individuals (except *Amietophrynus superciliaris*, which is listed as CITES Appendix I) were euthanized and prepared as voucher specimens (Supplemental Table 1) that are deposited at Cornell University Museum of Vertebrates (CUMV), the Museum of Comparative Zoology at Harvard University (MCZ), and North Carolina Museum of Natural Sciences (NCSM).

We followed the methods of Boyle et al. (2004) for *Bd* DNA extraction and qPCR detection of *Bd*. We processed

Table 1. Distribution of *Bd* across Anuran Families and Species Sampled in Monts de Cristal National Park[a]

Family	Genus	Species	*Bd*-positive/sampled	*Bd* intensity (GE)
Arthroleptidae	*Arthroleptis*	sp A	1/1	459.1
	Arthroleptis	sp B	0/1	–
	Cardioglossa	*elegans*	1/1	228.8
	Cardioglossa	*leucomystax*	0/2	–
	Leptopelis	*crystallinoron*	2/5	117.7 ± 161.9
	Leptopelis	*millsoni*	0/1	–
	Leptopelis	*notatus*	0/1	–
	Leptopelis	sp B	1/1	21.1
	Leptopelis	*zebra*	0/4	–
	Scotobleps	*gabonicus*	3/8	99.2 ± 84.2
	Trichobatrachus	*robustus*	0/1	–
Bufonidae	*Amietophrynus*	*superciliaris*	0/1	–
	Nectophryne	*batesii*	0/1	–
Hyperoliidae	*Afrixalus*	*paradorsalis*	0/1	–
	Hyperolius	*ocellatus*	1/2	409.9
	Hyperolius	*phantasticus*	0/1	–
	Hyperolius	*tuberculatus*	1/4	4.8
	Phlyctimantis	*leonardi*	0/4	–
Pipidae	*Silurana*	*epitropicalis*	0/1	–
Ranidae	*Amnirana*	sp A	1/3	2.0
	Amnirana	sp B	0/1	–
	Conraua	*crassipes*	0/1	–
	Petropedetes	*newtoni*	1/3	1.2
	Petropedetes	*palmipes*	2/5	5.9 ± 0.1
	Phrynobatrachus	*auritus*	3/3	2.4 ± 0.8
	Ptychadena	sp A	1/4	635.3
Rhacophoridae	*Chiromantis*	*rufescens*	1/3	116.6

Bd Batrachochytrium dendrobatidis, *GE* genome equivalents

[a]Data from the two sites surveyed within the park, the Kinguélé and Tchimbélé rivers, are combined. *Bd* infection intensity reported as average GE of positive samples

the qPCR experiments in 96-well plates on a real-time PCR machine (7900 HT; Applied Biosystems, Carlsbad, CA), and analyzed both the undiluted DNA extract and (1:10) dilutions of each sample to test for the possibility of PCR inhibition in undiluted extracts. Standards of known zoospore concentrations and negative controls were included in each plate. We evaluated the fluorescence levels of the samples and standards using SDS v 2.1 (Applied Biosystems). PCRs were repeated for all positive samples (both undiluted and diluted extracts) to confirm the presence of *Bd*. Samples were considered positive when a sigmoidal amplification occurred in both repeated PCR reactions and if the average infection intensity (inferred zoospore genome equivalents) between runs was greater than one. We estimated 95% confidence intervals for *Bd* prevalence at each site, using the modified Wald method, and used the average of the estimated zoospore genome equivalents to determine zoospore concentrations for individual samples.

Our final sample included 20 genera and 42 species of frogs from seven families. *Bd* was detected in four of the seven families (Arthroleptidae, Hyperoliidae, Ranidae, and Rhacophoridae), however, our sampling of Bufonidae, Pipidae, and Petropedetidae was limited (10 samples), thus we cannot reject the possibility that species in these families are also infected. Both national parks were *Bd*-positive at all elevations surveyed and across habitat types. Overall *Bd* prevalence for Monts de Cristal was 29.7%, and 13 of the 27 species sampled were *Bd*-positive (Table 1). The prevalence and *Bd* infection intensity varied slightly, but not significantly, between the two sites sampled at Monts de Cristal. At the Kinguélé river (elevational range 75–200 m), the prevalence was 25.6% (95% CI 12.2–39.0%) with a mean

Table 2. Distribution of *Bd* across Anuran Families and Species Sampled in the Vicinity of Ipassa Station in Ivindo National Park[a]

Family	Genus	Species	*Bd*-positive/sampled	*Bd* intensity (GE)
Arthroleptidae	*Arthroleptis*	*adelphus*	0/1	–
	Arthroleptis	sp A	1/8	97.0
	Astylosternus	*batesi*	0/2	–
	Leptopelis	*aubryi*	3/12	212.5 ± 181.7
	Leptopelis	*brevirostris*	1/4	293.3
	Leptopelis	*millsoni*	1/1	2.2
	Leptopelis	sp A	0/1	–
	Leptopelis	sp C	2/7	8.8 ± 1.1
	Leptopelis	*zebra*	0/1	–
Bufonidae	*Amietophrynus*	*camerunensis*	0/2	–
	Amietophrynus	*gracilipes*	0/2	–
	Nectophryne	*batesii*	0/2	–
Hyperoliidae	*Afrixalus*	*fulvovittatus*	1/6	7.4
	Afrixalus	*paradorsalis*	1/4	3.6
	Hyperolius	*bolifambae*	0/3	–
	Hyperolius	*cinnamomeoventris*	0/7	–
	Hyperolius	*kuligae*	0/1	–
	Hyperolius	*ocellatus*	3/8	3.3 ± 0.2
	Opisthothylax	*immaculatus*	0/2	–
Petropedetidae	*Dimorphognathus*	*africanus*	0/1	–
Ranidae	*Amnirana*	sp A	3/9	82.5 ± 129.2
	Amnirana	sp B	0/1	–
	Conraua	*crassipes*	1/7	1.3
	Ptychadena	sp A	0/4	–
	Ptychadena	sp B	1/1	2
Rhacophoridae	*Chiromantis*	*rufescens*	2/5	6.7 ± 1.9

Bd Batrachochytrium dendrobatidis, *GE* genome equivalents

[a]*Bd* infection intensity reported as average GE of positive samples

infection intensity of 223.7 genomic equivalents (GE) for infected individuals (range 2.4–635.3), whereas the prevalence at the Tchimbélé river (elevational range 450–560 m) was 37.9% (95% CI 20.3–55.6%) with a mean infection intensity of 22.0 GE for infected individuals (range 1.1–158.4). The overall *Bd* prevalence at Ivindo National Park (elevational range 480–530 m) was 23.5% (95% CI 15.3–31.7%), where 12 of the 26 species sampled were *Bd*-positive (Table 2). The *Bd* infection intensity at Ivindo was intermediate between the two sites at Monts de Cristal with a mean infection intensity of 66.6 GE (range 1.3–350.1) for *Bd*-positive individuals.

This study, to our knowledge, presents the first records of *Bd* in Gabon and the first record of infection for 19 species of African anurans. Infected frogs from four families (Arthroleptidae, Hyperoliidae, Ranidae, and Rhacophoridae) were common at all elevations (75–565 m) and all habitats surveyed (pristine forest, *bais*, disturbed forest). The two anuran communities sampled harbor similar species assemblages, with 12 species represented at both of the surveyed sites. Though the species composition overlaps by approximately 28%, our sample sizes are insufficient to infer whether certain species are more susceptible to *Bd* infection and whether differences in community assemblages might correspond to differences in *Bd* prevalence and infection intensity between sites.

As in many other *Bd* surveys of related species in African equatorial forests (Goldberg et al., 2007; Greenbaum et al., 2008; Kielgast et al., 2009), we sampled frogs from large breeding populations and did not find any moribund or dead frogs showing symptoms of chytridiomycosis. There are no historical population surveys to assess baseline population density and species richness of our focal sites, thus it is impossible to infer whether these populations experienced past declines. Though future efforts over longer sampling periods may identify diseased individuals, our short-term surveys did not find evidence of chytridiomycosis or significant mortality in this area.

Africa has been proposed as a potential source for the global expansion of *Bd* (Weldon et al., 2004), yet the dynamics of amphibian chytridiomycosis remain underexplored across the continent. Future efforts in Gabon, and Africa more generally, should focus on variation among hosts in susceptibility, possible variation in the virulence of the pathogen, and the environmental determinants of disease dynamics.

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References

Berger L, Speare R, Skerratt L (2005) Distribution of *Batrachochytrium dendrobatidis* and pathology in the skin of green tree frogs (*Litoria caerulea*) with severe chytridiomycosis. *Diseases of Aquatic Organisms* 68:65–70

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD (2004) Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using realtime Taqman PCR assay. *Diseases of Aquatic Organisms* 60:141–148

Doherty-Bone TM, Bielby J, Gonwouo NL, LeBreton M, Cunningham AA (2008) In a vulnerable position? Preliminary work fails to detect the amphibian chytrid pathogen in the highlands of Cameroon, an amphibian hotspot *Herpetological Journal* 18:115–118

Goldberg TL, Readel AM, Lee MH (2007) Chytrid fungus in frogs from an equatorial African montane forest in western Uganda. *Journal of Wildlife Diseases* 43:521–524

Greenbaum E, Kusamba C, Aristote MM, Reed K (2008) Amphibian chytrid fungus infections in *Hyperolius* (Anura: Hyperoliidae) from Eastern Democratic Republic of Congo. *Herpetological Review* 39:70–73

Hopkins S, Channing A (2003) Chytrid fungus in Northern and Southern Cape frog populations, South Africa. *Herpetological Review* 34:334–336

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, et al. (2007) Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. *Diseases of Aquatic Organisms* 73:175–192

Imasuen AA, Weldon C, Aisien MSO, Dupreez LH (2009) Amphibian chytridiomycosis: first report in Nigeria from the skin slough of Chiromantis rufescens. *Froglog* 90:6–8

Kielgast J, Rödder D, Veith M, Lötters S (2009) Widespread occurrence of the amphibian chytrid fungus in Kenya. *Animal Conservation* 13:1–8

Lips KR, Brem F, Brenes B, Reeve JD, Alford RA, Voyles J, et al. (2006) Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America* 103:3165–3170

Lötters S, Rödel MO, Burger M (2005) A new large tree frog from north-western Gabon (Hyperoliidae:Leptopelis). *Herpetological Journal* 15:149–152

Pauwels OSG, Rödel MO (2007) Amphibians and National Parks in Gabon, western Central Africa. *Herpetozoa* 19:135–148

Soto-Azat C, Clarke BT, Poynton JC, Cunningham AA (2010) Widespread historical presence of *Batrachochytrium dendrobatidis* in African pipid frogs. *Diversity and Distributions* 16:126–131

Weldon C, du Preez LH, Hyatt AD, Muller R, Speare R (2004) Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* 10:2100–2105