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Widespread Presence and High Prevalence of *Batrachochytrium dendrobatidis* in Gabon

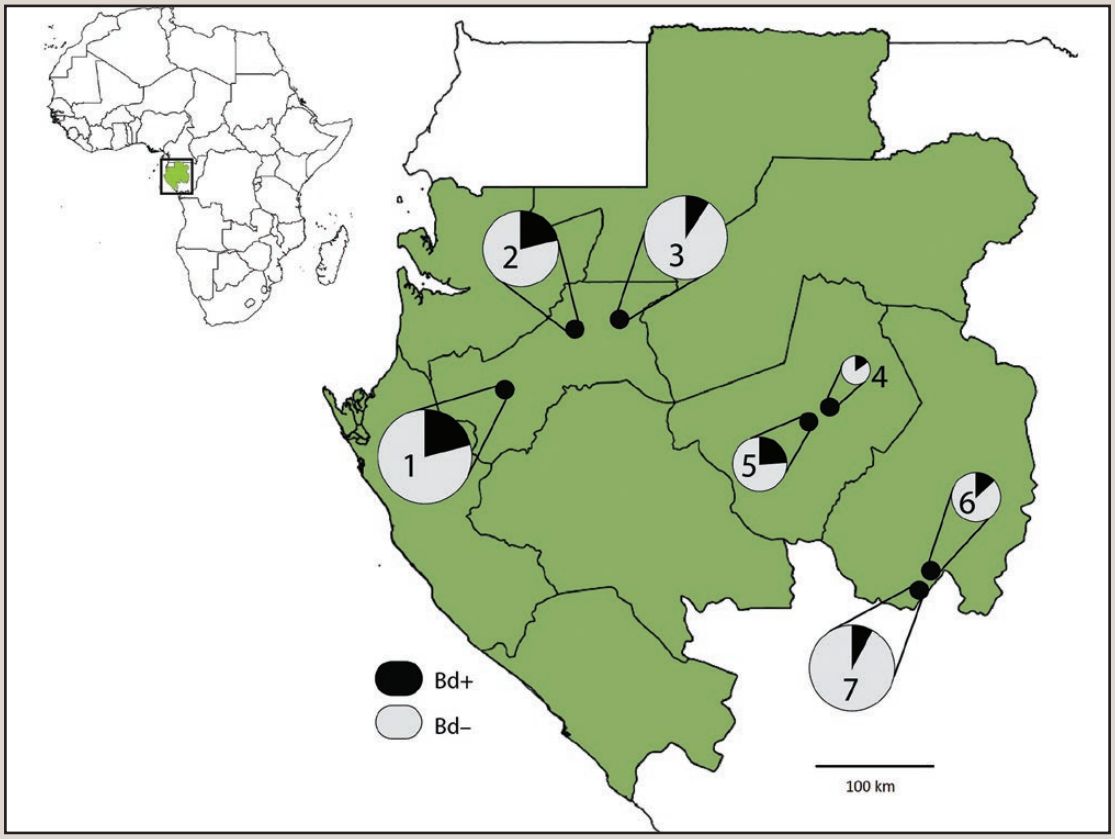

FIG. 1. Map of Gabon showing *Batrachochytrium dendrobatidis* (*Bd*) sampling locations for this study. Pie-charts are scaled by the total number of individuals swabbed at each site. Numbers refer to sites described in Table 1.

GREGORY F. M. JONGSMA*
New Brunswick Museum, 277 Douglas Avenue, Saint John, NB, Canada E2K 1E5
ABRAHAM BAMBA KAYA
JEAN-AIMÉ YOGA
JEAN-DANIEL MBEGA
JEAN-HERVÉ MVE BEH
Institut de Recherches Agronomiques et Forestières, Libreville, Gabon
ELIE TOBI
Center for Conservation and Sustainability Smithsonian Conservation Biology Institute, Gamba, Gabon
ANDREA M. EMRICH
141 Wentworth Ave., Saint John, NB, Canada E2L 2S7
GRAHAM P. DIXON-MACCALLUM
Department of Biology, University of Victoria, P.O. Box 3020, STN CSC, Victoria, BC, Canada V8W 3N5
DREW R. DAVIS
JACOB L. KERBY
Department of Biology, University of South Dakota, 414 E. Clark St., Vermillion, South Dakota 57069, USA
DAVID C. BLACKBURN
Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611, USA

*Corresponding author; e-mail: Gregor.Jongsma@gmail.com

Despite its small size, Gabon is a biodiversity hotspot with 198 mammal species, 680 bird species, 138 reptile species, 184 fish species, and 97 amphibian species (Lee et al. 2006; Pauwels and Vande weghe 2008). Emerging infectious diseases and habitat degradation pose the most immediate threats to amphibian diversity in Gabon. *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) are the causative agents of the amphibian disease chytridiomycosis which has been implicated in worldwide population decline and extinction (Berger et al. 1998; Lips et al. 2006; Wake and Vredenburg 2008; Martel et al. 2013). Across sub-Saharan Africa, *Bd* has been widely reported (Conradie et al. 2016). Despite extensive sampling, *Bd* has not been reported from West Africa (west of Nigeria; Penner et al. 2013). In Gabon, there have been four independent surveys for *Bd* with varied results. Bell et al. (2011) reported high prevalence of *Bd* from three sites in northern Gabon. Three subsequent studies at other sites across Gabon did not find *Bd* (Daversa et al. 2011; Gratwicke et al. 2011; Zimkus and Larson 2013). Here we present results from the most extensive survey for *Bd* in Gabon to date, based on the number of individuals, diversity of species, and geographic area sampled.

Gabon is a small equatorial country (267,667 km^2) that is dominated by continuous tropical moist forest (80% total land

TABLE 1. Summary information on sampling locations for *Batrachochytrium dendrobatidis* in Gabon. Site numbers correspond with numbers in Fig. 1.

#	Site	Province	Lat/Long	Elevation (m)	Dates	Habitat
1	Mitone	Moyen-Ogooué	00.6410°S, 10.2176°E	43	3–7 April 2015	Forest
2	Carivenville	Moyen-Ogooué	00.1770°S, 10.7795°E	44	8–13 April 2015	Forest
3	Junkville	Moyen-Ogooué	00.0621°S, 11.1587°E	86	15–18 April 2015	Forest-Savanna mosaic
4	Ogooué Cinq	Ogooué-Lolo	00.8105°S, 12.7995°E	419	4–5 May 2015	Forest
5	Madoukou	Ogooué-Lolo	00.8683°S, 12.6724°E	246	6–7 May 2015	Forest
6	Mboua	Haut-Ogooué	02.1532°S, 13.6398°E	504	26 April–1 May 2015	Forest
7	Doumaye	Haut-Ogooué	02.2402°S, 13.5812°E	526	21–25 April 2015	Forest-Savanna mosaic

cover; Lee et al. 2006). The dominant hydrological feature is the Ogooué River basin. There are four seasons: a long rainy season from January to May, a long cold dry season from June to September, a short rainy season from October to December, and a short dry season from December to January. The average annual temperature is 26°C (Lee et al. 2006).

We visited seven sites in three provinces: Moyen-Ogooué; Ogooué-Lolo; and Haut-Ogooué. We hand-caught individuals and stored them in separate plastic bags. The next morning, we swabbed live amphibians following protocols described by Hyatt et al. (2007) using sterile medical swabs (MW113; Medical Wire and Equipment Co., Wiltshire, UK). Our swab samples were stored in 1.5-mL snap-cap tubes with 95% EtOH and kept as cool as possible in the field. A subset of individuals was euthanized, tissued, stored in formalin and deposited at the California Academy of Sciences (CAS, San Francisco, California, USA) or in Gabon's national collection (curated by the Smithsonian Institution's Gabon Biodiversity Program in Gamba, Gabon).

Samples were extracted following the PrepMan Ultra extraction protocol (Hyatt et al. 2007) and diluted 1:10 with TE buffer to reduce potential inhibition during quantitative polymerase chain reaction (qPCR) analysis. Following fast qPCR methods described by Kerby et al. (2013), we used 10µL reactions (3µL DNA extract plus 7µL cocktail) to determine if samples were *Bd*-positive or *Bd*-negative. Additionally, each plate contained a negative control (nanopure water) and four synthetic DNA (sDNA) standards. Samples were run on a StepOnePlus qPCR machine and the number of *Bd* gene copies was quantified with StepOne software v2.3 (Applied Biosystems). All samples were run in triplicate and considered *Bd*-positive if: 1) amplification occurred in at least two of the three wells; and 2) the quantity was above 1.0. Samples were rerun if there were two wells with quantities near 1.0, or if independent values for a sample differed by an order of magnitude. All qPCR analyses were conducted at the Disease Testing Center at the University of South Dakota.

We swabbed a total of 463 frogs, representing 46 species from 18 genera in 10 families, across 7 sites (Table 1; Fig. 1). The overall prevalence across all sites was 18.6% with 60% of the sampled species testing *Bd*-positive, and *Bd*-positive results from all seven sites (across three provinces) (Table 2). Our results support Bell et al.'s (2011) findings that *Bd* is present in Gabon with high prevalence. In addition, we demonstrate that *Bd* is widespread across much of the country. Our raw data, including field and catalogue numbers are available at amphibiandisease.org: <https://n2t.net/ark:/21547/ANU2>.

There does not appear to be a strong association between the presence of *Bd* and specific taxa tested. The genus *Amnirana* (Ranidae) had both the highest prevalence and highest *Bd*

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FIG. 2. An apparently healthy adult male *Amnirana albolabris* (field# ORB-010) infected with *Batrachochytrium dendrobatidis* (43,422 zoospores equivalent).

gene copy number of any genus or family. One individual *Amnirana albolabris* (field# ORB-010) had a gene copy count of 43,422 (Fig. 2). Phrynobatrachidae was the only family that did not test positive for *Bd* during this study. However, because Skerratt et al. (2008) reported that >59 animals should be sampled to detect *Bd* when prevalence is low and our sampling includes only 23 individuals of *Phrynobatrachus*, this family may still be susceptible to *Bd* in Gabon. Bell et al. (2011) did report positive cases for three *Phrynobatrachus auritus* from northern Gabon. The Hyperoliidae, the most species-rich group sampled, had a prevalence of 18.7%. There were 6 species that made up 51% of the total sampling and each of these species had over 25 total individuals sampled (Table 2). *Scotobleps gabonicus* (19.2% prevalence), *Conraua crassipes* (19.2%), *Hyperolius cinnamomeoventris* (19.2%), *H. ocellatus* (16.7%) all had *Bd* prevalence close to the total prevalence. *Sclerophrys regularis* (10.7%) had the lowest prevalence of any highly sampled species, and *Amnirana albolabris* (38.7%) had the highest prevalence.

There was a lower prevalence of *Bd* at forest-savanna sites (N = 2 sites; mean = 9.4%, ± 1.1% SD) versus strictly forested sites (N = 5 sites; mean = 23.6%, ± 6.70% SD; t = 2.84, P = 0.036, d.f. = 5). Because there is no significant difference in the species composition of these sites based on a Sørensen similarity test, this suggests that either environmental variables are driving this difference in *Bd* prevalence, or there are transmission factors at play. For example, savanna-forest mosaic sites in Gabon are known to exhibit greater seasonal temperature fluctuations than surrounding forest sites (Bonnefille 2011). This temperature

TABLE 2. Amphibians sampled for *Batrachochytrium dendrobatidis* (*Bd*) in Gabon. Numbers indicate no. *Bd*-positive individuals/total no. sampled by species and site. Sites are numbered (1–7) in correspondence to Table 1.

Family/Species	Site							Total
	1	2	3	4	5	6	7	
Arthroleptidae								
Arthroleptis poecilonotus	-	-	-	-	2/3	-	0/2	2/5
Arthroleptis sylvaticus	0/1	1/1	-	-	-	-	-	1/2
Arthroleptis variabilis	0/2	1/3	1/2	-	-	-	0/1	2/8
Astylosternus batesi	-	-	-	-	-	0/1	0/6	0/7
Cardioglossa gracilis	0/1	-	-	-	-	-	0/3	0/4
Cardioglossa leucomystax	0/1	-	-	-	-	-	-	0/1
Leptodactylodon sp.	0/2	-	-	-	-	-	-	0/2
Leptopelis aubryi	0/2	0/5	1/2	-	-	-	-	1/9
Leptopelis calcaratus	-	-	-	-	-	-	0/3	0/3
Leptopelis millsoni	0/2	1/4	0/2	-	-	-	-	1/8
Leptopelis notatus	0/2	-	-	-	-	-	-	0/1
Leptopelis ocellatus	-	-	0/1	-	2/2	-	0/2	2/4
Leptopelis rufus	-	-	1/1	-	-	-	-	1/1
Leptopelis sp.	0/1	-	-	-	-	-	-	0/1
Scotobleps gabonicus	4/21	-	1/5	2/11	2/5	0/5	1/5	10/52
Bufonidae								
Sclerophrys gracilipes	1/2	0/1	-	-	-	-	0/1	1/4
Sclerophrys regularis	1/15	1/4	0/5	-	1/5	-	-	3/28
Sclerophrys superciliaris	-	-	-	-	-	-	0/1	0/1
Conrauidae								
Conraua crassipes	2/3	-	3/20	-	0/3	-	-	5/26
Dicroglossidae								
Hoplobatrachus occipitalis	-	-	-	-	-	-	0/2	0/2
Hyperoliidae								
Afrixalus dorsalis	0/2	-	1/10	-	-	-	-	1/12
Afrixalus paradorsalis	0/1	0/5	-	-	-	-	-	0/6
Cryptothylax greshoffii	-	-	-	-	-	1/3	-	1/3
Hyperolius adspersus	-	-	-	-	-	-	1/10	1/10
Hyperolius cinnamomeoventris	4/12	3/5	1/22	-	-	1/8	-	9/47
Hyperolius concolor	-	-	-	-	-	-	2/12	2/12
Hyperolius guttulatus	5/6	-	-	-	-	-	-	5/6
Hyperolius ocellatus	2/6	2/3	0/9	-	1/5	3/12	1/19	9/54
Hyperolius pardalis	-	-	-	-	1/5	-	1/8	2/13
Hyperolius phantasticus	-	-	-	-	-	-	0/3	0/3
Hyperolius platyceps	0/1	-	-	-	-	-	-	0/1
Hyperolius sp. 1	-	-	-	-	-	-	0/1	0/1
Hyperolius sp. 2	-	0/1	-	-	-	-	-	0/1
Hyperolius tuberculatus	0/3	5/15	0/5	-	-	-	-	5/23
Phlyctimantis leonardi	-	0/6	1/4	-	-	-	0/1	1/11
Phrynobatrachidae								
Phrynobatrachus africanus	0/6	-	-	-	0/1	-	0/5	0/12
Phrynobatrachus auritus	0/1	0/1	-	-	0/1	-	-	0/3
Phrynobatrachus sp.	-	-	-	-	-	-	0/5	0/5
Pipidae								
Xenopus cf. mellotropicalis	1/3	0/2	-	-	-	-	-	1/5
Xenopus amieti sp. group	-	2/4	0/2	-	-	-	-	2/6
Ptychadenidae								
Ptychadena perreti	-	0/2	0/1	-	1/4	-	-	1/7
Ranidae								
Amnirana albolabris	9/12	1/1	0/7	-	-	0/4	2/7	12/31
Amnirana amnicola	0/1	-	-	-	-	-	0/5	0/6
Amnirana lepus	-	-	-	-	-	-	1/3	1/3
Rhacophoridae								
Chiromantis rufescens	0/1	2/8	0/1	-	1/1	-	-	3/11
Total:	29/109	19/71	10/99	2/11	11/35	5/33	9/105	85/463
%:	26.6	26.8	10.1	18.2	31.4	15.2	8.6	18.6

fluctuation might be expected to influence the prevalence of *Bd* (Berger et al. 2004; Gaertner et al. 2012; Kinney et al. 2011; Kriger and Hero 2006; Whitfield et al. 2012). However, given our small sample sizes, many more forest and forest-savanna sites should be sampled to test this possible correlation.

All frogs that we encountered in the field appeared healthy. However, the high prevalence and widespread nature of *Bd* in Gabon demands future work to determine its impact on amphibians. A better understanding is needed of: 1) the historical presence of *Bd* in Gabon; 2) the contemporary genomic make-up of *Bd* in the country; and 3) which species, if any, exhibit symptoms of chytridiomycosis. Each of these will help to establish the extent to which *Bd* poses a threat to amphibians in tropical forests in Gabon and Central Africa.

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