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Baseline prevalence of *Batrachochytrium dendrobatidis* in a Central African rainforest amphibian community

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Batrachochytrium dendrobatidis (*Bd*), the fungal pathogen that causes chytridiomycosis, has been implicated in severe amphibian population declines and extinctions globally, yet the pathogen's distribution in Central Africa remains poorly documented. Gabon hosts exceptional amphibian diversity, but previous *Bd* surveys have been sparse or geographically restricted. We establish a baseline of *Bd* occurrence in Ngounié province, southern Gabon, by screening 276 adults, representing 57 species of anurans. Prevalence of *Bd* was 11.2% overall (31 individuals, 18 species). We report the first *Bd* detections in Gabon for *Cardioglossa leucomystax* and *Leptopelis notatus*, and the first global *Bd* records for *Amnirana amnicola* and *Arthroleptis taeniatus*. Infection was strongly associated with adult ecology: semi-aquatic and aquatic species showed significantly higher prevalence than arboreal or terrestrial taxa. Infection intensity was generally low, though one *Hyperolius tuberculatus* individual exhibited a very high load ($> 5 \times 10^5$ ITS copies) without visible signs of disease. We confirm *Bd* presence in southern Gabon and highlight aquatic habitats as *Bd* hotspots, providing baseline data for future monitoring and conservation in the region.

Keywords: Chytridiomycosis, Gabon, frog, reproductive mode, amphibian disease

Batrachochytrium dendrobatidis (*Bd*), l'agent fongique responsable de la chytridiomycose, a provoqué un déclin important et l'extinction de populations d'amphibiens du globe, mais la répartition de cet agent pathogène en Afrique centrale reste mal documentée. Le Gabon abrite une diversité exceptionnelle d'amphibiens, mais les études antérieures sur le *Bd* ont été rares ou géographiquement limitées. Nous établissons une base de référence de la présence du *Bd* dans la province de Ngounié, au sud du Gabon, en examinant 276 adultes, représentant 57 espèces d'anoures. La prévalence du *Bd* était de 11,2 % dans l'ensemble (31 individus, 18 espèces). Nous signalons les premières détections de *Bd* au Gabon pour les espèces *Cardioglossa leucomystax* et *Leptopelis notatus*, et les premières observations mondiales de *Bd* pour *Amnirana amnicola* et *Arthroleptis taeniatus*. L'infection était fortement associée à l'écologie des adultes: les espèces semi-aquatiques et aquatiques présentaient une prévalence significativement plus élevée que les arboricoles ou terrestres. L'intensité de l'infection était généralement faible, bien qu'un individu *Hyperolius tuberculatus* ait présenté une charge très élevée ($> 5 \times 10^5$ copies ITS) sans symptômes visibles de la maladie. Nous confirmons la présence de *Bd* dans le sud du Gabon et soulignons que les habitats aquatiques sont des zones sensibles au *Bd*, fournissant ainsi des données de référence pour la surveillance et la conservation futures dans la région.

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INTRODUCTION

The disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), is one of the proximate drivers of global amphibian declines (Luedtke et al., 2023). Discovered following mass mortality events in Australia and Central America in the 1990s (Berger et al., 1998), *Bd* has since contributed to declines in hundreds of amphibian species globally and extinctions in some (Scheele et al., 2019; Weldon et al., 2020). Experimental and field studies have shown that not only *Bd* presence, but also infection intensity, can strongly influence disease outcomes and mortality risk. For example, Vredenburg et al. (2010) demonstrated mass mortality events in *Rana muscosa* populations following *Bd* loads exceeding approximately 10,000 zoospore equivalents, suggesting that infection intensity may be a key predictor of chytridiomycosis impacts. Population monitoring, including disease monitoring and mitigation, is therefore critical for amphibian conservation (Luedtke et al., 2023). Initially thought to have originated in Africa (Weldon et al., 2004), updated genomic studies support an Asian origin for *Bd* (O'Hanlon et al., 2018), suggesting that *Bd* is a relatively novel pathogen and threat to amphibian biodiversity on the African continent (Doherty-Bone et al., 2020; Kouete et al., 2025). Nonetheless, monitoring continues to be scarce in parts of Africa, making it difficult to discern the geographic distribution of *Bd* and susceptibility to declines for African amphibian populations.

Widespread *Bd* prevalence has been noted in Central, Eastern and Southern Africa (Ghose et al., 2023). In Northern Africa, *Bd* has been detected in Morocco, the only country surveyed in this region to date (El Mouden et al., 2011; Ait El Cadi et al., 2019; Thumsová et al., 2022). In contrast, in tropical West Africa, little to no *Bd* has been detected, though it may have been historically present as early as 1969, based on recent tests of museum samples from Ghana and Benin (Penner et al., 2013; Nguyen et al., 2025). One hypothesis is that the Dahomey gap, a dry, unforested area spanning from western Nigeria to eastern Ghana, could be a biogeographic barrier to the spread of *Bd* in the region (Penner et al., 2013).

In nearby Central Africa, a retrospective study conducted by Hirschfeld et al. (2016) in western Cameroon showed a significant decline in species richness and abundance after *Bd* emergence on two mountains in the Cameroon Volcanic Line. This constitutes the first record of declines in Central Africa, raising concern for other montane habitats in the region. To date, no *Bd* surveys have been conducted in Angola, the Central African Republic, or Chad; however, environmental niche modelling suggests widespread prevalence across Central Africa, including coastal Angola, Cameroon, Equatorial Guinea and Gabon (Marshall et al., 2023; Zimkus et al., 2020).

Gabon hosts 107 amphibian species, representing some of the highest diversity of this group in Africa (Dewynter & Frétey, 2019). The few studies examining *Bd* prevalence and intensity in Gabon have yielded mixed

results; some showing no *Bd* presence (Daversa et al., 2011; Gratwicke et al., 2011; Zimkus & Larson, 2013) while others reveal moderate to high *Bd* prevalence across the country (Bell et al., 2011; Jongsma et al., 2016). Here, we provide baseline data on *Bd* prevalence and infection intensity in an amphibian assemblage from southern Ngounié province, Gabon. By documenting *Bd* infection status across 57 amphibian species, including several tested for the first time, our study provides baseline data for future monitoring and comparative research, contributing to a broader continental framework for understanding *Bd* distribution and conservation risk in Central Africa.

MATERIALS & METHODS

Study area

Fieldwork was conducted at five sampling sites within dense moist forest and a single marshland (Verhegghen et al., 2012) near and within the Baposso, Mambonga and Moukoubi communities in the Louetsi-Bibaka department of Ngounié province, Gabon (Lat 2° 4' 58.08" S, 12° 7' 43.248" E; 498–821 m a.s.l.; Fig. 1). Sampling took place in the vicinity of the Ramsar Site des Monts Birougou, part of the Chaillu Massif, a humid forest area of international importance for conservation. The Chaillu Massif is characterised by a humid equatorial climate, with year-round warm temperatures, high annual rainfall (> 2,000 mm) and pronounced rainfall seasonality. The area experiences a long wet season from October to May, peaking in October–November, and a short, drier season from June to September when precipitation is lowest. Sampling areas were situated near the headwaters of the Ogooué and Nyanga River basins, with some upper tributaries separated by less than 300 m (Fig. 1). The landscape comprises a matrix of old-growth forest, secondary forest of mixed ages, and plantations; however, we focused our sampling in interior old-growth areas with mature trees and continuous canopy cover.

Field methodology

Fieldwork was conducted between 22 August 2024 and 11 November 2024, with sampling at night between 18:00 and 24:00 h and opportunistic specimens collected during the day. We used ground- and canopy-access methods to sample a variety of habitats across the study site. Each evening, an eight-person team would divide into a sub-team that primarily surveyed stream, swamp and ground habitats, while a second sub-team used arborist methods (see Basham et al. (2024) for full methods) to survey canopy habitats. Opportunistic samples were also collected from trails and roads, as well as within campsite and community areas.

Specimens were captured by hand and stored individually in plastic bags. The date, time and associated microhabitat data were recorded at the time of capture. Frogs were handled through their individual plastic bags with nitrile-gloved hands to prevent cross-contamination and swabbed with a sterile, rayon-tipped swab (MW113, Medical Wire & Equipment), following standard protocols

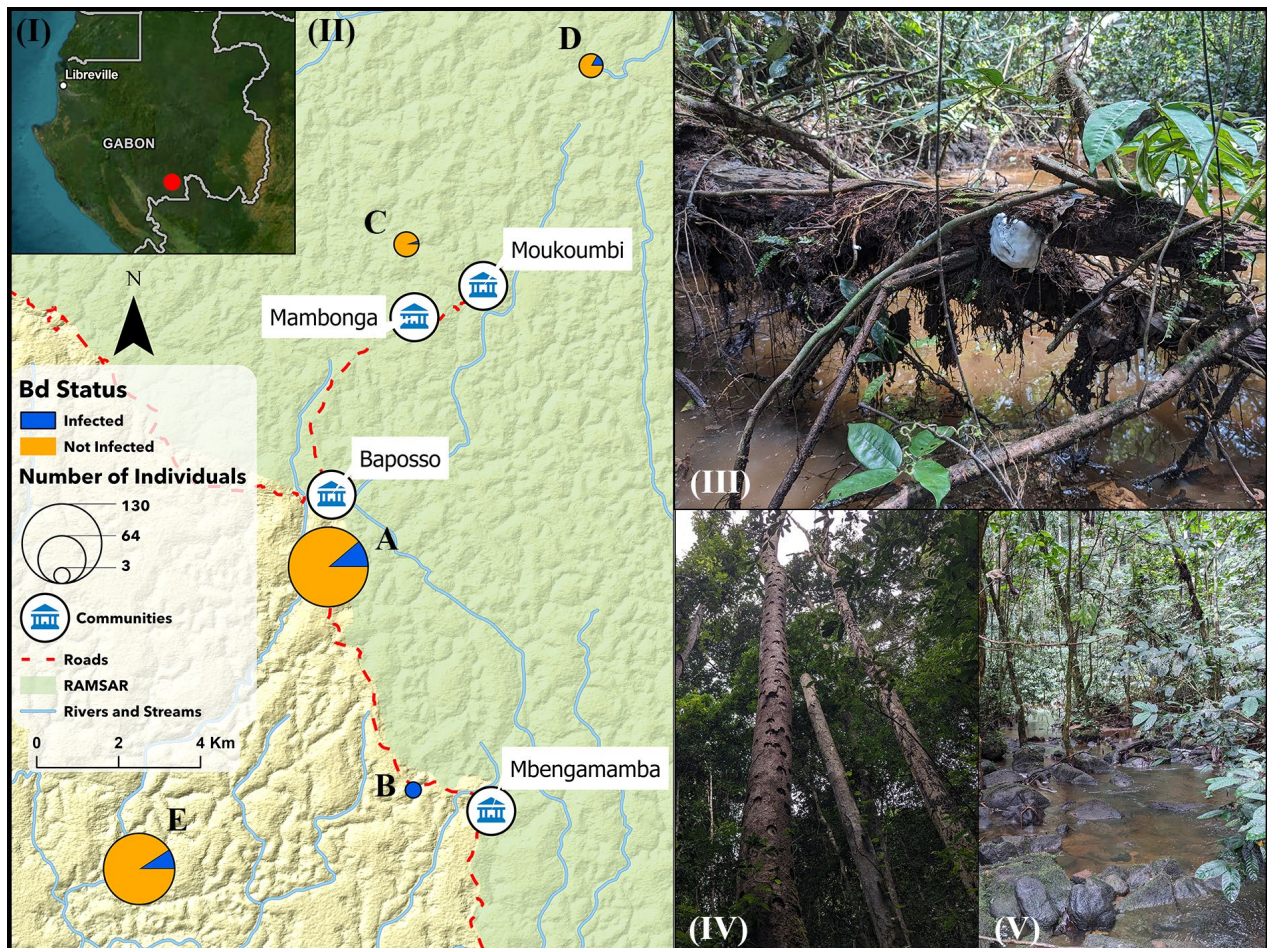


Figure 1. (I) Study area in the Louetsi-Bibaka Department of Ngounié Province, Gabon, (II) showing sampling locations near the communities of Baposso, Mbengamamba, Mambonga, and Moukoubi. Frog sampling for *Batrachochytrium dendrobatidis* (*Bd*) was conducted in – (III) interior forest ponds, (IV) the upper canopy, and (V) streams. Surveys were carried out in **A.** the adjacent forests of Baposso village, **B.** a marsh outside Mbengamamba and at field camps that facilitated access to remote areas: **C.** Mambonga Camp, **D.** Moukoubi Camp and **E.** Ngounié Camp.

outlined in Hyatt et al. (2007). We stored swabs in 1.5 mL Eppendorf tubes with 1 mL of 96% EtOH in a cool, dark room until refrigeration at 4 °C was possible, at which point they were maintained at approximately 4 °C until processing.

We categorised adult ecology of all species as arboreal, terrestrial, semi-aquatic and aquatic (Kouete et al., 2025). Due to limited sample sizes, semi-aquatic and aquatic categories were combined for statistical analyses. Reproductive modes followed Portik & Blackburn (2016): Aquatic eggs and aquatic larvae (AQAQ), terrestrial eggs and aquatic larvae (TEAQ), arboreal eggs and aquatic larvae (ARQA), direct development (DD) and the addition of a phytotelm breeding category (aquatic larvae develop in water held within vegetation; PHAQ; Basham et al., 2024).

Laboratory methods

We extracted DNA from 276 swabs of individual adult frogs using the Qiagen DNeasy Blood and Tissue Pro Kit following the manufacturer's animal tissues protocol (Qiagen, Germantown, MD, USA). To test extracts for *Bd*, we used qPCR with a TaqMan assay (Boyle et al., 2004),

with 1.2 µL each of primers ITS1-3 Chytr and 5.8S Chytr, which amplify a 146 bp fragment of the ribosomal ITS-1 region of *Bd*. We used Chytr MGB2 as our minor groove binder probe and added BSA to prevent PCR inhibition. We ran qPCRs on a QuantStudio™ 3 (Applied Biosystems). Cycling conditions were as follows: one 20 s Taq activation step at 95 °C, followed by 50 cycles of 95 °C for 1 s and 60 °C for 20 s. For all qPCRs, we used a serial dilution series of synthetic gBlock standards of the ITS region from 10^7 – 10^1 ITS copies. Each sample was initially screened in duplicate following standard *Bd* qPCR protocols (Boyle et al., 2004). Samples showing any amplification were subjected to additional replication to confirm infection status and reduce the likelihood of false positives near the detection limit. A small subset of samples were run with additional replicates if there was inconsistent amplification among replicates. Thus, the total number of qPCR replicates per sample ranged from two to seven. Infection status was determined using an established positivity threshold of >1 ITS copy (Kriger et al., 2006; Hyatt et al., 2007). For all final analyses, a sample was considered *Bd*-positive only if more than 50% of replicates exceeded 1 ITS copy (Arnott et al., 2024). For *Bd*-positive samples, infection

intensity was calculated as the mean ITS copy number across qPCR replicates. Three no-template negative controls were included in all qPCR runs; none amplified above the positivity threshold.

Species were preliminarily identified in the field based on external morphology and advertisement calls where applicable. Representative voucher specimens were collected and deposited at the New Brunswick Museum (NBM; Saint John, NB, Canada). Field identifications were subsequently confirmed by G. Jongsma (NBM). For individuals presenting difficult morphological identifications, species identity was further verified with molecular barcoding. We amplified a ~550 bp segment of the 16S rRNA gene from 152 swab or tissue extracts using the primers 16SA-L and 16SBH-NEW (Palumbi et al., 1991; Vences et al., 2024). We did not sequence all 276 samples because many represented common species already confirmed among the sequenced subset. Amplifications were performed in 12.5 µL reactions with the following thermal cycling conditions: 94 °C for 90 s, followed by 33 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 90 s. PCR products were purified and Sanger sequenced at Eton Bio (San Diego, CA 92121). Sequences were manually checked for quality and compared against a curated reference library of Central African amphibian 16S sequences (GJ), supplemented with GenBank BLAST searches where necessary. Samples clustering closely with reference sequences and showing low sequence divergence were considered reliably identified to species.

Statistical analysis

We used binomial Generalised Linear Models (GLMs) with a logit link function to assess the effects of host traits (adult ecology and reproductive mode) on the probability of frogs being *Bd*-positive. Overall effects of explanatory variables were tested using likelihood ratio tests (LRT) comparing full models to null (intercept-only) models. Post-hoc Tukey tests were conducted to perform pairwise comparisons. The package emmeans was used to generate predicted probabilities from the GLMs (Lenth

& Piaskowski, 2017). For reproductive mode, PHAQ was excluded from the GLM because all 10 PHAQ individuals were negative for *Bd* infection, which would result in complete separation if PHAQ were included in the binomial model. All statistical analyses were carried out using R version 4.2.2 (R Core Team, 2022). Many of the tested species had low abundances and therefore would require further sampling to confirm consistently positive or negative patterns (Skerratt et al., 2008). We did not analyse variation in infection intensity due to the limited number of positive samples ($n = 31$) spread among 18 species, which did not provide sufficient statistical power.

RESULTS

Prevalence

We tested a total of 276 individuals representing 57 host species in nine families, including four undescribed species (Table 1). The overall *Bd* prevalence was 11.2% (31 individuals) distributed among 18 species in seven families (Fig. 1). *Bd* was not detected in two families – Bufonidae and Conrauidae – however, the sample sizes for these families combined were less than 20 (Table 1; Fig. 1). The *Bd*-positive *Cardioglossa leucomystax* and *Leptopelis notatus* individuals are the first *Bd*-positive records in Gabon for these species (Fig. 2). To the best of our knowledge based on the compiled data available on AmphibiaWeb.org, we also present the first *Bd*-positive records for *Amnirana amnicola* and *Arthroleptis taeniatus* across their ranges (Fig. 2). Our testing of *Arthroleptis carquejai* and *Cardioglossa annulata* (*Bd*-negative) represent the first *Bd* tests for these species (AmphibiaWeb Project, 2020).

Bd prevalence varied by genus (Table 1), with *Ptychadena* (50%), *Hymenochirus* (100%), *Nyctibates* (100%) and *Kassina* (50%) having the highest prevalence; however, all had four or fewer individuals tested. Of the most abundant sampled genera, *Leptopelis* had a *Bd* prevalence of 11% ($n = 91$), followed by *Hyperolius* at 8% ($n = 50$).

Table 1. *Bd* prevalence data across sampled species, with adult ecology and reproductive mode categories. Asterisks denote reproductive modes that are currently unknown but can be inferred from closely related taxa.

Family	Genus/species	N	Positive (N)	Positive (%)	Adult Ecology	Reproductive Mode
Arthroleptidae		147	14	10		
	<i>Arthroleptis adelphus</i>	3	0	0	Terrestrial	DD
	<i>Arthroleptis carquejai</i>	2	0	0	Terrestrial	DD
	<i>Arthroleptis poecilonotus</i>	1	0	0	Terrestrial	DD
	<i>Arthroleptis</i> sp. 1	4	0	0	Terrestrial	DD*
	<i>Arthroleptis</i> sp. 2	1	0	0	Terrestrial	DD*
	<i>Arthroleptis sylvaticus</i>	4	0	0	Terrestrial	DD
	<i>Arthroleptis taeniatus</i>	6	1	17	Terrestrial	DD
	<i>Arthroleptis tuberosus</i>	1	0	0	Terrestrial	DD
	<i>Astylosternus batesi</i>	5	0	0	Terrestrial	AQAQ
	<i>Cardioglossa annulata</i>	3	0	0	Terrestrial	TEAQ
	<i>Cardioglossa elegans</i>	3	0	0	Terrestrial	TEAQ
	<i>Cardioglossa escalerae</i>	2	0	0	Terrestrial	TEAQ
	<i>Cardioglossa gracilis</i>	2	0	0	Terrestrial	TEAQ

Family	Genus/species	N	Positive (N)	Positive (%)	Adult Ecology	Reproductive Mode
Bufonidae	<i>Cardioglossa leucomystax</i>	6	1	17	Terrestrial	TEAQ
	<i>Leptopelis aubryi</i>	1	0	0	Arboreal	TEAQ
	<i>Leptopelis aubryioides</i>	16	0	0	Arboreal	TEAQ
	<i>Leptopelis boulengeri</i>	4	1	25	Arboreal	TEAQ
	<i>Leptopelis brevirostris</i>	18	2	11	Arboreal	DD
	<i>Leptopelis calcaratus</i>	26	4	15	Arboreal	TEAQ
	<i>Leptopelis millsoni</i>	21	2	10	Arboreal	TEAQ
	<i>Leptopelis notatus</i>	4	1	25	Arboreal	TEAQ
	<i>Leptopelis ocellatus</i>	1	0	0	Arboreal	TEAQ
	<i>Nyctibates corrugatus</i>	2	2	100	Terrestrial	AQAQ
	<i>Scotobleps gabonicus</i>	9	0	0	Terrestrial	AQAQ
	<i>Trichobatrachus robustus</i>	2	0	0	Terrestrial	AQAQ
		11	0	0		
	<i>Nectophryne afra</i>	6	0	0	Arboreal	PHAQ
	<i>Nectophryne batesii</i>	4	0	0	Arboreal	PHAQ
Conrauidae	<i>Wolterstorffina</i> sp.	1	0	0	Arboreal	Unknown
		1	0	0		
Hyperoliidae	<i>Conraua crassipes</i>	1	0	0	Semi-Aquatic	AQAQ
		80	7	9		
Phrynobatrachidae	<i>Afrixalus dorsalis</i>	3	0	0	Arboreal	ARAQ
	<i>Afrixalus laevis</i>	7	0	0	Arboreal	ARAQ
	<i>Afrixalus osorioi</i>	2	1	50	Arboreal	ARAQ
	<i>Afrixalus paradorsalis</i>	4	0	0	Arboreal	ARAQ
	<i>Hyperolius cinnamomeoventris</i>	2	0	0	Arboreal	ARAQ
	<i>Hyperolius kuligae</i>	3	0	0	Arboreal	ARAQ
	<i>Hyperolius obstetricans</i>	4	0	0	Arboreal	ARAQ
	<i>Hyperolius ocellatus</i>	5	0	0	Arboreal	ARAQ
	<i>Hyperolius pardalis</i>	3	0	0	Arboreal	ARAQ
	<i>Hyperolius platyceps</i>	8	0	0	Arboreal	ARAQ
	<i>Hyperolius</i> sp. 1	16	1	6	Arboreal	ARAQ*
	<i>Hyperolius</i> sp. 2	5	0	0	Arboreal	ARAQ*
	<i>Hyperolius</i> sp. 3	1	0	0	Arboreal	ARAQ*
	<i>Hyperolius tuberculatus</i>	3	3	100	Arboreal	AQAQ
	<i>Kassina maculosa</i>	4	2	50	Arboreal	AQAQ
	<i>Opisthothylax immaculatus</i>	6	0	0	Arboreal	ARAQ
	<i>Phlyctimantis verrucosus</i>	4	0	0	Arboreal	AQAQ
		14	1	7		
	<i>Phrynobatrachus africanus</i>	4	0	0	Terrestrial	AQAQ
	<i>Phrynobatrachus auritus</i>	7	0	0	Terrestrial	AQAQ
Pipidae	<i>Phrynobatrachus ikoundouensis</i>	3	1	33	Terrestrial	AQAQ
		10	4	40		
Ptychadenidae	<i>Hymenochirus boettgeri</i>	1	1	100	Aquatic	AQAQ
	<i>Xenopus andrei</i>	8	3	38	Aquatic	AQAQ
	<i>Xenopus mellotropicalis</i>	1	0	0	Aquatic	AQAQ
Ranidae		2	1	50		
	<i>Ptychadena aequiplicata</i>	1	0	0	Semi-Aquatic	AQAQ
Rhacophoridae	<i>Ptychadena perreti</i>	1	1	100	Semi-Aquatic	AQAQ
		7	3	43		
Rhacophoridae	<i>Amnirana amnicola</i>	4	3	75	Terrestrial	AQAQ
	<i>Amnirana lepus</i>	3	0	0	Terrestrial	AQAQ
Rhacophoridae		4	1	25		
	<i>Chiromantis rufescens</i>	4	1	25	Arboreal	ARAQ
Totals		276	31	11		

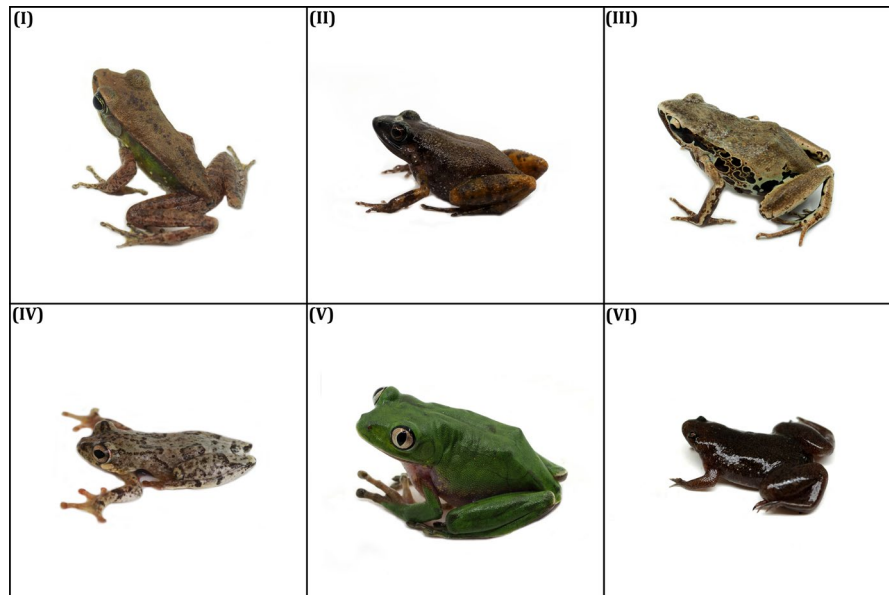


Figure 2. Photo panel of selected *Bd*-positive species – (I) *Amnirana amnicola*, (II) *Arthroleptis taeniatus*, (III) *Cardioglossa leucomystax*, (IV) *Hyperolius tuberculatus*, (V) *Leptopelis notatus*, and (VI) *Xenopus andrei*. *Amnirana amnicola* and *A. taeniatus* represent the first range-wide *Bd*-positive records for these species, while *C. leucomystax* and *L. notatus* are the first *Bd*-positive records from Gabon. *Hyperolius tuberculatus* included the individual with the highest observed *Bd* load ($>5 \times 10^5$ ITS copies). *Xenopus andrei*, a highly aquatic species, exhibited particularly high *Bd* prevalence (Table 1).

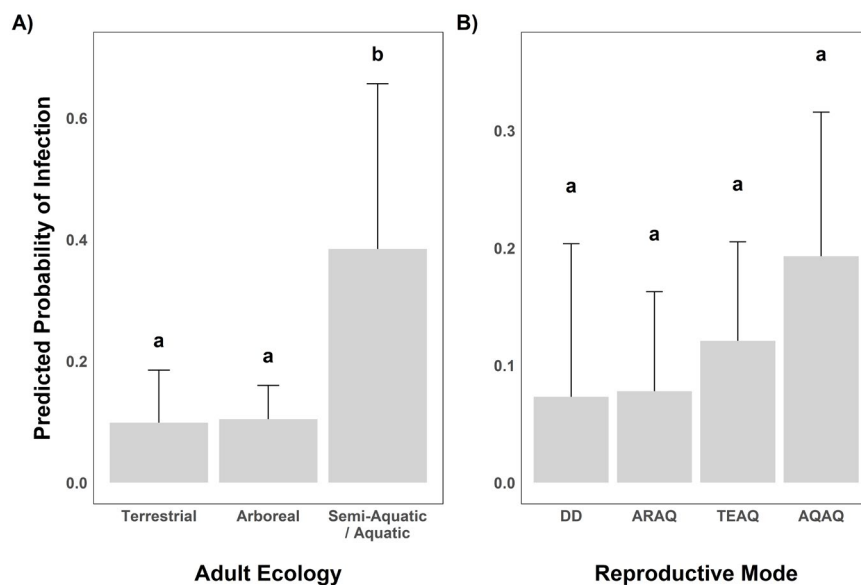


Figure 3. Predicted probability of *Bd* infection by – **A)** adult ecology and **B)** reproductive mode. Reproductive modes are classified as direct development (DD), arboreal eggs and aquatic larvae (ARAQ), terrestrial eggs and aquatic larvae (TEAQ) and aquatic eggs and aquatic larvae (AQAQ). Different letters indicate statistically significant differences among groups based on Tukey's HSD post-hoc tests ($\alpha = 0.05$); groups sharing the same letter do not differ significantly.

The effects of adult ecology

We found host adult ecology to have a significant overall effect on *Bd* prevalence (LRT $\chi^2 = 6.973$, $df = 2$, $P = 0.031$). A pairwise comparison Tukey test showed semi-aquatic/aquatic species had a significantly higher probability of infection than both arboreal and terrestrial species. Semi-aquatic/aquatic species had 5.7-fold higher odds of infection than arboreal and terrestrial species

(Fig. 3). Terrestrial and arboreal species represent the least infected frogs, with genera such as *Nectophryne*, *Opisthophyllax* and *Hyperolius* having zero or very few infected individuals. The aquatic genera *Hymenochirus* and *Xenopus* were among the most infected, with many or all the individuals testing positive (Table 1). There was no significant difference in infection probability between hosts with arboreal and terrestrial adult ecologies.

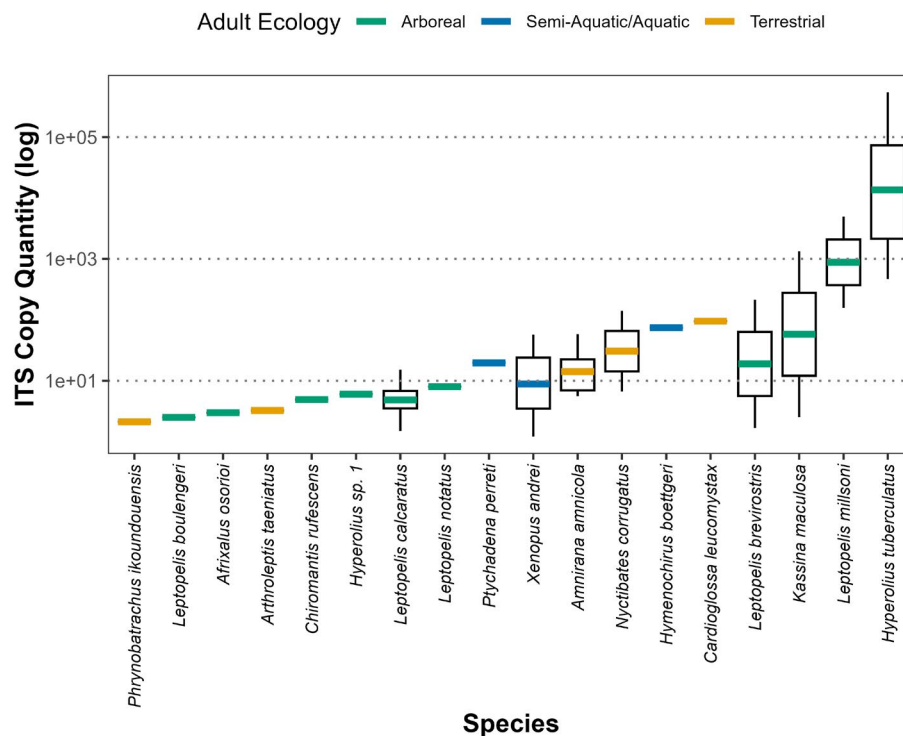


Figure 4. Boxplot of *Bd* infection intensity by *Bd*-positive species, on a logarithmic scale. Bold lines represent the mean of the distribution. Colours denote adult ecology category for each species.

The effects of host reproductive mode

GLM analysis showed no significant overall effect of reproductive mode on the probability of *Bd* infection, though the odds of infection were 2.8 times higher in AQAQ than in ARAQ (Fig. 3). The only reproductive mode in which *Bd* was not detected was in species where egg deposition and larval development occur in water-holding plants (PHAQ); however, this represents 10 tested individuals of two species in the genus *Nectophryne* (*N. afra* and *N. batesii*).

Infection intensity

Among the 31 individuals positive for *Bd*, intensity ranged from 1.2–545,000 ITS copies. The majority of positive individuals showed low levels of infection, while a small number, notably two *Hyperolius tuberculatus* individuals collected at the same pond on the same evening, presented orders of magnitude higher infection load (Figs. 2 & 4). Nonetheless, interpretation is limited by the small sample size, and it is not possible to distinguish whether the high intensity reflects a taxonomic pattern or a site-specific effect.

DISCUSSION

This study provides the first published positive records of *Batrachochytrium dendrobatidis* (*Bd*) from the Ngounié province of southern Gabon and confirms the pathogen's presence across multiple families. These results align with previous studies reporting low to moderate *Bd* prevalence in Gabon (Bell et al., 2011; Jongsma et al., 2016; Hydeman et al., 2017) and nearby Cameroon (Doherty-Bone et al., 2013; Miller et al., 2018), suggesting that *Bd*

persistence at modest prevalence may be characteristic of Central African forests. However, reports of much higher, long-term infection rates in Cameroon (Kouete et al., 2025) demonstrate that pathogen dynamics may vary regionally, emphasising the need for geographically expanded monitoring across the region.

We found that host ecology influenced infection patterns; the aquatic-associated families Pipidae (40%) and Ptychadenidae (50%) showed high prevalences. This aligns with well-established global links between *Bd* risk and prolonged contact with water, as zoospores persist and transmit most effectively in aquatic environments (Bielby et al., 2008; Li et al., 2021). Global meta-analyses further indicate that ecological association with water explains substantially more variation in *Bd* prevalence and infection intensity than host taxonomy or behavioural traits alone (Greenberg et al., 2017). Interestingly, the relationship between water dependence and infection risk seems to be nonlinear, with fully aquatic species often exhibiting lower *Bd* prevalence than semi-aquatic taxa (Greenberg et al., 2017). Our results partially reflect this pattern, as the highest prevalence occurred in Ptychadenidae, a semi-aquatic family. However, this finding is based on a small sample size and should be interpreted cautiously.

We also detected high prevalence in the fully aquatic Pipidae (40%), a similar result to Miller et al. (2018) who documented a similarly high prevalence (43%) in *Xenopus* in Cameroon, though from a limited sample size. Notably, our pattern contrasts with multiple studies from Central and other regions of Africa that reported a lower prevalence in fully aquatic species (Vredenburg et al., 2013; Jongsma et al., 2016; Hydeman et al., 2017;

Zimkus et al., 2020). Notably, the most sample-rich assessment to date, Kouete et al. (2025), reported Pipidae prevalence at only 12.8%, with several semi-aquatic families exhibiting a much higher prevalence, suggesting that prevalence levels in fully aquatic taxa is not typical for Central Africa. The suggestion that maximal aquatic specialisation may reduce infection risk while habitat generalism increases it (Greenberg et al., 2017) remains intriguing, as it implies a potential equilibrium in which extensive aquatic exposure combined with broad habitat use, yields the greatest vulnerability to *Bd*. Future studies with larger sample sizes will be required to clarify whether semi-aquatic species consistently exhibit higher infection risk than fully aquatic taxa in the Afrotropics.

Terrestrial direct-developing frogs exhibited a much lower prevalence but were not infection free, indicating that *Bd* can persist outside aquatic environments, likely via moist leaf litter or environmental reservoirs that receive seasonal inputs of zoospores (Kolby et al., 2015; Silva et al., 2019). During the wet season, large cohorts of metamorphosing juveniles emerge from ponds, shedding significant quantities of *Bd* into terrestrial habitats, potentially facilitating spillover into species with limited water contact (Kolby et al., 2015; Grogan et al., 2023). Thus, even low levels of terrestrial infection warrant attention, particularly for direct-developing species that may lack adaptive tolerance (Mesquita et al., 2017) and have experienced sharp declines following *Bd* emergence elsewhere (Hirschfeld et al., 2016).

Arboreal frogs exhibited very low prevalence, consistent with studies in nearby Cameroon (Kouete et al., 2025) and may be explained by canopy microclimates that are drier, warmer and less conducive to *Bd* growth (Burrowes et al., 2017). However, climate warming may increase canopy humidity and force some species downward to avoid heat stress (Basham & Scheffers, 2020), potentially elevating future *Bd* risk. We therefore recommend future surveillance that incorporates vertical microhabitat use, especially in regions where climate change may compress thermal niches.

Infection intensity was typically low, but one *H. tuberculatus* individual exhibited extremely high copy numbers, approximately 55 times the lethal limit suggested by Vredenburg et al. (2010). The absence of visible disease signs potentially indicates that African species may tolerate heavy infections without clinical chytridiomycosis (Reeder et al., 2012), a pattern consistent with hypotheses of long-term host-pathogen coexistence or lineage-specific tolerance (e.g. *Bd*-Cape; Belasen et al., 2022).

Taken together, our results indicate that *Bd* is present but currently at moderate prevalence in the forests of southern Gabon, with patterns shaped by dependence on aquatic habitats and life histories. Patterns of infection shown in semi-aquatic and aquatic taxa and low-prevalence terrestrial infections highlight the importance of water bodies as disease reservoirs, while also pointing to possible spillover into terrestrial environments. Our findings establish baseline data from

an understudied region in Gabon, provide new species-level infection records, and underscore the need for continued monitoring that includes vertical habitat use and potential environmental reservoirs.

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Author contributions

Edmund W. Basham and Gregory F.M. Jongsma conceptualised the project. Joseph J. Madden, Emily Forner, Patrick S. Champagne, Sverre Finney, Hermine Ina, Pierre Massala, Joris-Bloy Ngoulou and Edmund W. Basham conducted fieldwork. Edmund W. Basham, Gregory F.M. Jongsma and Abraham G. Bamba-Kaya organised logistics and fieldwork. Joseph J. Madden, Emily Forner, Patrick S. Champagne, Edmund W. Basham, Gregory F.M. Jongsma and Kelly R. Zamudio wrote the manuscript. Kelly R. Zamudio provided lab space.

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