Presence of the amphibian chytrid pathogen confirmed in Cameroon

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A fungal pathogen of amphibians, *Batrachochytrium dendrobatidis* (*Bd*), was detected in contemporary amphibian populations in the lowlands of the Congo Basin, Cameroon. The proportion of infection was low (1.4%; 1/70), and no clinical symptoms were observed. At a distant mountain site the survey failed to detect *Bd*. Given the likely origin of *Bd* on the African continent, the low prevalence and infection intensity could provide evidence for host-pathogen coevolution resulting in a partial resistance. Considering the suitable climate for *Bd* and the rich amphibian fauna, we suggest that the Cameroonian highlands should be further monitored.

Key words: Afromontane, chytridiomycosis, Congolian lowland rainforests, *Phlyctimantis, Xenopus*

The amphibian disease chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) is a dramatic example of an emerging infectious disease associated with population declines and extinctions. The fungus has been detected on all continents on which amphibians occur (Fisher et al., 2009), causing population declines and extinctions in America, Australia and Europe (Berger et al., 1998; Bosch et al., 2001; Bielby et al., 2009).

Africa is considered the most likely place of origin of *Bd* (Weldon et al., 2004). To date there is little evidence of population declines in African amphibians due to *Bd* (but see Weldon & du Preez, 2004; Channing et al., 2006; Measey, 2011), possibly due to host-pathogen coevolution (Kielgast et al., 2010) *Bd* has been detected in Africa in both historical (Weldon et al., 2004; Soto-Azat et al., 2010) and contemporary samples (e.g. Goldberg et al., 2007; Greenbaum et al., 2008; Kielgast et al., 2010; Bell et al., 2011). *Bd* has generally been found more commonly in eastern and southern Africa, while in western parts of Africa most attempts have yielded negative results (http://www.bd-maps.net). Cameroon is of particular importance as it lies at the natural border between the western and central African amphibian fauna (Penner et al., 2011), and is the region of origin of the historically oldest *Bd*-positive amphibian sample (a museum voucher specimen of *Xenopus fraseri* collected in 1933; Soto-Azat et al., 2010). However, very little effort has been made to survey contemporary amphibian populations for the presence of *Bd* in Cameroon, despite the exceptionally rich amphibian fauna in the Cameroonian highlands (Herrmann et al., 2005) from which new species are continuously being described (e.g. Zimkus, 2009; Barej et al., 2010; Blackburn et al., 2010b). Two studies from Mt. Oku, Bamenda Highlands failed to detect *Bd* in Cameroon (Doherty-Bone et al., 2008; Blackburn et al., 2010a). In neighbouring Nigeria, *Bd* was confirmed for lowland habitats but apparently absent in eastern mountain populations (Imasuen et al., 2009; Reeder et al., 2011). High *Bd*-prevalence (up to 37.9%) was recently detected in lowland forests of Gabon (Bell et al., 2011; but see also Daversa et al., 2011; Gratwicke et al., 2011). Based on *Bd* climate suitability modelling and high host species richness, Cameroon is an area with potential risk of future amphibian infections and subsequent declines (see Bielby et al., 2008; Rödder et al., 2009).

Here we describe results of surveys for the presence and proportion of infection of *Bd* in two different regions of Cameroon (Fig. 1). Samples were collected

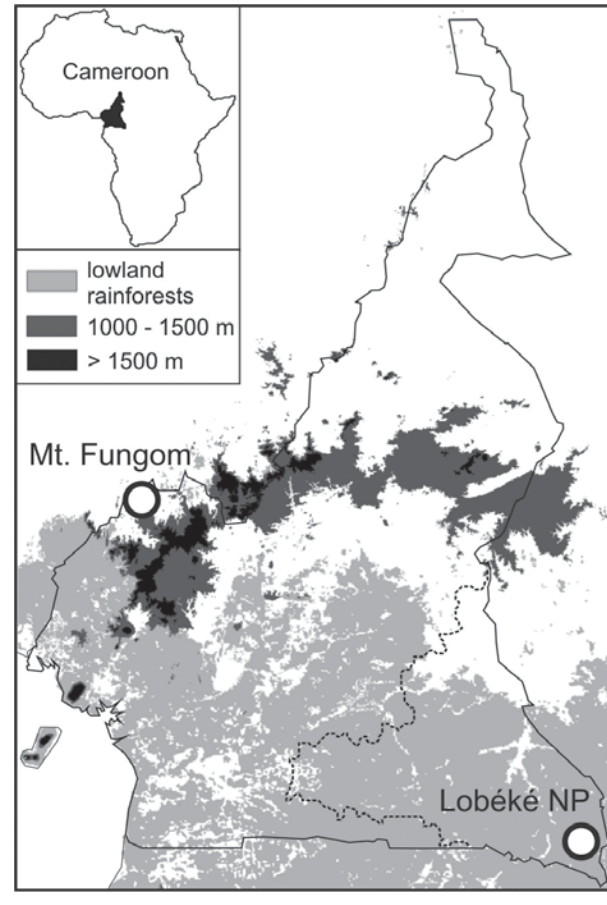

Fig. 1. Map of Cameroon showing the two sampled regions (Mt. Fungom and vicinity of the Lobéké National Park). Dashed line delimits border of the Congo Basin.

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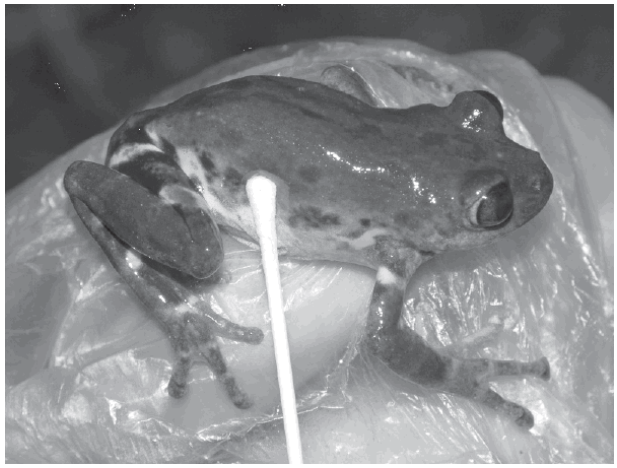

Fig. 2. A male of *Phlyctimantis leonardi* from locality PK27 during *Batrachochytrium dendrobatidis* (*Bd*) sampling by swabbing.

in May and June 2010 at the following localities: 1) Mt. Fungom, 06° 46.3' N, 09° 57.5' E, ~1200 m a.s.l., several microhabitats on the summit in submontane stream-side fringing forest and grassland mosaic; 2) five sites near Lobéké National Park; 2a) Mambele, 02° 26.5' N, 15° 25.8' E, ~470 m a.s.l., disturbed swampy edge of primary forest; 2b) Ngoum-Bandi (aka PK27), 02° 08.3' N, 15° 39.3' E, ~620 m a.s.l., small pond on the edge of primary forest; 2c) Kika, 01° 56.5' N, 15° 37.6' E, ~330 m a.s.l., swamp surrounded by farmbush; 2d) Malapa, 02° 06.1' N, 15° 21.3' E, ~390 m a.s.l., farmbush surrounded by disturbed primary forest; 2e) Mbimbé, 02° 05.2' N, 15° 24.5' E, ~450 m a.s.l., interior of pristine primary forest. All frogs were caught by hand using latex gloves or polypropylene bags during night encounter surveys. Swab samples were taken by firmly running sterile cotton swabs (Dryswab MW100 finetip) over the ventral surface, flanks and feet in the standardized manner (Hyatt et al., 2007). The examined specimens are either deposited in the herpetology collection of the National Museum in Prague, Czech Republic, or were released immediately after investigation.

DNA was extracted following Boyle et al. (2004). *Bd* detection was performed by real-time PCR (qPCR) with primers and Taqman probes specific to the pathogen on ABI prism 7500 Real-Time PCR System (Applied Biosystems) at the Institute of Zoology, Zoological Society of London. Extracted DNA samples were firstly pooled in pairs. If any of these pooled samples proved positive, samples were processed individually. A sample was considered positive if the genomic equivalent of one *Bd* zoospore reached at least 1.0. All detections were performed in duplicates.

Altogether, 104 samples of 21 frog species in 14 genera were collected in the two regions (34 samples in Mt. Fungom, 70 samples in the lowland forests; see details in Table 1). All negative controls proved negative. We detected one positive sample with a genomic equivalent value of 1.46 (mean of duplicate test; standard deviation=0.29) collected from a male *Phlyctimantis leonardi* (Hyperoliidae) from the lowland locality PK27 (Fig. 2). In total, 1.4% (95% confidence interval=0.1–7.5%) of individuals were infected within the lowland sample set and 1.0% (95% confidence interval=0.1–5.1%) from all tested specimens. We

Table 1. Amphibian species tested and results of our *Bd* screening in Cameroon. See text for locality details.

Habitat/Region	Family	Species	No. tested/ No. positive	Locality
Mountain/North-West Province	Arthroleptidae	*Astylosternus rheophilus*	3/0	Mt. Fungom
		Leptopelis modestus	5/0	Mt. Fungom
	Petropedetidae	*Petropedetes parkeri*	26/0	Mt. Fungom
Lowland/East Province	Arthroleptidae	*Leptopelis brevirostris*	2/0	Mbimbé
		Leptopelis notatus	1/0	Malapa
		Leptopelis ocellatus	5/0	Mambele, Mbimbé
	Bufonidae	*Amietophrynus maculatus*	3/0	Kika
	Dicroglossidae	*Hoplobatrachus occipitalis*	10/0	Kika
	Hyperoliidae	*Afrixalus "quadrivittatus"*	8/0	PK27
		Cryptothylax greshoffii	1/0	Kika
		Hyperolius cf. *balfouri*	2/0	PK27
		Hyperolius bolifambae	3/0	Mbimbé
		Hyperolius cf. *cinnamomeoventris*	1/0	Malapa
		Hyperolius ocellatus	1/0	Mambele
		Hyperolius pardalis	2/0	Mambele
		Phlyctimantis leonardi	7/1	PK27
	Pipidae	*Hymenochirus boettgeri*	2/0	Mambele
		Xenopus boumbaensis	14/0	Mambele, Malapa
	Ptychadenidae	*Ptychadena perreti*	1/0	PK27
	Ranidae	*Hylarana albolabris*	1/0	Mambele
	Rhacophoridae	*Chiromantis rufescens*	6/0	PK27

failed to detect *Bd* in the mountain locality. Fatal chytridiomycosis cases are known to occur when genomic equivalents reach about 10,000 (Vredenburg et al., 2010; Kinney et al., 2011). Low prevalence can be the result of an early stage of infection, or the asymptomatic presence of *Bd* (Swei et al., 2011). Studies that detected higher *Bd* prevalence in other African countries reported no mortalities (Kenya: Kielgast et al., 2010; Gabon: Bell et al., 2011), potentially supporting the African origin of *Bd* involving a host-pathogen coevolution (McCallum, 2005; Kielgast et al., 2010). It is interesting to note that no tested pipid frogs (*Xenopus*, *Hymenochirus*) were positive for *Bd* (as previously shown by Blackburn et al. (2010a) for another region in Cameroon). Similar to our results, Reeder et al. (2011) found *Bd* in eastern Nigeria only in a lowland site and without pathological symptoms. If the amphibians of the Cameroonian mountains are naive to *Bd* (see also Doherty-Bone et al. 2008), then concerning their predicted susceptibility the area is at high risk from the incursion of the chytrid from lowlands and should be further monitored.

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