Short Note

In a vulnerable position? Preliminary survey work fails to detect the amphibian chytrid pathogen in the highlands of Cameroon, an amphibian hotspot

T.M. Doherty-Bone[1], J. Bielby[2], N.L. Gonwouo[3], M. LeBreton[4] & A.A. Cunningham[2]

[1]*School of Biological Sciences, University of Aberdeen, UK*

[2]*Institute of Zoology, London, UK*

[3]*University of Yaoundé I, Laboratory of Zoology, Yaoundé, Cameroon*

[4]*UCLA/Johns Hopkins Cameroon Program, CRESAR, Yaoundé, Cameroon*

Establishing the presence in a region of the lethal amphibian pathogen *Batrachochytrium dendrobatidis* is important for predicting changes in amphibian assemblages, particularly in areas of high amphibian biodiversity. We examined 283 specimens of 26 anuran and one caecilian species inhabiting the highlands within North West Province, Cameroon, for the presence of *B. dendrobatidis* using real time PCR. All samples were negative for this pathogen. This part of Africa is a global hotspot for amphibian biodiversity. Our findings suggest that amphibians in this region are possibly naive to chytrid and are, therefore, at risk from the introduction of this pathogen.

Key words: Africa, amphibians, *Batrachochytrium dendrobatidis*, Biafran Highlands

The amphibian chytrid fungus (*Batrachochytrium dendrobatidis* – *Bd*), which causes the often fatal amphibian disease chytridiomycosis, has caused many amphibian population declines globally and is the likely cause of multiple amphibian species extinctions (Skerratt et al., 2007), particularly in mountainous areas, based on high rates of declines in these regions (Berger et al., 1998; Stuart et al., 2004; Pounds et al., 2006). This pathogen was contemporaneously discovered as the cause of amphibian mortality and population declines in Central America and Australia (Berger et al., 1998), possibly as a result of its introduction to naive species and populations (Morehouse et al., 2003; Rachowicz et al., 2005). Since its discovery, prospective and retrospective studies have identified chytridiomycosis as the cause of marked amphibian declines across five continents, and this disease is now considered to be one of the most important current threats to biodiversity (Daszak et al., 2003).

The status of *Bd* in Africa is cryptic. Retrospective studies of South African museum specimens have identified infection of the pipid frog *Xenopus laevis* with this pathogen since at least the 1930s (Weldon et al., 2004), and many workers consider Africa to be the natural endemic focus of *Bd*: *Bd* infection apparently has no ill effect on this species either at the individual or at the population level. The global trade in frogs, particularly *X. laevis*, from southern Africa in the early to mid 20th century is thought to have driven the international spread of the pathogen. In Africa, *Bd* infection has been identified in multiple amphibian species in the absence of reported declines (Weldon et al., 2004), but chytridiomycosis is considered to be the likely cause of at least one species extinction (the Kihansi spray toad, *Nectophrynoides asperginis*) on this continent (Weldon & du Preez, 2004).

Determining the presence or absence of *Bd* in other African regions is important for conservation planning to enable appropriate mitigating actions to be taken to conserve priority (i.e. endangered) species and sites of high amphibian diversity. One important region for amphibian diversity is the Biafran Highlands of West Africa, which extend from Bioko Island, Equatorial Guinea, along the Cameroon/Nigerian border (Gartshore, 1986; Myers et al., 2000; Bergl et al., 2007). Here we describe a survey in the Bamenda Highlands (a region of the Biafran Highlands in North West Cameroon) for the presence of *Bd*.

Field work was conducted between June and September 2006 in the North West Province of Cameroon, principally in the vicinity of the Kilum-Ijim Forests that surround Mount Oku. Weather conditions were monitored at base camp (1788 m a.s.l) daily at 0700 using a digital thermal hygrometer for temperature and humidity, and a standard rain meter for rainfall. Mean temperature was recorded daily at 17.6±0.7°C, with a weekly mean of 17.6±0.3°C. Mean daily humidity was 90±7.94%, with a mean daily rainfall of 9.8±10.53 mm and a weekly mean of 62.3±33.8 mm. The altitude of different survey areas ranged from 1534 to 2477 m a.s.l. These environmental conditions are considered favourable for the persistence of *Bd*, based on ecophysiological studies on this pathogen (Piotrowski et al., 2004; Woodhams & Alford, 2005; Kriger & Hero, 2007a). Habitats sampled included montane forest, grassland and cultivated land, all within a range of 10 km of one another.

Tissue samples, consisting of a toe clip for anurans or skin scrapings for caecilians, were collected from amphibians encountered. For each species sampled, all individuals were considered to belong to a single metapopulation. Species were sampled when encountered during a survey for affinities between forest and farmland, and were not sampled based on life history traits as in Rowley et al. (2007), although we recognize that *Bd* can be non-randomly distributed among hosts depending on various biological traits (Lips et al., 2003;

Correspondence: T.M. Doherty-Bone, Natural History Museum, Cromwell Road, London, SW7 5BD, UK.
E-mail: tommy_dbone@yahoo.com

Table 1. Overview of species studied. IUCN Red List status follows listings from the Global Amphibian Assessment (IUCN et al., 2006): LC – Least Concern; NT – Near Threatened; VU – Vulnerable; EN – Endangered; CR – Critically Endangered; DD – Data Deficient. "Restricted" describes ranges not extending beyond West Africa (i.e. range within the west and north of the Congo); "widespread" describes ranges extending beyond the Congo and the rest of Africa.

Species	Specimens sampled	IUCN Red List status	Range description
Afrixalus fulvovittatus	6	LC	Widespread
Arthroleptis adelphus	3	LC	Restricted
Arthroleptis cf. *poecilinotus*	6	–	Endemic
Arthroleptis poecilinotus	6	LC	Widespread
Arthroleptis variabilis	2	LC	Restricted
Astylosternus ranoides	2	EN	Endemic
Astylosternus rheophilus	4	VU	Endemic
Bufo maculatus	2	LC	Widespread
Bufo regularis	1	LC	Widespread
Cardioglossa oreas	5	EN	Endemic
Crotaphatrema lamotteii (caecilian)	1	DD	Endemic
Hyperolius hieroglyphicus	20	VU	Endemic
Leptodactylodon bicolor	3	VU	Endemic
Leptodactylodon perreti	3	EN	Endemic
Leptopelis modestus	1	LC	Widespread
Phrynobatrachus cf. *natalensis*	1	–	Endemic
Phrynobatrachus cf. *steinadachneri*	30	–	Endemic
Phrynobatrachus cf. *werneri*	25	–	Endemic
Phrynobatrachus sp.	4	–	–
Phrynobatrachus steinadachneri	39	VU	Endemic
Phrynobatrachus werneri	10	LC	Endemic
Ptychadena bibrioni	1	LC	Widespread
Trichobatrachus robustus	5	LC	Restricted
Werneria bambutensis	4	EN	Endemic
Xenopus amieti	5	NT	Endemic
Xenopus laevis	1	LC	Widespread
Xenopus longipes	74	CR	Endemic
Xenopus sp.	2	–	–
Unidentified frogs	17	–	–
Total	283		

Kriger & Hero, 2007b; Bielby et al., 2008). Tissue samples were fixed in ethanol and stored individually in labelled Eppendorf tubes (1.5 ml). These were exported to the UK with permission from the Cameroonian government (Ministry of Forests and Wildlife, Ref. 7431632). In the laboratory, DNA was extracted from tissue samples and was amplified using real-time PCR with *Bd*-specific primers (based on the methods of Boyle et al., 2004). *Bd*-negative and *Bd*-positive control samples were included with each analysis. Internal positive controls were not available for this study.

Tissue samples were taken from 283 amphibian specimens of 27 species (282 toe clips from anurans, one skin scraping from a caecilian; Table 1). None of the individuals tested were positive for *Bd* infection. Positive controls containing different concentrations of *Bd* DNA showed positive amplifications, while negative controls showed no amplification. Real-time PCR screening for chytrid is highly sensitive, being capable of detecting at least one fungus genome equivalent in a tissue sample (Boyle et al., 2004).

Ideally, a minimum of 59 animals of each species would have been sampled in order to detect *Bd* with a 0.95 probability assuming a 5% prevalence of infection (Thrusfield, 1995). This was not possible for any species other than *Xenopus longipes* (for which 74 animals were tested). As all 283 amphibians tested were sympatric and assuming each species is equally likely to be infected with *Bd* should it be present, this number of animals tested would have detected a 2% prevalence of *Bd* with a 0.95 probability (Thrusfield, 1995). As Weldon et al. (2004) reported an average prevalence of 2.7% in South African *Xenopus* spp. and Goldberg et al. (2007) reported a 22% prevalence of *Bd* infection in a multi-species assemblage in Uganda, it is likely that we would have detected *Bd* had it been present in our study area. However, these results should be considered as preliminary, as numerous factors could limit the ability of real-time PCR to detect infection by *Bd*. Such factors include: the failure of real-time PCR to detect early stages of infection, damage to the pathogen's DNA, or the presence of PCR inhibitors (Hyatt et al., 2007). As already discussed, *Bd* infection varies among host spe-

cies, and thus future survey work should include those species with traits that make them more susceptible to the impact of *Bd* infection. This would include stream-breeding montane endemic species such as *Cardioglossa* sp., *Astylosternus* sp. and *Leptodactylodon* sp.

This study is the first assemblage-wide survey for *Bd* infection in African amphibians west of the Congo Basin. Of the species represented, 16 are restricted to the Biafran Highlands of West Africa and, of these, all except one are listed by the IUCN as being of conservation concern (Table 1). Ten of the species tested have ranges extending beyond the Biafran Highlands. These wider-ranging species represent possible routes of *Bd* incursion to the region via contiguous transmission between populations in infected areas. The presence of chytrid in these, and sympatric, species in other regions should be investigated and considered when assessing the risk of *Bd* spreading to the mountain endemics of the Biafran Highlands. This is especially of concern as amphibians that are naive to *Bd* in high altitude, tropical regions appear to be particularly susceptible to population declines and extinctions following the emergence of this pathogen (Berger et al., 1998; Stuart et al., 2004; Pounds et al., 2006; Bielby et al., 2008).

Acknowledgements. This manuscript was greatly improved following comments by Stefan Lötters and one anonymous reviewer. Fieldwork was carried out as part of the Belo Community Herpetological and Conservation Project. Work was facilitated by staff of the Belo Rural Development Project (BERUDEP), the Cameroon Herpetological Project (CamHerp) and the University of Aberdeen and funded by the British Herpetological Society, the University of Aberdeen, the Carnegie Trust for the Universities of Scotland, the Royal Zoological Society of Scotland, the Trinity and Patrick Award from the Royal Geographical Society (with the Institute of British Geographers), the North of England Zoological Society, the Gordon Foundation and the Explorer's Club of New York. Assistance was afforded in the lab by Matt Perkins. Sincere thanks are due to field assistants Roseanne Miller, Ngane Benjamin Kome, Yongahbi Fred Talah and Fouepi Eric Landrie, logistical facilitator Godwin Mahow, forest managers Sanih Thaddeus (Abuh Community Forest) and Tosam David Amoh (Anyafouma Community Forest) and forest guide Khimal Peter Yuli. Thanks are also due to Innocent Wultof, David Blackburn, Stefan Lötters, Barry Clarke and David Gower. The Cameroon Ministry of Forests and Wildlife granted research and export permits for this study, for which we express our deepest gratitude.

REFERENCES

Berger, L., Speare, R., Daszak, P., Green, E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G. & Parkes, H. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences USA* 95, 9031–9036.

Bergl, R.A., Oates, J.F. & Fotso, R. (2007). Distribution and protected area coverage of endemic taxa in West Africa's Biafran forests and highlands. *Biological Conservation* 134, 195–208.

Bielby, J., Cooper, N., Cunningham, A.A., Garner, T.J. & Purvis, A. (2008). Predicting susceptibility to future declines in the world's frogs. *Conservation Letters* 1, 82–90.

Boyle, D.G., Boyle, D.P., Olsen, V., Morgan, J.A.T. & Hyatt, A.D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real time Taqman PCR assay. *Diseases of Aquatic Organisms* 60, 141–148.

Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2003). Infectious disease and amphibian population declines. *Diversity and Distributions* 9, 141–150.

Gartshore, M.E. (1986). The status of the montane herpetofauna of the Cameroon highlands. In *Conservation of Cameroon Montane Forests*, 204–240. Stuart, S.N. (ed.). Cambridge: International Council for Bird Preservation.

Goldberg, T.L., Readel, A.M. & Lee, M.H. (2007). Chytrid fungus in frogs from an equatorial African montane forest in western Uganda. *Journal of Wildlife Diseases* 43, 521–524.

IUCN, Conservation International & NatureServe (2006). Global Amphibian Assessment. www.globalamphibians.org. Downloaded on 17 January 2008.

Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., Berger, L., Obendorf, D., Datton, A., Kriger, K., Hero, M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F. & Colling, A. (2007). Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73, 175–192.

Kriger, K.M. & Hero, J.M. (2007a). Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology* 271, 352–359.

Kriger, K.M. & Hero, J.M. (2007b). The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Diversity and Distributions* 13, 781–788.

Lips, K.R., Reeve, J.D. & Witters, L.R. (2003). Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* 17, 1078–1088.

Morehouse, E.A., James, T.Y., Ganely, A.R.D., Vilgalys, R., Berger, L., Murphy, P.J. & Longcore, J.E. (2003). Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Molecular Ecology* 12, 395–403.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A.B. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.

Piotrowski, J.S., Annis, S.L. & Longcore, J.E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96, 9–15.

Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Marca, E., Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sánchez-Azofeifa, G.A., Still, C.J. & Young, B.E.

(2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439, 161–167.

Rachowicz, L.J., Hero, J.M., Alford, R.A., Taylor, J.W., Morgan, J.A.T., Vredenburg, V.T., Collins, J.P. & Briggs, C.J. (2005). The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. *Conservation Biology* 19, 1441–1448.

Rowley, J.J.L., Chan, S.K.F., Tang, W.S., Speare, R., Skerratt, L.F., Alford, R., Cheung, K.S., Ho, C.Y. & Campbell, R. (2007). Survey for the amphibian chytrid *Batrachochytrium dendrobatidis* in Hong Kong in native amphibians and in the international amphibian trade. *Diseases of Aquatic Organisms* 78, 87–95.

Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., Phillott, A.D., Hines, H.B. & Kenyon, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth*, DOI: 10.1007/s10393-007-0093-5.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischmann, D.L. & Waller, R.W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786.

Thrusfield, M. (1995). *Veterinary Epidemiology*, 2nd edn, 187–191. Oxford: Blackwell Science.

Weldon, C. & du Preez, L.H. (2004). Decline of Kihansi spray toad, *Nectophrynoides asperginis*, from the Udzungwa mountains, Tanzania. *Froglog* 62, 2–3.

Weldon, C., du Preez, L.H., Hyatt, A.D., Muller, R. & Speare, R. (2004). Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* 10, 2100–2105.

Woodhams, D.C. & Alford, R.A. (2005). Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. *Conservation Biology* 19, 1449–1459.

Accepted: 15 November 2008