Frost, T., and O. Testa (eds.). 2009. Speleological Projects 2007 and 2008 to Gabon (Central Africa) - Expéditions Spéléologiques 2007 et 2008 au Gabon (Afrique Centrale). Berliner Höhlenkundliche Berichte:35–68.

Goldberg, T. L., A. M. Readel, and M. H. Lee. 2007. Chytrid fungus in frogs from an equatorial African montane forest in western Uganda. J. Wildl. Dis. 43:521–524.

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

Imasuen, A. A., C. Weldon, M. S. O Aisien, and L. H. Du Preez. 2009. Amphibian chytridiomycosis: first report in Nigeria from the skin slough of *Chiromantis rufescens*. Froglog 90:6–8.

IUCN [International Union for the Conservation of Nature] Species Survival Commission. 2004. IUCN Global Amphibian Assessment. Conservation International Center for Applied Biodiversity Science, NatureServe. Available at http://www.globalamphibians.org (accessed 8 Aug 2010).

Kielgast, J., D. Rödder, M. Veith, and S. Lötters. 2010. Widespread occurrence of the amphibian chytrid fungus in Kenya. Anim. Conser. 13:1–8.

Lips K. R. 1998. Decline of a tropical montane amphibian fauna. Conserv. Biol. 12:106–17.

———, F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc. Natl. Acad. Sci. USA. 103:3165–3170.

———, D. E. Green, and R. Papendick. 2003. Chytridiomycosis in wild frogs from southern Costa Rica. J. Herpetol. 37:215– 218.

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

Plana, V. 2004. Mechanisms and tempo of evolution in the African Guineo–Congolian rainforest. Phil. Trans. R. Soc. Lond. B 359:1585–1594.

Reeder, N. M. M., T. Cheng, V. T. Vredenburg, and D. C. Blackburn. Survey of the chytrid fungus *Batrachochytrium dendrobatidis* from montane and lowland frogs in eastern Nigeria. Herpetology Notes. In press.

Rödder, D., J. Kielgast, J. Bielby, S. Schmidtlein, J. Bosch, T. W. J. Garner, M. Veith, S. Walker, M. C. Fisher, and S. Lötters. 2009. Global amphibian extinction risk assessment for the panzootic chytrid fungus. Diversity 1:52–66.

Skerratt, L. F., L. Berger, H. B. Hines, K. R. McDonald, D. Mendez, and R. Spear. 2008. *Survey protocol for detecting chytridiomycosis in all Australian frog populations.* Dis. Aquat. Org. 80:85–94.

Soto-Azat, C., B. T. Clarke, J. C. Poynton, and A. A. Cunningham. 2010. Widespread historical presence of *Batrachochytrium dendrobatidis* in African pipid frogs. Diversity and Distributions 16:126–131.

Weldon, C., L. H. Preez, A. D. Hyatt, R. Muller, and R. Speare. 2004. Origin of the amphibian chytrid fungus. Emerging Infectious Diseases 10: 2100–2105.

White, L. J. T., and K. Abernethy. 1997. A Guide to the Vegetation of the Lopé Reserve, Gabon. 2nd ed., Wildlife Conservation Society, Multipress, Libreville.

Herpetological Review, 2011, 42(1), 69–71.

Batrachochytrium dendrobatidis Not Detected on Amphibians from Two Lowland Sites in Gabon, Africa

Batrachochytrium dendrobatidis (*Bd*) is behaving as an invasive pathogen in many parts of the new world where it has been associated with massive amphibian declines in native populations (Berger et al. 1998; Lips et al. 2006; Lips et al. 2008; Soto-Azat et al. 2010). *Bd* has also been found on various Pipidae species in South Africa, Uganda, Malawi and Cameroon from as early as the 1930s, suggesting a widespread historical prevalence on the continent (Soto-Azat et al. 2010; Weldon et al. 2004). Despite these observations, many areas in Africa have not been systematically surveyed for *Bd*. Contemporary *Bd* distribution data are sorely lacking in West and Central Africa, including for Gabon (http://www.bd-maps.net). Yet, such data may prove critical in assessing the risk of *Bd* infection to amphibians on the continent, and identifying its ultimate origin.

In November to December 2009, we surveyed amphibians for *Bd* at two lowland areas in Gabon (Fig. 1). The first site was located in the SW region of the country around the town of Gamba (2.78676°S, 0.04551°E; elevation 0–100 m). The area is a coastal plain covered by a mosaic of rainforest patches interspersed with open, seasonally inundated grasslands (Alonso et al. 2006). The second site was at the Libreville campus and surrounding areas of L'Ecole Nationale des Eaux et Forêts training center in the NW region of the country (0.6030167°N, 9.3372667°E; elevation 0–100 m). It is covered by primary and secondary rainforest with many streams, but most amphibians at this site were found in temporary wetlands formed in open grassy areas adjacent to the forest. At each site we opportunistically searched for amphibians in forests, streams, wetlands and ditches. Amphibians were captured with a gloved hand and were brought indoors for processing where they were swabbed with a sterile cotton swab (10 strokes on the belly and thigh,

B. GRATWICKE*
A. ALONSO
T. ELIE
J. KOLOWSKI
J. LOCK
N. ROTZEL
J. SEVIN
R. C. FLEISCHER
Smithsonian Conservation Biology Institute, 3001 Connecticut Ave., NW Washington, DC 20008, USA

** Corresponding author; e-mail: brian.gratwicke@gmail.com*

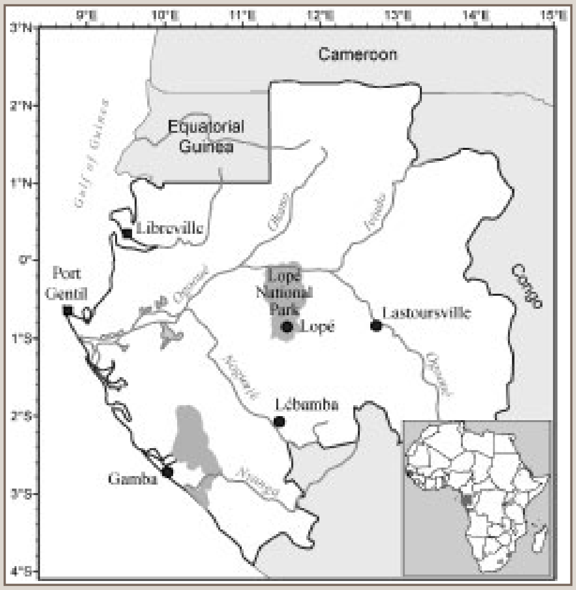

Fig. 1. Locations of Libreville and Gamba, Gabon, where amphibians were sampled for *Batrachochytrium dendrobatidis*.

and five strokes on each foot). Swabs were stored in tissue buffer prior to DNA extraction. Specimens were identified to the extent possible in the field using a small reference collection of specimens housed on site at Gamba. We retained 129 specimens for the Smithsonian's National Museum of Natural History (USNM 578099 – 578232) Washington, DC, USA. We swabbed and released an additional 24 *Hyperolius* spp. at Libreville.

Each swab was incubated in an oscillating thermal incubator at 56°C and 350 rpm in 400 µl of buffer ATL (Qiagen) and proteinase K (Qiagen) for 24 h. After incubation, DNA was extracted from the lysate solutions using a Qiagen Biosprint 96 DNA Blood Kit according to the manufacturer's instructions for "extraction from buccal swabs." Extracted DNA was eluted in 200 µl of AE buffer (Qiagen) and stored at 4°C prior to further analysis. To ensure successful DNA extraction, a fragment of 16s mRNA was amplified for each sample, including negative controls (N = 10), and visualized on 2% agarose gels. Testing for the presence of *Bd* was performed using a Qiagen QuantiTect SYBR Green PCR Kit using the primers ITS1-3 Chytr and 5.8s Chytr, developed by Boyle et al. (2004). To identify potential false positive and false negative results, two positive and two negative controls were included in every PCR reaction; false positive/negative results were not obtained.

A total of 86 frogs from Gamba and 66 frogs from Libreville were sampled, represented by five families (Table 1); all were *Bd*- negative. We used a binomial distribution to calculate the maximum potential prevalence rate using a 95% confidence limit with our sample of 0 infected swabs (successes) from 152 frogs (trials). Assuming 100% *Bd* detectability on all animals swabbed, we can be 95% confident that—if *Bd* is present in wild frogs at these two sites—prevalence rates are lower than 2%. While we cannot rule out non-detection as a reason for its supposed absence, we can say that if *Bd* is present, its prevalence in this region is extremely low and thus unlikely to be having any significant ecological impact on the sampled populations. These data as well as those of Daversa et al. (2011, this issue) therefore lend support to climate models which predict *Bd* has a low probability of affecting amphibian populations in lowland areas (Rödder et al. 2009).

Table 1. Amphibian species tested for *Batrachochytrium dendrobatidis* (*Bd*) at two sites (Gamba and Libreville) in Gabon.

Species	Gamba	Libreville	*Bd* positive
Afrixalus dorsalis	3	7	0
Amietophrynus regularis	4	0	0
Chiromantis rufescens	0	5	0
Hoplobatrachus occipitalis	6	0	0
Hyperolius spp.	10	32	0
Hyperolius tuberculatus	2	9	0
Hyperolius cf. *cinnamomeoventris*	3	1	0
Hyperolius nasutus	25	2	0
Hyperolius phantasticus	15	0	0
Hyperolius platyceps	1	0	0
Leptopelis aubryi	1	1	0
Leptopelis sp. (juvenile)	2	0	0
Phlyctimantis leonardi	6	2	0
Phrynobatrachus auritus	1	0	0
Ptychadena sp.	1	5	0
Xenopus epitropicalis	6	2	0
Totals	86	66	0

Acknowledgments.—We are grateful to field and financial support from Shell Gabon, and the Smithsonian Conservation Biology Institute's Center for Conservation Education and Sustainability. Ecole Nationale des Eaux et Forêts provided logistical support. We also thank the Government of Gabon for giving us permission to conduct the study and to export the specimens, and M. Butler, T. Leuteritz, and O. Pauwels for providing valuable logistical advice and assistance. This is publication #134 of the Gabon Biodiversity Program.

Literature Cited

Alonso, A., M. E. Lee, P. Campbell, O. S. G. Pauwels and F. Dallmeier, eds. 2006. Gamba, Gabon: Biodiversity of an Equatorial African Rainforest. Bull. Biol. Soc. Washington 12:1–448.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, and K. R. McDonald. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Nat. Acad. Sci. 95:9031–9036.

Boyle, D. G., D. B. Boyle, V. Olsen, J. A. T. Morgan, and A. D. Hyatt. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis. Aquat. Org. 60:141–148.

Daversa, D., J. Bosch, and K. Jeffery. 2011. First survey of the disease-causing fungus, *Batrachochytrium dendrobatidis*, in amphibian population of tropical areas of Gabon, Africa. Herpetol. Rev. 42: [this issue].

Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc. Nat. Acad. Sci. 103:3165–3170.

———, J. Diffendorfer, J. R. Mendelson III, and M. W. Sears. 2008. Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. PLoS Biology 6:e72.

Rödder, D., J. Kielgast, J. Bielby, S. Schmidtlein, J. Bosch, T. Garner, M. Veith, S. Walker, M. Fisher, and S. Lötters. 2009. Global amphibian extinction risk assessment for the panzootic chytrid fungus. Diversity 1:52–66.

Soto-Azat, C., B. T. Clarke, J. C. Poynton, and A. A. Cunningham. 2010. Widespread historical presence of *Batrachochytrium dendrobatidis* in African pipid frogs. Diversity and Distributions 16:126–131.

Weldon, C., L. H. Preez, A. D. Hyatt, R. Muller, and R. Speare. 2004. Origin of the amphibian chytrid fungus. Emerg. Inf. Dis. 10:2100–2105.

Herpetological Review, 2011, 42(1), 71–75.

First Record of the Chytrid Fungus *Batrachochytrium dendrobatidis* in North Africa

An important driver of amphibian declines is chytridiomycosis, a disease caused by the pathogen *Batrachochytrium dendrobatidis* (*Bd*; Berger et al. 1998; Daszak et al. 1999, 2003; Ron and Merino-Viteri 2000; Bosch et al. 2001; Collins and Storfer 2003, Ron et al. 2003; La Marca et al. 2005; Wake and Vredenburg 2008; Walker et al. 2010). *Bd* is now known to be an emerging pathogen that is rapidly expanding its global range (Fisher et al. 2009) and now has attained a global distribution on all continents that contain amphibians (www.bd-maps.net). Research by Weldon et al. (2004) on the potential origin of *Bd* suggests that the panzootic originated in South Africa and has perhaps been dispersed by international trade in amphibians, becoming established around the word (Rödder et al. 2009). To date, the rapid and widespread distribution of *Bd* infection across hundreds of amphibian species is alarming.

In Spain, outbreaks of chytridiomycosis and mass mortalities has been reported in many species and in different regions of the country (Bosch et al. 2001; Bosch and Martínez-Solano 2006; Walker et al. 2008, 2010). In the south of Spain, *Bd* is widely distributed (www.bd-maps.net); Bosch et al., unpubl. data), suggesting that its occurrence in the north of Morocco is plausible. The two regions are spatially proximate with similar Mediterranean ecologies, however are separated by the straits of Gibraltar. In addition, ecological niche modelling has shown that the environmental envelope in the north of Morocco is suitable for the establishment of *Bd* where susceptible amphibians occur (Ron 2005; Rödder et al. 2009). The legal and illegal increase in the transport of animals for pet trade (Fisher and Garner 2007), and the possibility that the fungus could be vectored into Morocco on the feathers of water birds, are potential modes of transmission between Spain and Morocco. However, no systematic surveillance for *Bd* has been undertaken across this region, where several endemic species occur with a high ecological value. The aim of the present study is to conduct the first survey for *Bd* in north Morocco in order to determine the presence, prevalence and intensity of infection in the amphibian populations that occur within region. These data will allow us to understand the distributional patterns of *Bd*, providing essential data for the effective management and control of this emergent pathogen.

Surveys for *Bd* were conducted between October 2005 and April 2009. During this period, we prospected the north of Morocco which is primarily a mountainous area (central-western Rif and north part of middle Atlas). Samples from the plain of Gharb and the Mammora regions were provided by P. de Pous (University of Applied Science van Hall-Larenstein, the Netherlands). The Rif region forms a mountainous barrier that is relatively low in elevation (less than 2500 m) with approximately half of the surface occurring above 500 m. It is an extension of the Baetic Cordillera, which includes the Sierra Nevada in the south of Spain. The area is a sub-wet zone with an average annual rainfall between 800 and 1400 mm (Bab Taza: 1361 mm/year), and probably reaches 2000 mm on the highest summits, and wetlands are common. In comparison, the coastal fringe is dry, where rainfall does not reach 500 mm (Oued Laou, 473 mm). Across the coast, the summer is moderate in temperature (Oued Laou, mean maximum temperature of the hottest month: 28.6°C), however weather stations located inland re-

E. H. EL MOUDEN
T. SLIMANI
Laboratoire de Biodiversité et Dynamique des Ecosystèmes
Département de Biologie, Faculté des Sciences-Semlalia
Université Cadi Ayyad, B.P. 2390, Marrakech, Morocco
D. DONAIRE
Mar Egeo 7, Jerez de la Frontera, 11407 Cádiz, Spain
S. FERNÁNDEZ-BEASKOETXEA
Departamento de Biodiversidad y Biología Evolutiva
Museo Nacional de Ciencias Naturales CSIC
José Gutiérrez Abascal 2, 28006 Madrid, Spain
M. C. FISHER
Department of Infectious Disease Epidemiology
Imperial College London W2 1PG, UK
J. BOSCH*
Departamento de Biodiversidad y Biología Evolutiva
Museo Nacional de Ciencias Naturales CSIC
José Gutiérrez Abascal 2, 28006 Madrid, Spain

**Corresponding author; e-mail: bosch@mncn.csic.es*