# AMPHIBIAN AND REPTILE DISEASES

*Herpetological Review*, 2020, 51(4), 726–732.


## Low Prevalence of *Batrachochytrium dendrobatidis* Detected in Amphibians from Vietnam's Highest Mountains

**BENJAMIN TAPLEY***
*Zoological Society of London, Regent's Park, London, NW1 4RY, UK*
*e-mail: ben.tapley@zsl.org*
**PHILLIP JERVIS**
*Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK; MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, UK*
*e-mail: phil.jervis2010@gmail.com*
**LUAN THANH NGUYEN**
*Asian Turtle Program of Indo-Myanmar Conservation, R.1806, 18th F., CT1 Bac Ha C14 Building To Huu St., Nam Tu Liem District, Ha Noi, Vietnam; e-mail: nguyenluanbio@gmail.com*
**CHRISTOPHER PORTWAY**
*Australian Museum Research Institute, Australian Museum, 1 William St, Sydney, NSW, 2010, Australia*
*e-mail: christopher.portway@austmus.gov.au*
**CHUNG THANH NGUYEN**
*e-mail: chung.crco@gmail.com*
**HAO VAN LUONG**
*Hoang Lien National Park, Sa Pa, Lao Cai, Vietnam*
*e-mail: haoluongvan@gmail.com*
**DANIEL KANE**
*Zoological Society of London, Regent's Park, London, NW1 4RY, UK*
*e-mail: daniel.kane@zsl.org*
**LOLA BROOKES**
*Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK. MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, UK; Department of Production and Population Health, The Royal Veterinary College, Hawkshead Lane, Herts AL9 7TA, UK*
*e-mail: lola.brookes@ioz.ac.uk*
**MATTHEW W. PERKINS**
*Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK; e-mail: matthew.perkins@ioz.ac.uk*
**PRIA GHOSH**
*MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, UK; Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa*
*e-mail: pria.ghosh13@imperial.ac.uk*
**CLAUDIA WIERZBICKI**
*Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK; MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, UK*
*e-mail:c.wierzbicki@imperial.ac.uk*

Chytridiomycosis, caused by the amphibian chytrid fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*), is an infectious disease implicated in the declines of over 500 amphibian species worldwide (Scheele et al. 2019). Amphibian population declines associated with the disease have been reported from all continents where amphibians occur, with the exception of Asia (Mutnale et al. 2018). Current evidence suggests that *Bd* originated in Asia (O'Hanlon et al. 2018) and five of the six known lineages of *Bd* have been detected on the continent (Farrer et al. 2011; Schloegel et al. 2012; Bataille et al. 2013; Rodriguez et al. 2014; Byrne et al. 2019).

The Hoang Lien Range in northwestern Vietnam is within the Indo-Burma biodiversity hotspot (Myers et al. 2000) and contains the last remnants of native forest of the northern Vietnamese highlands (Hoang et al. 2014). The region is within the suspected native range of *Bd* and supports a diverse amphibian fauna with more than 80 species of amphibians currently described (Ohler et al. 2000; Matsui et al. 2017; Tapley et al. 2017a, Tapley et al. 2017b; Tapley et al. 2018). This includes two microendemic, Critically Endangered megophryid frogs, *Oreolalax sterlingae* (Nguyen et al. 2013; IUCN 2015a) and *Leptobrachella botsfordi* (Rowley et al. 2013a; IUCN 2015b), the former is restricted to Mount Fansipan, Vietnam's highest mountain (Nguyen et al. 2020) and the latter is only known from Mount Fansipan and Mount Pu Ta Leng (Tapley et al. 2020). There are several other threatened frog species from the region and three newly described *Megophrys* species from the area are likely also threatened (van Dijk et al. 2004; Lu et al. 2014; Tapley et al. 2017b; Tapley et al. 2018). As a result, Mount Fansipan

**JENNIFER SHELTON**
*MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, UK*
*e-mail: j.shelton@imperial.ac.uk*
**MATTHEW C. FISHER**
*MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, UK*
*e-mail: matthew.fisher@imperial.ac.uk*
**JODI J. L. ROWLEY**
*Australian Museum Research Institute, Australian Museum, 1 William St, Sydney, NSW, 2010, Australia; Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney NSW 2052, Australia; e-mail: jodi.rowley@austmus.gov.au*

*Corresponding author

TABLE 1. Full list of the 601 *Batrachochytrium dendrobatidis*-ITS qPCR results organized by sampling year and site from the Hoang Lien Range, Vietnam. Bold rows indicate surveys where *Batrachochytrium dendrobatidis* (*Bd*) was detected. Protocol numbers and all metadata (species, age class, location, site, date, and genomic equivalents) are available from doi:10.5061/dryad.cjsxksn3v. *Batrachochytrium salamandrivorans* (*Bsal*) sample sizes are also indicated; *Bsal* was not detected at any site.

| Year | Site number | Site | *Bd* Prevalence | *Bd* Prevalence C.I. (95%) | *Bsal* sample size per site |
|---|---|---|---|---|---|
| 2015 | 1 | 2800 m, Mt Fansipan 22.3146°N, 103.7657°E | 0/15 | 0–21% | 0 |
| **2015** | **2** | **2200 m, Mt Fansipan 22.3283°N, 103.7818°E** | **2/23** | **1–30%** | **0** |
| 2015 | 3 | 1900 m, Mt Fansipan 22.3507°N, 103.7714°E | 0/13 | 0–25% | 0 |
| 2015 | 10 | 1026 m, Ban Hoa 22.2586°N, 103.9643°E | 0/1 | 0–98% | 0 |
| **2016** | **1** | **2800 m, Mt Fansipan 22.3146°N, 103.7657°E** | **2/22** | **1–29%** | **0** |
| 2016 | 9 | 1770 m, 8 km NW Sapa 22.3821°N, 103.7870°E | 0/7 | 0–41% | 0 |
| 2016 | 3 | 1900 m, Mt Fansipan 22.3507°N, 103.7714°E | 0/4 | 0–60% | 0 |
| 2016 | 4 | 1200 m, Mt Fansipan 22.3226°N, 103.8286°E | 0/2 | 0–84% | 0 |
| 2017 | 3 | 1900 m, Mt Fansipan 22.3507°N, 103.7714°E | 0/45 | 0–8% | 0 |
| 2017 | 4 | 1200 m, Mt Fansipan 22.3226°N, 103.8286°E | 0/24 | 0–14% | 0 |
| 2017 | 2 | 2200 m, Mt Fansipan 22.3283°N, 103.7818°E | 0/31 | 0–11% | 0 |
| 2017 | 7 | 2800 m, Mt Ky Quan San 22.5005°N, 103.6040°E | 0/9 | 0–34% | 0 |
| 2017 | 6 | 2100 m, Mt Ky Quan San 22.5061°N, 103.6152°E | 0/6 | 0–5% | 0 |
| 2017 | 1 | 2600 m, Mt Fansipan 22.3146°N, 103.7657°E | 0/33 | 0–11% | 0 |
| 2018 | 8 | 2000 m, Mt Pu Ta Leng 22.4325°N, 103.6300°E | 0/17 | 0-20% | 17 |
| 2018 | 2 | 2200 m, Mt Fansipan 22.3283°N, 103.7818°E | 0/60 | 0–6% | 43 |
| 2018 | 3 | 1900 m, Mt Fansipan 22.3507°N, 103.7714°E | 0/48 | 0–7% | 48 |
| **2018** | **4** | **1200 m, Mt Fansipan 22.3226°N, 103.8286°E** | **1/6** | **0–64%** | **6** |
| 2018 | 1 | 2600 m, Mt Fansipan 22.3146°N, 103.7657°E | 0/45 | 0–8% | 35 |
| 2018 | 5 | 2500 m, Mt Fansipan 22.2959°N, 103.8042°E | 0/31 | 0–11% | 31 |
| **2019** | **3** | **1900 m, Mt Fansipan 22.3507°N, 103.7714°E** | **1/52** | **0–10%** | **0** |
| 2019 | 2 | 2200 m, Mt Fansipan 22.3283°N, 103.7818°E | 0/44 | 0–8% | 0 |

TABLE 1. Continued.

| Year | Site number | Site | *Bd* Prevalence | *Bd* Prevalence C.I. (95%) | *Bsal* sample size per site |
|---|---|---|---|---|---|
| 2019 | 1 | 2600 m, Mt Fansipan 22.3146°N, 103.7657°E | 0/6 | 0–5% | 0 |
| 2019 | 5 | 2500 m, Mt Fansipan 22.2959°N, 103.8042°E | 0/22 | 0–15% | 0 |
| 2019 | 4 | 1200 m, Mt Fansipan 22.3226°N, 103.8286°E | 0/35 | 0–10% | 0 |

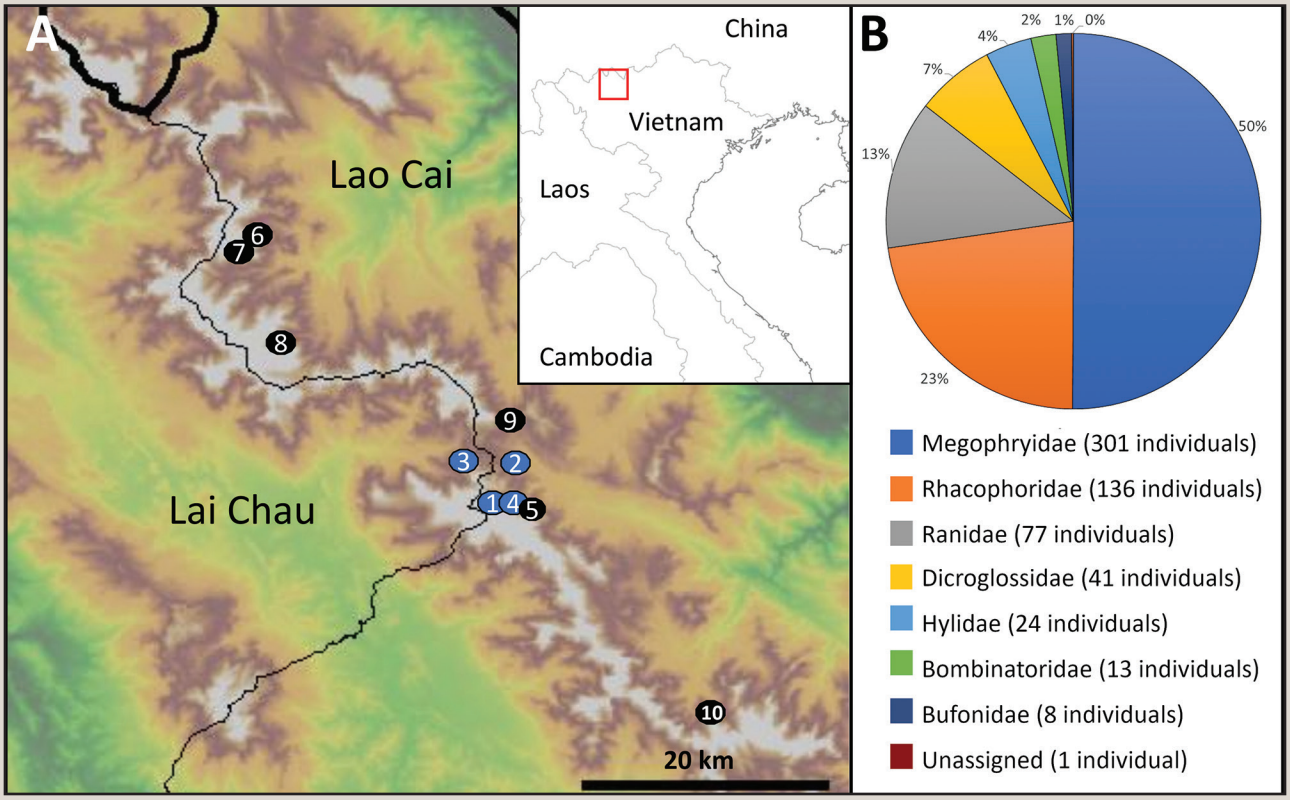


FIG. 1. (A) Map of amphibian sampling sites for *Batrachochytrium dendrobatidis* (*Bd*) in the Hoang Lien Range, Vietnam; black dots indicate sites where *Bd* was not detected and blue dots indicate sites where *Bd* was detected at least once over the five-year study period. (B) Proportion of amphibian individuals (N = 601) of 40 species sampled by family.

has been identied as a priority site by the Alliance for Zero Extinction (AZE 2016).

Previously, efforts have been made to determine the presence and prevalence of *Bd* in the Hoang Lien Range at elevations between 600 m and 900 m; *Bd* was not detected in any of the 82 samples analyzed (Swei et al. 2011). This study aims to investigate the presence of *Bd* in the Hoang Lien Range in more detail and at higher elevations, as well as any patterns in *Bd* infection in space, time, and host species over a five-year sampling period. This study also aims to investigate the presence of *Bsal* in the Hoang Lien Range.

Visual encounter surveys were undertaken between 1900 h and 2300 h by 2–7 persons in the Vietnamese Winter (December 2017), Spring (March 2018 and April 2019), Summer (June 2016, June 2018), and Autumn (September 2015, September 2017, and September 2018). Surveys were undertaken in Lao Cai Province (Sa Pa and Bat Xat districts) and Lai Chau Province (Tam Duong District), at Mount Fansipan, Mount Pu Ta Leng and Mount Ky Quan San (Table 1). Surveys at four sites (sites 1–4, Fig. 1A) were conducted across an elevation gradient and were repeated up to four times each year, whereas the other surveys were opportunistic. Each amphibian was identified to the species level (where possible) and swabbed with a sterile dry swab (Dryswab MW100, Medical Wire & Equipment, Corsham, Wiltshire, SN13 9RT, UK) before release. Post-metamorphic amphibians were swabbed in a standardized way, with a total of 30 strokes; five times on the underside of each thigh, shank, and hind foot. Tadpoles were swabbed by placing the swab against their mouthparts and rotating the swab 360° ten times. Animals were handled with powder-free nitrile gloves which were changed between each sampled individual. Swabs were then sealed in their accompanying sleeves and kept at room temperature before being transferred to diagnostic laboratories in the UK.

Molecular analyses were performed in laboratories at Imperial College and the Zoological Society of London (ZSL) Institute of Zoology (Method 1) and the ZSL Institute of Zoology (Method 2) according to the following protocols; only one method was used per sample: in Method 1 (421 samples from 421 individual amphibians), we performed *Bd*-ITS quantitative PCR using methods and primers described in Boyle et al. (2004). Briefly, DNA was

TABLE 2. List of species samples (individuals) for *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) in the Hoang Lien Range, Vietnam, including infection intensity, collection data, extinction risk and breeding habitat association. Bold rows indicate species for which individuals were *Bd*-positive. Extinction risk data (IUCN 2017); life history data (IUCN 2017; and personal observations of the authors). Key to life history information U = unknown; FGL = forest and grassland; MFGL = montane forest and grassland; MF = montane forest; EFGL = evergreen forest and grassland; OWH = open wet habitats; PB = pond breeding; SB = stream breeding; DD = direct developing; and THB = tree hole breeding.

| Family | Species | Extinction risk | Life history | Proportion of samples with *Bd* detected | Date *Bd* detected | Infection intensity (avg GE) | Proportion of samples with *Bsal* detected |
|---|---|---|---|---|---|---|---|
| Bombinatoridae | ***Bombina microdeladigitora*** | **Not assessed** | **MFGL, U** | **1/13** | **Sept 2015** | **0.1 (N = 1)** | **0/5** |
| Bufonidae | ***Duttaphrynus melanostictus*** | **LC** | **FGL, PB** | **1/8** | **Jun 2018** | **2.16 (N = 1)** | **0/1** |
| Dicroglossidae | *Fejervarya limnocharis* | LC | OWH, PB | 0/9 | - | - | - |
| | *Limnonectes nguyenorum* | Not assessed | FGL, U | 0/2 | - | - | 0/2 |
| | *Nanorana aenea* | LC | MF, SB | 0/4 | - | - | - |
| | *Nanorana yunnanensis* | EN | MFGL, SB | 0/2 | - | - | - |
| | *Quasipaa* sp. | N/A | MFGL, SB | 0/24 | - | - | 0/13 |
| Hylidae | *Hyla annectans* | LC | FGL, PB | 0/24 | - | - | 0/14 |
| Megophryidae | *Leptobrachella botsfordi* | CR | MF, SB | 0/26 | - | - | 0/15 |
| | *Leptobrachella bourreti* | DD | MF, SB | 0/52 | - | - | 0/15 |
| | *Leptobrachella pluvialis* | EN | MF, SB | 0/19 | - | - | 0/7 |
| | *Leptobrachium ailaonicum* | NT | MF, SB | 0/14 | - | - | 0/4 |
| | ***Megophrys fansipanensis*** | **Not assessed** | **MF, SB** | **2/40** | **Jun 2016** | **0.12–0.32 (N = 2)** | **0/24** |
| | *Megophrys gigantica* | VU | MF, SB | 0/1 | - | - | 0/1 |
| | ***Megophrys hoanglienensis*** | **Not assessed** | **MF, SB** | **1/37** | **Sept 2015** | **0.1 (N = 1)** | **0/10** |
| | *Megophrys jingdongensis* | LC | MF, SB | 0/4 | - | - | - |
| | *Megophrys maosonensis* | Not assessed | MF, SB | 0/2 | - | - | - |
| | *Megophrys rubrimera* | Not assessed | MF, SB | 0/5 | - | - | - |
| | *Megophrys* sp. | N/A | MF, SB | 0/6 | - | - | - |
| | *Oreolalax sterlingae* | CR | MF, SB | 0/95 | - | - | 0/19 |
| Ranidae | *Amolops* cf. *chunganensis* | LC | MF, SB | 0/5 | - | - | - |
| | *Amolops* cf. *minutus* | EN | MF, SB? | 0/7 | - | - | 0/3 |
| | *Amolops ottorum* | Not assessed | MF, SB? | 0/3 | - | - | 0/3 |
| | *Amolops splendissimus* | VU | MF, SB? | 0/1 | - | - | 0/1 |
| | *Amolops* sp. 1 | N/A | MF, SB? | 0/5 | - | - | 0/5 |
| | *Amolops* sp. 2 | N/A | MF, SB? | 0/1 | - | - | 0/1 |
| | *Babina chapaensis* | LC | FGL, PB | 0/20 | - | - | 0/8 |
| | *Hylarana* sp. | N/A | U, U | 0/1 | - | - | - |
| | *Odorrana* cf. *chapaensis* | NT | MF, SB? | 0/9 | - | - | - |
| | *Odorrana* sp. 1 | N/A | MF, SB? | 0/13 | - | - | 0/4 |
| | *Odorrana* sp. 2 | N/A | MF, SB? | 0/12 | - | - | 0/1 |
| Rhacophoridae | ***Gracixalus sapaensis*** | **Not assessed** | **MF, U** | **1/13** | **Apr 2019** | **0.2 (N = 1)** | **0/1** |
| | *Gracixalus yunnanensis* | Not assessed | MF, U | 0/2 | - | - | 0/2 |
| | *Kurixalus odontarsus* | LC | FGL, PB | 0/12 | - | - | 0/7 |
| | *Polypedates megacephalus* | LC | FGL, PB | 0/23 | - | - | 0/4 |
| | *Raorchestes* cf. *parvulus* | LC | MFGL, DD | 0/8 | - | - | 0/2 |
| | *Zhangixalus dorsoviridis* | DD | MFGL, PB | 0/16 | - | - | - |
| | *Zhangixalus duboisi* | DD | MFGL, PB | 0/51 | - | - | 0/7 |
| | *Zhangixalus feae* | LC | EFGL, PB | 0/8 | - | - | - |
| | *Zhangixalus nigropunctatus* | NT | FGL, PB | 0/1 | - | - | - |
| | *Theloderma bicolor* | EN | MF, THB | 0/2 | - | - | - |
| Unassigned | — | - | NA | UNK | 0/1 | - | 0/1 |

extracted from swabs using PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, California, USA). The Ct threshold was set to ΔRn = 0.1. Dilutions of 1:10 were run in duplicate and considered positive if both wells amplified and were over 0.1 GE after accounting for the dilution factor (genomic equivalents where 1 GE is the quantity of genetic material present in a single zoospore). These samples were run against duplicate standards of 0.1, 1, 10, and 100 GE of *Bd* (globally-dispersed pandemic lineage; isolate IA042 from the Spanish Pyrenees). Samples were run in duplicate with two negative controls (where the DNA template was replaced with filtered water) per plate. In Method 2 (180 samples from 180 individual amphibians), DNA was extracted from the swabs using Qiagen DNeasy following Boyle et al. (2004) and analyzed by qPCR, using *Bd* and *Bsal* specific primers and probes, following Blooi et al. (2013) duplex qPCR. Bovine serum albumin (BSA) was included in reactions to reduce amplification inhibition (Garland et al. 2010). In combination with samples, positive controls of known *Bd* and *Bsal* concentrations (100, 10, 1, and 0.1 zoospore genomic equivalents: GE) and negative controls were included in the assays. Samples were run in duplicate on PCR plates and repeated until both wells gave identical positive or negative results. The Clopper-Pearson 95% confidence intervals in prevalence data were calculated using the binom.test function in base R (R Core Team 2013).

A total of 601 samples (individuals) from 40 species of amphibians were analyzed for *Bd* over a five-year period. This represents one of the most intensive surveys for *Bd* undertaken in mainland southeast Asia to date. There were six *Bd* detections from these samples which gives an overall prevalence of 1% (0.37–2.2% (CI) (Table 1) and infection intensity was low (average 0.5 GE, range 0.1–2.16 GE). *Bd* was only detected in post-metamorphic amphibians. Method 2 was only run for some of the samples collected in 2018 and 2019, and we did not detect *Bsal* in any of the 180 samples (individuals) from 27 species of amphibians tested. Through a combination of low infection prevalence and intensity, our results show that it is challenging to accurately determine *Bd* prevalence within this region. A low prevalence of the pathogen requires large sample sizes to be confident of detection (Gray et al. 2017), whereas low intensity of infection increases the probability of false negatives (Sabino-Pinto et al. 2019).

Given the low prevalence of *Bd* within this dataset it is not possible to infer temporal trends. There were two *Bd* detections in each of the first two years, and despite an increase in sampling effort there were only two *Bd* positives in the remaining three years of the study (2017–2019). We found *Bd* to be present at 4 of 10 (40%) sites sampled (Table 1). Given the low overall prevalence and lack of repeated *Bd* detections in these positive sites over successive years, we cannot exclude the possibility that *Bd* is present at sites where we have not detected the pathogen.

Half of the individual frogs swabbed were from the family Megophryidae (Table 2 and Fig. 1B). The six *Bd*-positive individuals were from five species of frog occurring between 1200 m and 2800 m in elevation. Two of these species were newly described and potentially highly threatened megophryid frogs (Table 2). We did not detect *Bd* in any of the Critically Endangered species (Table 2) and we did not encounter any amphibians exhibiting clinical signs of disease.

With recent work demonstrating that *Bd* originated in East Asia (O'Hanlon et al. 2018), there will likely be an increased interest in the ecology and distribution of *Bd* within its native range. We found a typical endemic pattern of low-prevalence and low-intensity of infection which is consistent with other studies on the continent (Kusrini et al. 2008; Mendoza et al. 2011; Savage et al. 2011; Swei et al. 2011; Gilbert et al. 2012; Rowley et al. 2013b; Le et al. 2017; Mutnale et al. 2018). We also found that the majority of *Bd*-positive animals were high-elevation, stream-associated species (Table. 2) which has been a pattern observed in the declines of amphibian communities in the Neotropics where *Bd* has been introduced (Berger et al. 1998; Daszak et al. 1999; Lips et al. 2006).

The international trade of amphibians has been implicated in the introduction, and accelerating the spread, of pathogenic amphibian chytrids (Garner et al. 2009; Schloegel et al. 2009; Wombwell et al. 2016; Fitzpatrick et al. 2018). Wild-caught *Bombina microdeladigitora* (Fire Bellied Toad) are exported to exotic pet markets in Europe (Nguyen et al. 2017). Our findings show *B. microdeladigitora* were *Bd* positive within their native range, complimenting evidence from Nguyen et al. (2017) who found *B. microdeladigitora* in the District of Sa Pa positive for *Bsal*. Furthermore, some of the *B. microdeladigitora* exported from Vietnam to Germany for the international pet trade have tested positive for *Bsal* (Nguyen et al. 2017). Together these findings reinforce the pathogen-pollution risk of the wildlife trade from northern Vietnam, and more generally.

While the overall *Bd* infection dynamics of the Hoang Lien Range imply that there is little immediate cause for conservation concern, the impact of the pathogen within Asian amphibian communities remains unknown and we encourage continued surveillance of the region, especially at sites where range-restricted and highly threatened amphibians are present. It should be noted that climate change is expected to alter disease dynamics in montane systems through a shift in host-parasite interactions leading to cold-adapted populations of amphibians being more susceptible to lethal chytridiomycosis during unusually warm conditions (Xie et al. 2016; Cohen et al. 2017; Sauer et al. 2020). Further work will be needed to understand whether the pathogen could become a future threat in this region.

*Acknowledgments.*—We are extremely grateful for the support of Nguyen Huu Hanh, Vice Chief of Lao Cai FPD who arranged the office's work at Ba Xat. We also thank Nguyen Dinh Thang from Lao Cai FPD and Nghiem Trong Tan, both of whom provided support for the field work on Mount Ky Quan San. We thank the staff at Hoang Lien National Park for their assistance and collaboration. In particular we would like to thank Nguyen Huu Hanh (Director) and Nguyen Quang Vinh (Former Director) for continued support and partnership. The Vietnamese Ministry of Agriculture and Rural Development and staff at Hoang Lien National Park kindly facilitated surveys and issued permissions (permit numbers 19/BTTNVN and A13041165/A72-P2). We thank Tim Cutajar and Luke Harding for their assistance in the field and we thank Trenton W. J. Garner for comments on the draft manuscript the use of laboratory facilities. Ethical approval was granted by the Zoological Society of London's ethics committee (project ZFP1). We would also like to extend our thanks to the People's Committee of Lao Cai Province for supporting this programme of research. This work was supported by Ocean Park Conservation Foundation Hong Kong and an EDGE Fellowship for LTN from the Zoological Society of London. MCF, CW, PG, JS were supported by the Morris Animal Foundation, The Leverhulme Trust (RPG-2014-273), the Natural Environmental Research Council (NERC NE/E006841/1), and a PhD DTP award by the ICL Grantham Institute. MCF is a fellow in the CIFAR 'Fungal Kingdoms' program

## Literature Cited

AZE (Alliance for Zero Extinction). 2016. http://www.zeroextinction.org/. Accessed 11 March 2020.

Bataille, A., J. J. Fong, M. Cha, G. O. Wogan, H. J. Baek, H. Lee, M. -S. Min, and B. Waldman. 2013. Genetic evidence for a high diversity and wide distribution of endemic strains of the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* in wild Asian amphibians. Mol. Ecol. 22:4196–4209.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, and H. B. Hines. 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.

Blooi, M., F. Pasmans, J. E. Longcore, A. Spitzen-van der Sluijs, F. Vercammen, and A. Martel. 2013. Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. J. Clin. Microbiol. 51:4173–4177.

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.

Byrne, A. Q., V. T. Vredenburg, A. Martel, F. Pasmans, R. C. Bell, D. C. Blackburn, M. C. Bletz, J. Bosch, C. J. Briggs, R. M. Brown, and A. Catenazzi. 2019. Cryptic diversity of a widespread global pathogen reveals expanded threats to amphibian conservation. Proc. Nat. Acad. Sci. 116:20382–20387.

Cohen, J. M., M. D. Venesky, E. L. Sauer, D. J. Civitello, T. A. McMahon, E. A. Roznik, and J. R. Rohr. 2017. The thermal mismatch hypothesis explains outbreaks of an emerging infectious disease. Ecol. Lett. 20:184–193.

Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. Emerg. Infect. Dis. 5:735–748.

Farrer, R. A., L. A. Weinert, J. Bielby, T. W. J. Garner, F. Balloux, F. Clare, J. Bosch, A. A. Cunningham, C. Weldon, L. H. du Preez, L. Anderson, S. L. Kosakovsky Pond, R. Shahar-Golan, D. A. Henk, and M. C. Fisher. 2011. Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. Proc. Natl. Acad. Sci. U.S.A. 108:18732–18736.

Fitzpatrick, L. D., F. Pasmans, A. Martel, and A. A. Cunningham. 2018. Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. Sci. Rep. 8:1–10.

Garland, S., A. Baker, A. D. Phillott, and L. F. Skerratt. 2010. BSA reduces inhibition in a TaqMan® assay for the detection of *Batrachochytrium dendrobatidis*. Dis. Aquat. Org. 92:113–116.

Garner, T. W. J., I. Stephen, E. Wombwell, and M. C. Fisher. 2009. The amphibian trade: bans or best practice? EcoHealth 6:148–151.

Gilbert, M., D. Bickford, L. Clark, A. Johnson, P. H. Joyner, L. O. Leatts, K. Khammavong, L. V. Nguyen, A. Newton, T. P. W. Seow, S. Roberton, S. Silithammavong, S. Singhalath, A. Yang, and T. A. Seimon. 2012. Amphibian pathogens in Southeast Asian frog trade. EcoHealth 9:386–398.

Hoang, H. T. T., V. Vanacker, A. Van Rompaey, K. C. Vu, and A. T. Nguyen. 2014. Changing human-landscape interactions after development of tourism in the northern Vietnamese Highlands. Anthropocene 5:42–51.

IUCN SSC Amphibian Specialist Group. 2015a. *Leptolalax botsfordi*. The IUCN Red List of Threatened Species 2015: https://www.iucnredlist.org. Accessed 11 March 2020.

———. 2015b. *Oreolalax sterlingae*. The IUCN Red List of Threatened Species 2015: https://www.iucnredlist.org. Accessed 11 March 2020.

IUCN. 2017. https://www.iucnredlist.org/. Accessed 19 April 2020.

Kusrini, M. D., L. F. Skerratt, S. Garland, L. Berger, and W. Endarwin. 2008. Chytridiomycosis in frogs of Mount Gede Pangrango, Indonesia. Dis. Aquat. Org. 82:187–194.

Le, D. T. T., J. J. L. Rowley, and H. D. Hoang. 2017. Preliminary monitoring of amphibian populations at a montane site in Vietnam with the presence of *Batrachochytrium dendrobatidis*. Herpetol. Rev. 48:557–560.

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.

Lu, S., D. Yang, P. P. van Dijk, And S. Swan. 2004. *Nanorana yunnanensis*. The IUCN Red List of Threatened Species 2004: https://www.iucnredlist.org. Accessed 20 May 2020.

Matsui, M., A. Ohler, K. Eto, and T. T. Nguyen. 2017. Distinction of *Gracixalus carinensis* from Vietnam and Myanmar, with description of a new species. Alytes 33:25–37.

Mendoza II, J. A., J. P. Gaertner, J. Holden, M. R. J. Forstner, and D. Hanh. 2011. Detection of *Batrachochytrium dendrobatidis* on amphibians in Pursat Province, Cambodia. Herpetol. Rev. 42:542–545.

Mutnale, M. C., S. Anand, L. M. Eluvathingal, J. K. Roy, G. S. Reddy, and K. Vasudeven. 2018. Enzootic frog pathogen *Batrachochytrium dendrobatidis* in Asian tropics reveals high ITS haplotype diversity and low prevalence. Sci. Rep. 8:10125.

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

Nguyen, L. T., B. Tapley, T. Cutajar, C. T. Nguyen, C. Portway, L. Harding, H. Van Luong, and J. J. Rowley. 2020. A description of the tadpole of the Critically Endangered Botsford's leaf-litter frog (*Leptobrachella botsfordi*) with comments on the distribution and conservation status of the species. Zootaxa 4860:293–300.

Nguyen, T. T., T. V. Nguyen, T. Ziegler, F. Pasmans, and A. Martel. 2017. Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. Amphibia-Reptilia 38:554–556.

Nguyen, T. Q., T. M. Phung, M. D. Le, T. Ziegler, and Böhme, W. 2013. First record of the genus *Oreolalax* (Anura: Megophryidae) from Vietnam with description of a new species. Copeia 2013:213–222.

O'Hanlon, S. J, A. Rieux, R. A. Farrer, G. M. Rosa, B. Waldman, A. Bataille, T. A. Kosch, K. A. Murray, B. Brankovics, M. Fumagalli, M. D. Martin, N. Wales, M. Alvarado-Rybak, K. A. Bates, L. Berger, S. Böll, L. Brookes, F. Clare, E. A. Courtois, A. A. Cunningham, T. M. Doherty-Bone, P. Ghosh, D. J. Gower, W. E. Hintz, J. Höglund, T. S. Jenkinson, C-F. Lin, A. Laurila, A. Loyau, A. Martel, S. Meurling, C. Miaud, P. Minting, F. Pasmans, D. S. Schmeller, B. R. Schmidt, J. M. G. Shelton, L. F. Skerratt, F. Smith, C. Soto-Azat, M. Spagnoletti, G. Tessa, L. F. Toledo, A. Valenzuela-Sánchez, R. Verster, J. Vörös, R. J. Webb, C. Wierzbicki, E. Wombwell, K. R. Zamudio, D. M. Aanensen, T. Y. James, M. T. P. Gilbert, C. Weldon, J. Bosch, F. Balloux, T. W. J. Garner and M. C. Fisher. 2018. Recent Asian origin of chytrid fungi causing global amphibian declines. Science 360:621–627.

Ohler, A., O. Marquis, S. Swan, and S. Grosjean. 2000. Amphibian biodiversity of Hoang Lien Nature Reserve (Lao Cai Province, northern Vietnam) with description of two new species. Herpetozoa 13:71–87.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at https://www.R-project.org/. Accessed 15 May 2020.

Rodriguez, D., C. G. Becker, N. C. Pupin, C. F. B. Haddad, and K. R. Zamudio. 2014. Long-term endemism of two highly divergent lineages of the amphibian-killing fungus in the Atlantic Forest of Brazil. Mol. Ecol. 23:774–787.

Rowley, J. J. L., V. Q. Dau, and T. T. Nguyen. 2013a. A new species of *Leptolalax* (Anura: Megophryidae) from the highest mountain in Indochina. Zootaxa 3737:415–428.

———. H. D. Hoang, D. T. T. Le, V. Q. Dau, T. Neang, and T. T. Cao. 2013. Low prevalence or apparent absence of *Batrachochytrium dendrobatidis* infection in amphibians from sites in Vietnam and Cambodia. Herpetol. Rev. 44:466–469.

Sabino-Pinto, J., E. Krause, M. C. Bletz, A. Martel, F. Pasmans, S. Steinfartz, and M. Vences. 2019. Detectability vs. time and costs in pooled DNA extraction of cutaneous swabs: a study on the amphibian chytrid fungi. Amphibia-Reptilia 40:29–39.

Sauer, E. L., J. M. Cohen, M. J. Lajeunesse, T. A. McMahon, D. J. Civitello,

S. A. Knuti, K. Nguyen, E. A. Roznik, B. F. Sears, S. Bessler, and B. K. Delius. 2020. A metaanalysis reveals temperature, dose, life stage, and taxonomy influence host susceptibility to a fungal parasite. Ecology 101:e02979.

Savage, A. E., L. L. Grismer, S. Anuar, K. O. Chan, J. L. Grismer, E. Quah, M. A. Muin, N. Ahmad, M. Lenker, and K. R. Zamudio. 2011. First record of *Batrachochytrium dendrobatidis* infecting four frog families from peninsular Malaysia. EcoHealth 8:121–128.

Scheele, B. C., F. Pasmans, L. F. Skerratt, L. Berger, A. Martel, W. Beukema, A. A. Acevedo, P. A. Burrowes, T. Carvalho, A. Catenazzi, and I. De la Riva. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363:1459–463.

Schloegel, L. M., A. M. Picco, A. M. Kilpatrick, A. J. Davies, A. D. Hyatt, and P. Daszak. 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). Biol. Conserv. 142:1420–1426.

Schloegel, L. M., L. F. Toledo, J. E. Longcore, S. E. Greenspan, C. A. Vieira, M. Lee, S. Zhao, C. Wangen, C. M. Ferreira, M. Hipolito, and A. J. Davies. 2012. Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. Mol. Ecol. 21:5162–5177.

Swei, A., J. J. Rowley, D. Rödder, M. L. L. Diesmos, A. C. Diesmos, C. J. Briggs, R. Brown, T. T. Cao, T. L. Cheng , R. A. Chong, B. Han, J. M. Hero, H. D. Hoang, M. D. Kusrini, D. T. Le, J. A. McGuire, M. Meegaskumbura, M. S. Min, D. G. Mulcahy, T. Neang, S. Phimmachak, D. Q. Rao, N. M. Reeder, S. D. Schoville, N. Sivongxay, N. Drei, M. Stöck, B. L. Stuart, L. S. Torres, D. T., Tran, T. S. Tunstall, D. Vietes and V. T. Vredenburg. 2011. Is chytridiomycosis an emerging infectious disease in Asia? PLoS ONE 6:e23179.

Tapley, B., T. Cutajar, S. Mahony, C. T. Nguyen, V. Q. Dau, A. M. Luong, D. T. Le, D., T. T. Nguyen, T. Q. Nguyen, C. Portway, H. V. Luong, and J. J. L. Rowley. 2018. Two new and potentially highly threatened *Megophrys* horned frogs (Amphibia: Megophryidae) from Indochina's highest mountains. Zootaxa 4508:301–333.

———, T. Cutajar, S. Mahony, C. T. Nguyen, V. Q. Dau, T. T. Nguyen, H. V. Luong, and J. J. L. Rowley. 2017b. The Vietnamese population of *Megophrys kuatunensis* (Amphibia: Megophryidae) represents a new species of Asian horned frog from Vietnam and southern China. Zootaxa 4344:465–492.

———, L. T. Nguyen, C. Portway, T. Cutajar, C. T. Nguyen, H. V. Luong, D. Kane, Harding, L. and J. J. L. Rowley. 2020. A point endemic no more; a range extension for Oreolalax sterlingae (Nguyen et al., 2013) in Bat Xat District, Lao Cai Province, northern Vietnam. Herpetol. Notes 13:497–500.

———, J. J. L. Rowley, C. T. Nguyen, and L. V. Luong. 2017a. A conservation action plan for the amphibians of the Hoang Lien Mountain range. http://www.amphibians.org/wp-content/uploads/2017/03/An-action-plan-for-the-amphibians-of-the-Hoang-Lien-Mountain-Range-2017-2021.pdf. Accessed 28 July 2020.

van Dijk, P. P., A. Ohler, and K. Kunz. 2004. *Theloderma bicolor* (errata version published in 2018). The IUCN Red List of Threatened Species 2004: https://www.iucnredlist.org. Accessed 20 May 2020.

Wombwell, E. L., T. W. J. Garner, A. A. Cunningham, R. Quest, S. Pritchard, J. M. Rowcliffe, and R. A. Griffiths. 2016. Detection of *Batrachochytrium dendrobatidis* in amphibians imported into the UK for the pet trade. EcoHealth 13:456–466.

Xie, G. Y., D. H. Olson, and A. R. Blaustein. 2016. Projecting the global distribution of the emerging amphibian fungal pathogen, *Batrachochytrium dendrobatidis*, based on IPCC climate futures. PLoS ONE 11:p.e0160746.

*Herpetological Review*, 2020, 51(4), 732–737.


# *Batrachochytrium dendrobatidis* from Amphibian and eDNA Samples in the Peace Region of British Columbia, Canada

**JESSICA BRUNET**
**BRENDAN A. GRAHAM**
**JAIMIE KLEMISH**
**THERESA M. BURG**
*Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, T1K 3M4, Canada*
**DAN JOHNSON**
*Department of Geography & Environment, University of Lethbridge, Lethbridge, Alberta, T1K 3M4, Canada*
**STEVE WISEMAN***
*Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, T1K 3M4, Canada; Water Institute for Sustainable Environments, University of Lethbridge, Lethbridge, T1K 3M4, Alberta, Canada*

** Corresponding author; e-mail: steve.wiseman@uleth.ca*

The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), a leading cause of worldwide amphibian declines (Stuart et al. 2004), has been recorded in Canada since the 1960s (Ouellet et al. 2005) and is widespread within Alberta and British Columbia (Slough 2009; Govindarajulu et al. 2010; Stevens et al. 2012; Richardson et al. 2014). In 2008 and 2009, in response to concern about effects of chytridiomycosis on native species of amphibians, the Ministry of Environment of the Province of British Columbia, Canada, conducted a province-wide survey of the distribution of *Bd* (Govindarajulu et al. 2010). The survey determined that *Bd* was widespread and recommended additional monitoring of effects on amphibian populations (Govindarajulu et al. 2010). A survey of *Bd* occurrence in captive animals in British Columbia advanced provincial knowledge of the pathogen's occurrence in trade markets, with one non-native frog from a pet store testing *Bd*-positive (Govindarajulu et al. 2017). However, limited sampling has been conducted within the Peace Region, and therefore the extent of *Bd* in this region is not well known. Five native amphibian species occur in the Peace Region of British Columbia: Columbia Spotted Frogs (*Rana luteiventris*), Western Toads (*Anaxyrus boreas*), Wood Frogs (*Lithobates sylvaticus*), Boreal Chorus Frogs (*Pseudacris maculata*), and Long-Toed Salamanders (*Ambystoma macrodactylum*). Of these five species, Columbia Spotted Frogs, Western Toads, and Wood Frogs are known to be susceptible to *Bd* infection (Russell et al. 2010, 2019; Gahl et al. 2012). The goal of this