

First record of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* in Thailand

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The presence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) was reported only recently from Asia. Based on known New World occurrences, Ron (2005) predicted the habitat suitability for *Bd* and concluded that South East Asia is particularly suitable for the establishment of the pathogen. Rödder et al. (2010) used species distribution models to predict the future distribution of *Bd* driven by climate change and found that bioclimatic conditions in Asia are suitable for the possible emergence of the fungus.

While the emergence of chytridiomycosis has contributed to amphibian population declines in other continents within the last 20 years (Berger et al., 1998; Bosch and Martínez-Solano, 2006; Fischer et al., 2009; Kilpatrick et al. 2009), there are still little data known from Asia. A lethal outbreak of the disease was discovered first in Japan (Une et al., 2008; Goka et al., 2009), followed by reports of the occurrence of *Bd* from Indonesia (Kusrini et al., 2008; Swei et al., 2011), South Korea (Yang et al., 2009), China (Bai et al., 2010), Laos (Swei et al., 2011), Malaysia (Savage et al., 2011; Swei et al., 2011) and Cambodia (Gaertner et al., 2011; Mendoza et al., 2011) (Fig.1.). Swei et al. (2011) completed a survey in Asia and adjoining Papua New Guinea region, collecting 3363 samples from 15 countries over 9 years. They detected *Bd* in 6 countries with an overall 2.35% infection rate.

Despite the climate and habitat suitability throughout the South East Asian region *Bd* remained undetected from Thailand and Myanmar (Fig.1.). The occurrence of the chytrid fungus in Thailand was surveyed by

McLeod et al. (2008) by histological screening of museum specimens collected in the last 60 years from 13 provinces. Testing 123 amphibian specimens representing 28 species *Bd* was not observed in any of the examined samples. However histology is not the most powerful method for detection of *Bd* (Boyle et al., 2004; Kriger et al., 2006).

Here we report the first data on the occurrence of *Bd* from Peninsular Thailand, from an adult specimen of the lesser toad, *Ingerophrynus parvus*. During a field trip surveying the amphibian fauna of the Prince of Songkla University (PSU) Protected Area on the 4th and the 6th of August 2011, we collected skin swabs from six adult specimens of *I. parvus*. The protected area on Kho-Hong Hill is covered by secondary tropical forest, primarily comprising an abandoned rubber plantation with 3 to 4 metre high trees, some under-storey of shrubs, copious leaf litter and some seasonal streams. The annual mean temperature is 28.3° C, the mean humidity is 72%, and mean rainfall is 2118 mm (Bumrungsri et al., 2006). The specimens were found on rocks near a stream at about 100 m elevation (07° 00' 23"N, 100° 30' 38"E).

We used sterile dry swabs (Biolab) to sample the back, belly, sides and legs of the specimens. The samples were then transferred to Hungary and stored at +4° C until processing in the Laboratory for Molecular Taxonomy of the Hungarian Natural History Museum and the Veterinary Diagnostic Directorate of the National Food Chain Safety Office. The individuals were euthanized, preserved and deposited in the Herpetological Collection of the Prince of Songkla University with catalogue numbers PSUZC-AM 2011.11, PSUZC-AM 2011.12, PSUZC-AM 2011.13, PSUZC-AM 2011.14, PSUZC-AM 2011.15 and PSUZC-AM 2011.16.

The PSU Protected Area contains 16 amphibian species, representing 6 families (Ichthyophidae (1), Megophryidae (2), Bufonidae (2), Ranidae (7), Microhylidae (4), Rhacophoridae (1)) (Wangkulangkul et al., 2008). The studied species, *I. parvus* inhabits tropical forests of Peninsular Thailand, Peninsular

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Figure 1. Map of South East Asia showing countries where *Bd* has been detected (dark grey) and has yet to be detected (light grey).

Malaysia, southern Myanmar and Indonesia, and has isolated populations in southeastern Thailand and Cambodia (Van Dijk and Iskandar, 2004). It does not occur at high elevations, preferring lowland areas with slow-moving streams suitable for laying its eggs. It is a common species in the PSU Protected Area.

To detect *Bd* we used the real-time PCR protocol by Boyle et al. (2004). We ran every sample in duplicate



Figure 2. *Ingerophrynus parvus* specimen tested positive for *Bd* (Photo: S. Wangkulangkul).

with a dedicated internal positive control (TaqMan Exogenous Internal Positive Control Reagents) for each sample that helps to identify inhibitors present in the DNA extractions. The templates were run and analyzed on a Rotor-Gene 6000 real-time rotary analyzer (Corbett Life Science). Genomic equivalents (GE) for all positive samples were estimated from standard curves based on known positive controls, considering 0.1 as the minimum value indicative of infection.

One out of the six sampled individuals tested positive for *Bd* with low infection intensity (mean genomic equivalent = 0.38, SD = 0.12). The specimen did not show the most common clinical signs of infection, such as discoloured skin, shedding of skin, abnormal postures, unnatural behaviour or seizures (Fig.2.).

The presence of *Bd* in Peninsular Thailand could be expected as in the two states of Peninsular Malaysia that border with Thailand (Kedah and Perak) and in two more states (Penang and Pahang) with similar climatic conditions to Hatyai region *Bd* was found in 10 amphibian species representing 4 families, although with low prevalence (Savage et al., 2011).

The reason why *Bd* does not cause mass die-offs in Asia still remains obscure. However, more data on the global distribution of the fungus will help us to have a better understanding of the threat of *Bd* to amphibians within the region. Further comprehensive research on the distribution of *Bd* in Thailand is required.

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