

## Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe

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**Abstract.** Pathogen pollution has caused dramatic losses of amphibian diversity on a global scale. The recently emerged chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) has been hypothesized to have its origin in Asian urodelan populations, from which it may have been introduced to Europe through the trade in live urodelans. We here show that *Bsal* is present on wild small-webbed fire-bellied toads (*Bombina microdeladigitora*) from Vietnam and on representatives of the same species that have recently been imported in Germany. This finding suggests that the installment of measures to mitigate the *Bsal* threat through the amphibian trade should not be limited to urodeles, but should equally take anurans into account.

**Keywords:** animal trade, anura, Asia, *Batrachochytrium salamandrivorans*.

Anthropogenic spread of disease agents may threaten amphibian survival. The chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) emerged in Europe, causing extirpation events in fire salamanders (Martel et al., 2013; Spitzen et al., 2016), and posing a potential threat to the survival of most western Palearctic urodelans (Martel et al., 2014). The origin of *Bsal* is hypothesized to be in Asia, where the fungus appears to be endemic in urodelan populations (Martel et al., 2014; Laking et al., 2017). Although wild amphibians traded for use as pets have been proposed as possible vectors bringing *Bsal* into Europe, actual evidence for this claim

is scarce. Recently, *Bsal* was shown to be capable of infecting anurans as well (Stegen et al., 2017), which would have major implications for *Bsal* epidemiology.

The hypothesis that trade in urodelans has been the main vector of *Bsal* has resulted in several countries (USA, Canada, Switzerland) blocking the importation of salamanders and newts from Asia and others (EU) exploring sanitary measures to avoid further introduction of this and other amphibian pathogens (Gray et al., 2015). Although recently demonstrated to be potential *Bsal* vectors, the role of anurans in this fungus' epidemiology is not clear. We here provide results of a sampling of small-webbed fire-bellied toads (*Bombina microdeladigitora*) both in wild Vietnamese populations and in recently imported animals in the pet trade in Europe. This species occurs near the Vietnamese-Chinese border region and the origin was labelled by the importer to be Vietnam.

Eighty and 57 post metamorphic *B. microdeladigitora* were sampled in in Sa Pa, Lao Cai Province, Vietnam, May and July, 2017 respectively. Each animal was handled using a new pair of gloves and swab samples were collected using cotton tipped swabs and processed for qPCR as described by Blooi et al. (2013). In

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June 2017, 36 *B. microdeladigitora* that were recently imported as Vietnamese fire-bellied toads to a German pet store were sampled using the same protocol. PCR amplicons from all positive qPCR assays were sequenced to confirm identity of *Bsal*. In the wild specimens, *Bsal* DNA was detected at low loads (14,20GE  $\pm$  7,00) in 19/80 samples in May and in 0/57 samples in July. In the traded animals, *Bsal* was detected in 3/36 animals. All PCR amplicons showed 100% similarity with the sequence of the *Bsal* type strain AMFP13/1 (Martel et al., 2013).

An increasing number of reports describes occurrence of *Bsal* in pet urodelans (Sabino-Pinto et al., 2015; Fitzpatrick et al., 2016). The current lack of options to mitigate this disease once a wild population is being hit (Stegen et al., 2017) renders prevention of disease introduction in naïve areas pivotal. Our finding confirms that Asian anurans have vectored *Bsal* into Europe at least on this one occasion. Although the toads suffered from mild infections, with low fungal loads, Stegen et al. (2017) recently demonstrated that very low numbers of *Bsal* zoospores are lethal for susceptible salamander species and an environmentally resistant spore remains infective for at least one month, suggesting the potential of transmission of low level infections to highly susceptible species. Small-webbed toads are imported rarely and in small numbers. However, the closely related *Bombina orientalis* is traded in massive numbers with over 3.5 million specimens traded in the USA between 2001-2009 (Herrel and van der Meijden, 2014), posing a potentially significant threat of disease introduction. For mitigating the disease threat of *Bsal* to *Bsal* naïve regions, we therefore call for the instalment of proper sanitary measures (quarantine, entry controls) in the live amphibian trade from Asia, not only pertaining to urodeles, but also including anurans. Moreover, installing such measures for the trade in wildlife could reduce the risk of pathogen spillover to native fauna, livestock and even humans.

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