SEPARC
Information Sheet #18

***B*ATRACHOCHYTRIUM SALAMANDRIVORANS**

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<u>Amphibian Die-offs</u>:

The amphibian skin disease chytridiomycosis has been identified as a major driver of amphibian declines and extinctions worldwide (Berger et al. 1998; Daszak, Cunningham, and Hyatt 2003; Lips et al. 2006). Until recently, chytridiomycosis was thought to be exclusively caused by the fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*) (see Information Sheet #2R). In 2013, a novel chytridiomycosis-inducing pathogen, *Batrachochytrium salamandrivorans* (*Bsal*) was isolated from the skin of infected European fire salamanders (*Salamandra salamandra*) following a mass die-off event in Bunderbos, Netherlands (Martel et al. 2013; Sluijs et al. 2013). Unlike *Bd* which can infect both anuran and urodelan taxa, *Bsal* colonization appears to be specific to salamander hosts (Martel et al. 2014).

Though occurrence of *Bsal* in wild populations has been limited to regions of East Asia and Western Europe (Martel et al. 2014), this novel pathogen poses a significant threat to North American salamanders, especially those inhabiting the southeastern United States (Yap et al. 2015) (Figure 1).

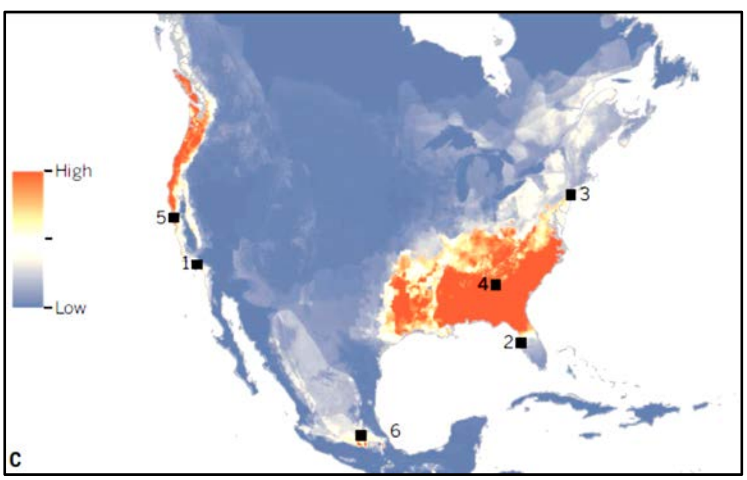

Figure 1. Mapping the threat of *Bsal* to North American salamanders. Salamander *Bsal* vulnerability model illustrating the high susceptibility of salamanders inhabiting the southeastern United States to *Bsal*. Black squares indicate major ports for salamander imports. Port 6 represents Mexico City (Yap et al. 2015).

Pathogen Characteristics:

Chytridiomycosis is caused by two chytrid taxa: *Batrachochytrium dendrobatidis* (*Bd*) (Longcore et al. 1999) and *Batrachochytrium salamandrivorans* (*Bsal*) (Martel et al. 2013). The life cycle of each fungi is characterized by two stages: a motile zoospore stage (i.e., infective stage) and a sedentary zoosporangium stage (i.e., growth stage) (Berger et al. 2005; Martel et al. 2013). Similar to *Bd*, zoospores of *Bsal* are equipped with a single posterior flagellum and are released from discharge tubes of colonial (thalli containing multiple zoosporangia) or monocentric (single thallus forming a single zoosporangium) thalli (Figure 2.A). Unlike *Bd*, mature zoosporangia of *Bsal* form germ tubes and colonial thalli tend to be more abundant than those observed in *Bd* cultures (Figure 2). *Bsal* also has lower thermal growth characteristics than *Bd* and experiences optimal growth between 10 ˚C and 15 ˚C. Death of *Bsal* occurs at temperatures equal to or above 25 ˚C (Martel et al. 2013).

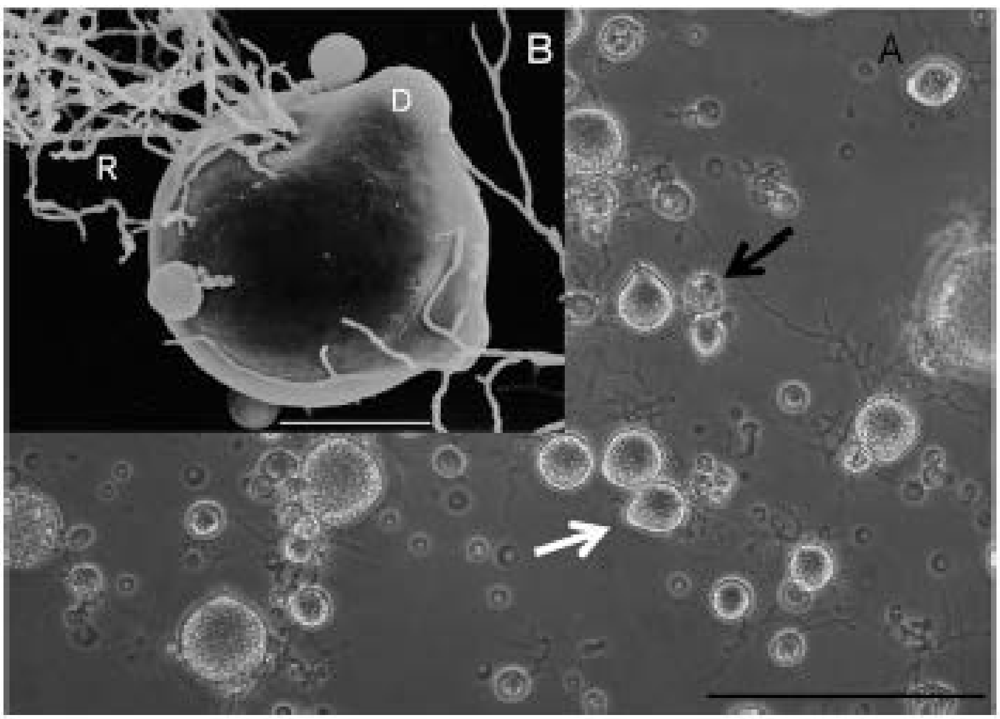

Figure 2. Culture of *Bsal* in vitro grown on TGhL broth at 15 ˚C. (A) Colonial thalli indicated by black arrow and discharge tubes indicated by white arrow. (Scale bar, 100 µm). (B) Scanning electron micrograph of mature zoosporangium showing rhizoids (R), discharge tubes (D), and germ tube formation (Scale bar, 10 µm) (Martel et al. 2013).

Transmission:

Bsal can be transmitted across urodelan hosts via direct contact (Martel et al. 2014). Infectivity experiments indicated that Asian salamander species such as the Japanese fire-bellied newt (*Cynops pyrrhogaster)*, Blue-tailed fire-bellied newt (*C. cyanurus*), and Tam Dao salamander (*Paramesotriton deloustali*) may serve as reservoir hosts because these species were able to limit or clear infection by *Bsal* (Martel et al. 2014). New world salamanders including members of Salamandridae (e.g., *Notophthalmus* and *Taricha*) and Plethodontidae (e.g., *Hydromantes*) appeared to be highly susceptible to *Bsal* and experienced rapid mortality when exposed to pathogen concentrations of 5,000 zoospores/mL (Martel et al. 2014).

Signs of Disease and Diagnostic Testing:

Salamanders suffering from *Bsal*-induced chytridiomycosis exhibit anorexic, apathetic, and ataxic behaviors (Martel et al. 2013). Unlike *Bd*-induced chytridiomycosis, which is characterized by epidermal hyperkeratosis (increased thickness of the stratum corneum) and hyperplasia (Berger et al. 2005), *Bsal*-induced chytridiomycosis is primarily ulcerative. These skin lesions present as multifocal skin erosions and deep ulcerations across the body (Martel et al. 2013) (Figure 3.A). On microscopic examination, necrotic keratinocytes (i.e., epidermal cells) containing colonial or monocentric thalli are often found along the periphery of skin lesions (Figure 3.B and 3.C) (Martel et al. 2013).

Based on the infectivity experiments conducted by Martel et al. (2014), mortality of infected salamanders was frequent and occurred within 10 to 54 days post-inoculation (Martel et al. 2014, Supplementary Table S1).

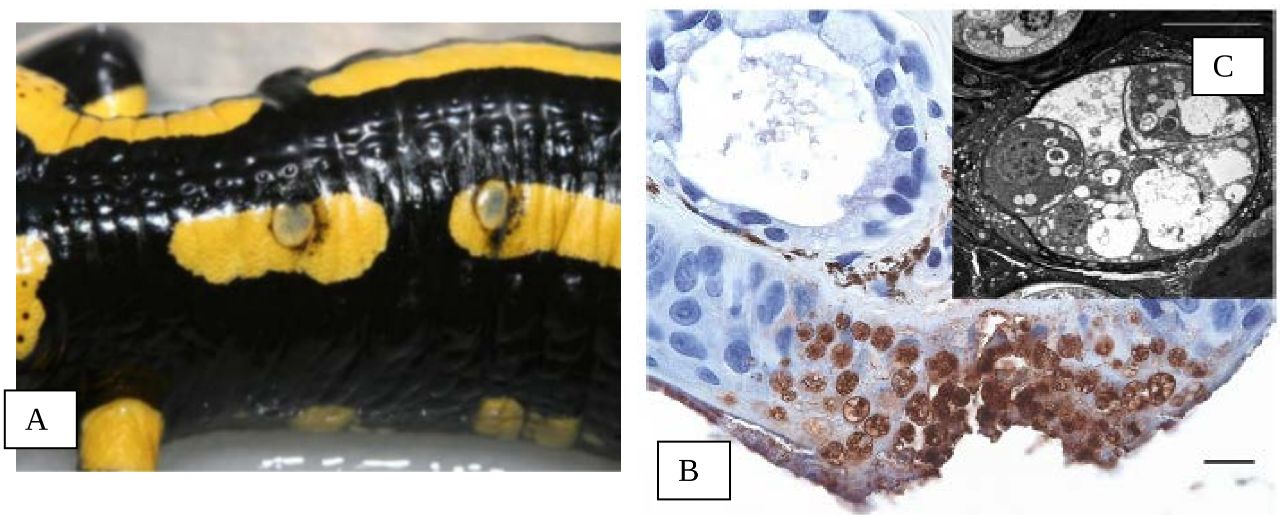

Figure 3. (A) Dorsal view of fire salamander (*Salamandra salamandra)* with skin lesions caused by *Bsal* infection (Blooi et al. 2015); (B) Micrograph of the skin of a fire salamander that died from infection with *Bsal* (A) Immunohistochemical staining of 5 µm skin section. Intracellular colonial thalli abundant throughout the epidermis surrounding erosive skin lesions. (Scale bar, 20 µm). (B) Transmission electron microscopy micrograph of an intracellular colonial thallus of *Bsal* within a keratinocyte (Scale bar, 4 µm) (Martel et al. 2013).

Diagnosis of *Bsal* can be achieved using immunohistochemical staining and/or molecular methodology. Blooi et al. (2013) provide a protocol for the rapid simultaneous detection of *Bd* and *Bsal* using Real-Time Polymerase Chain Reaction (PCR). Diagnosis of clinical disease, chytridiomycosis is made if an infection load of 10,000 genetic (zoospore) equivalents per swab is detected (Kinney et al. 2011; Blooi et al. 2013).

Factors Contributing to Emergence:

Outbreaks of *Bsal*-induced chytridiomycosis in wild populations have been limited to a 2010 occurrence in the Netherlands (Martel et al. 2013; Sluijs et al. 2013) and 2013/2014 occurrences in Belgium. *Bsal*-induced mortality has also been reported for several captive salamander populations. Specifically, an investigation is currently underway to determine the origin of several undisclosed species of imported/ captive-bred amphibians that died from *Bsal* in the United Kingdom (Cunningham et al. 2015). Additionally, a private keeper in Germany reported a rapid mortality event of several fire salamander species including *Salamandra salamandra, S. algira, S. Corsica,* and *S. infraimmaculata*. The presence of *Bsal* was confirmed in the German collection using Real-Time PCR (Sabino-Pinto et al. In Press). In a robust study conducted by Martel et al. (2014), over 5,000 wild amphibian specimens from Asia, Africa, North America and Europe were screened for *Bsal* using qPCR. Positive test results for *Bsal* were found only in East Asian (Thailand, Vietnam, and Japan) and European samples. Zhu et al. (2014) screened an additional 665 amphibians (both urodeles and anurans) from 15 provinces of China. All wild, captive, and museum specimens tested negative for *Bsal* indicating that *Bsal* may be confined to only certain regions of Asia (Zhu et al. 2014). To date, apparent disease has not been observed in East Asian salamander populations. Molecular data suggests that *Bsal* originated in East Asia and has long been an endemic of the area. The historical presence of *Bsal* in East Asian salamander communities may explain why some Asian salamanders (e.g., *Cynops pyrrhogaster*, *C. cyanurus*, *Paramesotriton deloustali*) are able to tolerate and/or clear *Bsal* infections serving as potential reservoir hosts (Martel et al. 2014).

Commercial trade of Asian salamanders is the greatest factor contributing to the spread of *Bsal* and has been implicated in pathogen transmission to naïve urodelan hosts (Martel et al. 2014). Over 750,000 Asian salamanders were imported into the United States during the years 2010 to 2014 (Yap et al. 2015). The majority of imported salamanders were of the genera *Cynops* and *Paramesotriton* (Yap et al. 2015). This mass importation of animals will likely facilitate the spread of *Bsal* to North America, if it has not done so already.

Preventative Measures:

Infected salamanders can be effectively cleared of *Bsal* infections by exposing animals to 25 ˚C for 10 days (Blooi et al. 2015). However, host thermal tolerance must be considered prior to thermal treatment (see Hutchison 1961 and Bury 2008). Blooi et al. (2015) provide a detailed protocol for an alternative treatment appropriate for temperature sensitive species using voriconazole, polymyxin E combined with low temperature exposure (http://www.nature.com/article-assets/npg/srep/2015/150630/srep11788/extref/srep11788-s1.pdf).

Specific guidelines to minimize transmission of *Bsal* are not yet available. However, Phillott et al. (2010) provide a thorough review of hygiene protocols to minimize the risk of human mediated pathogen transmission to naïve amphibian populations. More information on disinfection protocols for field equipment can be found in Information Sheet #10R.

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