The fungal pathogen *Batrachochytrium salamandrivorans* is not detected in wild and captive amphibians from Mexico

M. Delia Basanta[1,2,3], Víctor D. Ávila-Akerberg[4], Allison Q. Byrne[5], Gabriela Castellanos-Morales[6], Tanya M. González Martínez[3,4], Yurixhi Maldonado-López[7], Erica Bree Rosenblum[5], Ireri Suazo[8], Gabriela Parra-Olea[9]*, Eria A. Rebollar[1]*

[1] Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México.
[2] Department of Biology, University of Nevada Reno, Reno, Nevada, United States of America
[3] Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, México.
[4] Instituto de Ciencias Agropecuarias y Rurales, Universidad Autónoma del Estado de México, Toluca, Estado de México, México.
[5] Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA, United States.
[6] Departamento de Conservación de la Biodiversidad, El Colegio de la Frontera Sur, Unidad Villahermosa, Villahermosa, México.
[7] CONACYT-Instituto de Investigaciones sobre los Recursos Naturales, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, México.
[8] Instituto de Investigaciones sobre los Recursos Naturales, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, México.
[9] Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, México.

*Corresponding Authors:
Eria A. Rebollar[1]
Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Cuernavaca,
Morelos 62210, México.
Email address: rebollar@ccg.unam.mx

Gabriela Parra-Olea[9]
Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México 04510, México
Email address: gparra@ib.unam.mx

Abstract

The recent emergence of the pathogen *Batrachochytrium salamandrivorans* (*Bsal*) is associated with rapid population collapses of salamanders in Europe and its arrival to new areas could cause dramatic negative effects on other amphibian populations and species. Areas with high amphibian diversity such as Mexico could be highly threatened due to the arrival of *Bsal*, particularly salamander species which are more susceptible to chytridiomycosis caused by this pathogen. Thus, immediate surveillance is needed as a ~~proactive~~ strategy to efficiently contend with an emerging infectious disease such as chytridiomycosis. In this study, we surveyed 490 wild and captive amphibians from 48 species across 76 sites in the North, Central, and South of Mexico to evaluate the presence of *Bsal*. Amphibians were sampled in sites with variable degrees of amphibian richness and suitability for *Bsal* according to previous studies. From the 76 sampling sites, 10 of them were located in areas with high amphibian richness and potential moderate to high *Bsal* suitability. We did not detect *Bsal* in any of the samples, and no signs of the disease were observed in any individual at the time of sampling. Our results suggest that *Bsal* has not yet arrived at the sampled sites or could be at low prevalence within populations with low ~~detection~~ occurrence probability. This is the first study that evaluates the presence of *Bsal* in different regions and amphibian species in Mexico, which is the second most diverse country in salamander species in the world. We highlight the risk and the importance of continuing surveillance of *Bsal* in Mexico and discuss control strategies to avoid the introduction and spread of *Bsal* in the country.

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Introduction

Chytridiomycosis is an emerging disease caused by the fungal pathogens *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) and is considered one of the principal causes of amphibian population declines worldwide (Longcore, Pessier & Nichols, 1999; Martel et al., 2013; Scheele et al., 2019). While *Bd* has been associated with worldwide population declines since the 1970s (Cheng et al., 2011; Scheele et al., 2019), the recent emergence of *Bsal* is associated with rapid population collapses of the European salamander *Salamandra salamandra* (Martel et al., 2014; Stegen et al., 2017). Currently, the known distribution of *Bsal* is restricted to several countries in Asia and Europe (Martel et al., 2014; Laking et al., 2017; Yuan et al., 2018; Lötters et al., 2020). Amphibians from Asia have shown resistance and tolerance to *Bsal* infections, whereas European salamanders have suffered population declines and have shown severe symptoms due to *Bsal* infections (Martel et al., 2013; Martel et al., 2014~~, 2014~~). The tolerance and/or resistance to *Bsal* infections in Asiatic amphibians may be due to a long co-evolution between the pathogen and their hosts, while a recent introduction of this pathogen in naïve salamander populations could be the reason for the declines detected in Europe (Martel et al., 2014; Lacking et al., 2017).

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The pet trade has been suggested as one of the primary causes for the spread of *Bsal* to naïve areas, and experimental studies have demonstrated that *Bsal* infections are lethal to several North American salamanders such as *Notophthalmus viridescens*, *Chiropterotriton* spp. and *Aquiloeurycea cephalica* (Martel et al., 2014; North American *Bsal* Task Force, 2022). The pathogen *Bsal* has been detected in imported amphibians from Asia to Europe that did not show signs of disease (Cunningham et al., 2015; Gray et al., 2015; Nguyen et al., 2017; Yuan et al., 2018). Anurans have been considered more tolerant to *Bsal* infections than salamanders, acting as reservoirs and carrier species of the pathogen (Martel et al., 2014; Stegen et al. 2017; North American *Bsal* Task Force, 2022). However, recent evidence has shown that anurans can also be susceptible to *Bsal* infections under an experimental setting (Towe et al., 2021). Additionally, experimental co-infections of *Bd* and *Bsal* have shown higher mortalities than infections caused by only one of the pathogens (Longo, Fleischer & Lips, 2019). Considering the potential threat that *Bsal* represents for amphibian species in naïve regions, there is a considerable concern that *Bsal* will arrive in new areas such as North America, causing devastating impacts on amphibian diversity (North American *Bsal* Task Force, 2022).

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Mexico ranks second in the world in the number of salamander species per country, and seventh in the number of amphibian species in general (AmphibiaWeb, 2022). However, according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, 62% of amphibian species in this country are in some category of risk (IUCN, 2022). It is highly likely that the potential introduction of *Bsal* to Mexico would represent a serious threat to local and global amphibian diversity. Previous studies have predicted that regions of Mexico such as the Trans Mexican Volcanic Belt, Sierra Madre del Sur, Sierra Madre Oriental, and Northern Oaxaca are highly suitable for *Bsal* (Basanta, Rebollar & Parra-Olea, 2019; García-Rodríguez et al., 2022). These studies also have found that the potential suitable areas for *Bsal* corresponded to areas of high salamander diversity, increasing the risk of species loss in case the pathogen arrives. Thus, *Bsal* surveillance directed to areas of high suitability for *Bsal* and high amphibian diversity could be a good strategy to take immediate ~~proactive~~ conservation actions. Recent efforts to monitor the presence of *Bsal* in the USA and Northern Mexico have not detected the pathogen (Bales et al., 2015; Klocke et al., 2017; Parrott et al., 2017; Newman et al., 2019; Hardman et al., 2020; Waddle et al., 2020; Hill et al., 2021). However, surveillance of *Bsal* presence in amphibians across Mexico is still lacking.

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In this study, our main goal was to survey wild and captive amphibian populations in Mexico to search for the presence of *Bsal*. We sampled amphibians from different sites across the country with variable degrees of amphibian diversity and *Bsal* suitability areas based on previous studies. This information could be informative for future surveillance efforts in areas of potential risk and for identify new areas of where this pathogen arrival may be more likely to occur in Mexico.

Materials & Methods

Sampling design

We sampled wild amphibian species from North, Central and South Mexico. Additionally, we sampled individuals of *Ambystoma mexicanum* from a captivity center in Mexico City that includes different populations derived from pet owners and captive reproduction programs. All surveys took place between the years of 2015 to 2021. All wild and captive amphibians were captured using a new inverted plastic bag or sterile plastic container and manipulated them with a new pair of nitrile gloves to avoid cross-contamination (Phillott et al., 2010). Swab sampling was performed using standardized methods (Hyatt et al., 2007) and sterile swabs (MW113 rayon swabs, Medical Wire and Equipment, Corsham, UK).

Molecular methods

Chytrid DNA was extracted from swabs using two extraction methods that are widely used for chytrid detection: Prepman Ultra and the Qiagen DNeasy Blood and Tissue Kit. For the Prepman extractions, we followed the protocol of Boyle et al. (2004) for 43 samples. Extractions from Prepman were diluted to a concentration of 1:10 to avoid any inhibition during the qPCR process (Boyle et al., 2004). Given that the Qiagen extraction result in a higher quantity and quality of extracted DNA (Cheng et al., 2011), we switched for this approach for 447 samples and followed the protocol of the manufacturer with an additional lysozyme step (Rebollar et al., 2016).

We used the extracted DNA from swabs to detect *Bsal* through two different methods: quantitative TaqMan polymerase chain reaction (qPCR) as described in Martel et al. (2013), and an amplicon sequencing approach using the Fluidigm Juno system as described in Byrne et al., (2017) (Table S1). From 490 samples analyzed, 384 were tested with qPCR and 106 were sequenced via Fluidigm. For the qPCR, each sample was assayed in duplicate with one negative control (sterile water), one positive control (DNA extraction of *Bsal* isolate ~~kindly~~ donated by Dr. Vance T. Vredenburg, San Francisco State University), and four standards of DNA made with a synthetic fragment of the 5.8S-ITS1 region of *Bsal* (Martel et al., 2013): 1, 100, 1000, and 10 000 ITS *Bsal* equivalent copies. We considered a positive detection of *Bsal* DNA if a detectable signal existed at 40 or fewer qPCR cycles. For the amplicon sequencing approach, samples were sequenced as described in Basanta et al. (2021). Briefly, DNA extracts were first cleaned using an isopropanol precipitation and preamplified using two pools of 96 primer pairs. Samples were then loaded into a Fluidigm Juno LP 192.24 IFC (Fluidigm, Inc.) which performed microfluidic PCR amplification of 192 amplicons, one of which was designed to amplify the ITS1 region of *Bsal*. After amplification, samples were barcoded and pooled for sequencing on an Illumina MiSeq Lane using the Micro 300 bp paired-end kit.

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Salamander richness and Bsal *suitability models*

We estimated the amphibian richness for each site sampled. For this, we performed a richness map at a resolution of 30 arcseconds (~1 km2) using the distribution range maps of amphibians from Mexico (IUCN, 2022) and the *fasterize* package (Ross et al., 2020) in R v.3.6.1 (Core Team, 2019). Then, we extracted the richness values for each site using QGIS v3.8.3 (www.qgis.org). Amphibian richness can be used as a predictor of the diversity in the sampled sites and could inform how many species could be threatened by *Bsal*. We also revised the risk category according to the IUCN (IUCN, 2022) for all amphibian species analyzed. Additionally, we estimated the potential risk of *Bsal* on each sampled site by extracting the *Bsal* suitability values from the model obtained in García-Rodríguez et al. (2022) using QGIS v3.8.3 (www.qgis.org). Because the logistic output of the suitability map of *Bsal* ranges from 0 to 1, with 0 indicating unsuitable habitat and 1 indicating the highest suitability, we also categorized them into four categories of suitability (not suitable, low suitability, moderate suitability, high suitability) according to Basanta, Rebollar & Parra-Olea (2019).

Results

We collected 490 samples of 48 species from 9 families across 76 sites in Mexico (Table 1, Table S1). Samples were collected across six years between 2015 and 2021. We did not detect *Bsal* in any of the 490 samples. No signs of the disease were observed in any individual at the time of sampling.

Of the 48 species sampled, 24 of them were endemic to Mexico, and according to the risk categories by the IUCN (IUCN, 2022), 23 are Critically Endangered (CR), Endangered (EN), Vulnerable (VU), or Near Threatened (NT) (Table S1). Our sampled sites were located in areas where 163 amphibian species are distributed (39% of the total amphibian diversity in Mexico). Of the 76 sampled sites, 50 where from areas of high species richness, hosting between 13 and 28 amphibian species (Table S1). The rest of the sites (n= 26) were from areas of low-medium richness hosting between 7 to 12 amphibian species (Table S1). Additionally, six species (84 individuals out of the 490) were sampled in 10 sites with moderate to high *Bsal* suitability. Finally, four sites had high species richness and moderate/high Bsal suitability. The species surveyed in these four sites were the species *Ambystoma granulosum* (EN), *A. rivulare* (DD), *Chiropterotriton totonacus* (CR) and *Rana montezumae* (LC), which are distributed in the Trans-Mexican Volcanic Belt region and are all endemic to Mexico (Table S1).

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Discussion

This is the first study that evaluates the presence of *Bsal* in amphibian species across different regions of Mexico. We did not detect *Bsal* in any of the amphibian skin samples obtained from North, Central and Southern Mexico. The non-detection of *Bsal* in those areas suggests that to date, *Bsal* has not yet arrived to these areas. In agreement with our results, previous studies have not detected *Bsal* in the USA and Northern Mexico (Bales et al., 2015; Klocke et al., 2017; Parrott et al., 2017; Newman et al., 2019; Hardman et al., 2020; Waddle et al., 2020; Hill et al., 2021), which could indicate that *Bsal* is still absent in North America, or that *Bsal* is at very low prevalence within these populations and has a low detection probability. Because the risk of this pathogen is high for amphibians and its arrival to America is imminent, we highlight the importance of continuous surveillance of *Bsal* in areas of potential risk and new areas where the pathogen arrival is more likely to occur.

To date, *Bsal* remains restricted to Europe and Asia (Martel et al., 2014; Laking et al., 2017; Yuan et al., 2018; Lötters et al., 2020), and amphibians from the pet trade from Asia have been found infected by *Bsal* (Cunningham et al., 2015; Nguyen et al., 2017; Yuan et al., 2018; Martel et al., 2020;). Thus, individuals from farms and markets are considered as the principal potential source for the spread of this pathogen to naïve areas (Gray et al., 2015). In our study, we sampled captive individuals of *Ambystoma mexicanum*. This species is one of the most traded amphibians worldwide (Carpenter et al., 2014), and the potential presence of *Bsal* in this species could threaten not only amphibians from Mexico but also from other countries that have a high amphibian trade such as the USA and Canada. Future surveillance should include the screening of amphibians from pet stores and farms that were potentially imported.

Considering the high amphibian richness present in Mexico and the high number of species in some category of risk, the introduction of *Bsal* into the country should be considered a major threat to amphibian biodiversity. Our survey includes regions that have a high amphibian taxonomic and functional diversity (Ochoa-Ochoa et al., 2020; García-Rodriguez et al., 2022), and/or have a high/moderate *Bsal* suitability: the Sierra Madre Oriental, the extreme eastern Trans-Mexican Volcanic Belt, southeastern Sierra Madre del Sur, mountains of Chiapas in Mexico (García-Rodríguez et al., 2022). One example are the Mexican bolitoglossine salamanders *Chiropterotriton* spp. and *Aquiloeurycea cephalica* which are highly susceptible to *Bsal* as evidenced by their high mortality in experimental infections (North American *Bsal* Task Force, 2022). Thus, the arrival of *Bsal* could cause drastic population collapses and could dramatically reduce salamander bolitoglossine biodiversity.

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Moreover, we sampled 39 of the 103 amphibian species that have tested positive to *Bd* infection in Mexico (López-Velazquez, 2018; Basanta et al., 2021). The potential co-infection of *Bd* and *Bsal* on these species could affect them severely (Longo, Fleischer & Lips, 2019). We suggest continuing the surveillance and increasing the efforts in the areas and species with high risk (i.e., areas with high *Bsal* suitability, high amphibian richness, and *Bd* presence), however, it is also of high relevance to start strict surveillance and monitoring at all entry points for exotic species for trade.

Understanding the potential entry sites and invasion routes of *Bsal* may help in designing efficient surveillance and conservation strategies. Pathogen transmission could be influenced by the amphibian susceptibility to *Bsal*. Thus, knowledge of species susceptibility to *Bsal* and information on its spatial transmission (Kearney & Porter, 2009; Malagon et al. 2020) may help predicting potential spreading routes in the country if it is introduced. Previous experimental studies have shown that salamanders infected by *Bsal* show clinical signs of chytridiomycosis, while anurans ~~were~~ are asymptomatic and may act as potential reservoirs of the pathogen (Martel et al., 2014; North American *Bsal* Task Force, 2022). In contrast, a recent study found that the Cuban treefrog *Osteopilus septentrionalis* is highly susceptible to *Bsal* infections (Towe et al., 2021), demonstrating that infections of *Bsal* could also affect anuran species under specific scenarios. Experimental research on host *Bsal* susceptibility in Mexican anuran and salamander species is urgently needed.

Conclusions

The emergence of infectious diseases such as chytridiomycosis in amphibians poses a major threat to wildlife populations (Martel et al., 2013; Scheele et al., 2019). To date, there are no effective treatments for *Bsal* infections in natural populations. The non-detection of *Bsal* in our study suggests that to date *Bsal* has not yet arrived in these regions in Mexico. The major challenges are the design of *in situ* disease containment and mitigation post *Bsal* arrival (Canessa et al., 2018; Thomas et al., 2019). Control strategies should focus on preventive measures to reduce the introduction risk such as the implementation of biosecurity measures in amphibian trade, and surveillance in areas of potential introduction and high risk. For example, the United States Fish and Wildlife and the government of Canada restricted salamander imports from 2016 and 2017 respectively, which has likely prevented the arrival of *Bsal* to these countries (https://www.ecfr.gov/current/title-50/part-16; Government of Canada, 2017; North American *Bsal* Task Force, 2022). It is imperative for a swift legislative change to prevent pathogen spread and implement a strategic plan to prevent and control *Bsal* invasion in Mexico (North American *Bsal* Task Force, 2022). For this, it is urgently necessary to establish a working network between Mexican authorities, research groups, pet shops, and local communities to report any signs of disease in any organism to stop the possible spread of *Bsal* in Mexico.

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References

AmphibiaWeb. 2022. AmphibiaWeb: Information on amphibian biology and conservation. [Web application]. http://www.amphibiaweb.org.

Bales EK, Hyman OJ, Loudon AH, Harris RN, Lipps G, Chapman E, et al, Terrell KA. 2015. Pathogenic chytrid fungus *Batrachochytrium dendrobatidis*, but not *B. salamandrivorans*, detected on eastern hellbenders. *PloS one* 10(2): e0116405 DOI: 10.1371/journal.pone.0116405.

Basanta MD, Byrne AQ, Rosenblum EB, Piovia- Scott J, Parra- Olea G. 2021. Early presence of *Batrachochytrium dendrobatidis* in Mexico with a contemporary dominance of the global panzootic lineage. *Molecular Ecology* 30(2): 424–437. DOI: 10.1111/mec.15733.

Basanta MD, Rebollar EA, Parra-Olea G. 2019. Potential risk of *Batrachochytrium salamandrivorans* in Mexico. *PLoS One* 14(2):1–13. DOI: 10.1371/journal.pone.0211960.

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of aquatic organisms* 60(2):141-148.

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Byrne AQ, Rothstein AP, Poorten TJ, Erens J, Settles ML, Rosenblum EB. 2017. Unlocking the story in the swab: A new genotyping assay for the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. *Molecular ecology resources* 17(6): 1283-1292.

Canessa S, Bozzuto C, Campbell GEH, Cruickshank SS, Fisher MC, Koella JC, et al., Schmidt BR. 2018. Decision- making for mitigating wildlife diseases: From theory to practice for an emerging fungal pathogen of amphibians. *Journal of Applied Ecology* 55(4):1987-1996. DOI:10.1111/1365-2664.13089.

Carpenter AI, Andreone F, Moore RD, Griffiths RA. 2014. A review of the international trade in amphibians: the types, levels and dynamics of trade in CITES-listed species. *Oryx* 48(4): 565-574. DOI: 10.1017/S0030605312001627.

Cheng TL, Rovito SM, Wake DB, Vredenburg VT. 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences* 108(23):9502-9507.

Comentado [MGG13]: DOI

Cunningham AA, Beckmann K, Perkins M, Fitzpatrick L, Cromie R, Redbond J, O'Brien MF, Ghosh P, Shelton J, Fisher MC. 2015. Surveillance: Emerging disease in UK amphibians. *Veterinary Record* 176(18): 468. DOI: 10.1136/vr.h2264.

García- Rodríguez A, Basanta MD, García- Castillo MG, Zumbado- Ulate H, Neam K, Rovito S, et al., Parra- Olea G. 2022. Anticipating the potential impacts of *Batrachochytrium salamandrivorans* on Neotropical salamander diversity. *Biotropica* 54(1):157-169. DOI: 10.1111/btp.13042.

Government of Canada. 2017. Customs Notice 17-17—Environment and Climate Change Canada (ECCC)'s Import Restrictions on Salamanders—Wild Animal and Plant Trade Regulations. https://www.cbsa-asfc.gc.ca/publications/cn-ad/cn17-17-eng.html.

Gray MJ, Lewis JP, Nanjappa P, Klocke B, Pasmans F, Martel AN, Stephen C, Parra Olea G, Smith SA, Sacerdote-Velat A, Christman MR, Williams JM, Olson DH. 2015. *Batrachochytrium salamandrivorans*: The North American response and a call for action. *PLoS Pathogens* 11(12):1–9. DOI: 10.1371/ journal.ppat.1005251.

Hardman RH, Sutton WB, Irwin KJ, McGinnity D, Reinsch SD, Freake M, Colclough P, Miller BT, Da Silva Neto JG, Souza M, et al. 2020. Geographic and individual determinants of important amphibian pathogens in hellbenders (*Cryptobranchus alleganiensis*), in Tennessee and Arkansas, USA. *Journal of Wildlife Diseases* 56:803–814.

Hill AJ, Hardman RH, Sutton WB, Grisnik MS, Gunderson JH, Walker DM. 2021. Absence of *Batrachochytrium salamandrivorans* in a Global Hotspot for Salamander Biodiversity. *The Journal of Wildlife Diseases* 57(3): 553-560. DOI: 10.7589/JWD-D-20-00218.

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Heros M, Hines H, Phillot R, Campbell R, Marantelli G, Gleason F, Coiling A. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73:175–192. DOI: 10.3354/dao073175.

IUCN 2022. The IUCN Red List of Threatened Species. Version 2021-3. <https://www.iucnredlist.org> Downloaded on 6th May, 2022.

Kearney M, Porter W. 2009. Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters* 12(4):334–350. DOI: 10.1111/j.1461-0248.2008.01277.x.

Comentado [MGG14]: DOI

Klocke B, Becker M, Lewis J, Fleischer RC, Muletz-Wolz CR, Rockwood L, et al., Gratwicke B. 2017. *Batrachochytrium salamandrivorans* not detected in US survey of pet salamanders. *Scientific Reports* 7(1):1-5. DOI: 10.1038/s41598-017-13500-2.

Laking AE, Ngo HN, Pasmans F, Martel A, Nguyen TT. 2017. *Batrachochytrium salamandrivorans* is the predominant chytrid fungus in Vietnamese salamanders. *Scientific Reports* 7(44443):1–5. DOI: 10.1038/srep44443.

Longcore JE, Pessier AP, Nichols DK 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91(2):219-227.

Longo AV, Fleischer RC, Lips KR. 2019. Double trouble: co-infections of chytrid fungi will severely impact widely distributed newts. *Biological Invasions* 21(6):2233-2245.

López-Velázquez A. 2018. Ocurrencia de la quitridiomicosis en México. In López-Velázquez A, Basanta MD, Ochoa-Ochoa LM, eds. *Quitridiomicosis en México*. Mexico City: Publicación Especial de la Sociedad Herpetológica Mexicana N° 5, 35–52.

Lötters S, Wagner N, Albaladejo G, Böning P, Dalbeck L, Düssel H, Feldmeier S, Guschal M, Kirst K, Ohlhoff D, Preissler K, Reinhardt T, Schlüpmann M, Schulte U, Schulz V, Steinfartz S, Twietmeyer S, Veith M, Vences M, Wegge J. 2020. The amphibian pathogen *Batrachochytrium salamandrivorans* in the hotspot of its European invasive range: Past–present–future. *Salamandra* 56(3):173–188.

Malagon DA, Melara LA, Prosper OF, Lenhart S, Carter ED, Fordyce JA, Peterson AC, Miller DL, Gray MJ. 2020. Host density and habitat structure influence host contact rates and *Batrachochytrium salamandrivorans* transmission. *Scientific Reports* 10(1):1–11. DOI: 10.1038/s41598-020-62351-x.

Martel A, Blooi M, Adriaensen C, Rooij PV, Beukema W, Fisher MC. 2014. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science* 346(6209):630–631. DOI: 10.1126/science.1258268.

Martel A, Spitzen-van der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC, Woeltjes A, Bosman W, Chiers K, Bossuyt F, Pasmans F. 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences* 110(38):15325–15329. DOI: 10.1073/pnas.1307356110.

Martel AN, Vila-Escale M, Fernández-Giberteau D, MartinezSilvestre A, Canessa S, Van Praet S, Pannon P, Chiers K, Ferran A, Kelly M, Picart M, Piulats D, Li Z, Pagone V,

Comentado [MGG15]: DOI

Comentado [MGG16]: DOI

Comentado [MGG17]: DOI

PérezSorribes L, Molina C, Tarragó-Guarro A, Velarde-Nieto R, Carbonell F, et al., Pasmans F. 2020. Integral chain management of wildlife diseases. *Conservation Letters* 13(2):e12707. DOI: 10.1111/conl.12707.

Newman JC, Mota JL, Hardman RH, Dillman JW, Barrett K. 2019. Pathogen detection in green salamanders in South Carolina, USA. *Herpetological Review* 50:503–505.

Comentado [MGG18]: DOI

Nguyen TT, Nguyen TV, Ziegler T, Pasmans F, Martel A. 2017. Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. *Amphibia Reptilia* 38(4):554–556. DOI: 10.1163/15685381-00003125.

North American *Bsal* Task Force. 2022. A North American Strategic Plan to Prevent and Control Invasions of the Lethal Salamander Pathogen *Batrachochytrium salamandrivorans*. https://www.salamanderfungus.org/wp-content/uploads/2022/03/Bsal-Strategic-Plan_March-2022_FINAL.pdf.

Ochoa-Ochoa LM, Mejía-Domínguez NR, Velasco JA, Dimitrov D, Marske KA. 2020. Dimensions of amphibian alpha diversity in the new world. *Journal of Biogeography* 47(11):2293–2302. DOI: 10.1111/jbi.13948.

Parrott JC, Shepack A, Burkart D, LaBumbard B, Scimè P, Baruch E, Catenazzi A. 2017. Survey of pathogenic chytrid fungi (*Batrachochytrium dendrobatidis* and *B. salamandrivorans*) in salamanders from three mountain ranges in Europe and the Americas. *EcoHealth* 14(2):296–302. DOI: 10.1007/s1039 3-016-1188-7.

Phillott AD, Speare R, Hines HB, Skerratt LF, Meyer E, McDonald KR, Cashins SD, Mendez D, Berger L. 2010. Minimising exposure of amphibians to pathogens during field studies. *Diseases of Aquatic Organisms* 92(2–3):175–185. DOI: 10.3354/ dao02162.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. Vienna, Austria. URL: R Foundation for Statistical Computing. https://www.R-project.org.

Rebollar EA, Hughey MC, Medina D, Harris RN, Ibáñez R, Belden LK. 2016. Skin bacterial diversity of Panamanian frogs is associated with host susceptibility and presence of *Batrachochytrium dendrobatidis*. *The ISME journal* 10(7):1682-1695.

Comentado [MGG19]: DOI

Ross N, Sumner M, EcoHealth Alliance. 2020. fasterize: Fast polygon to raster conversion. R package version, 1(3).

Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, Acevedo AA, Burrowes PA, Carvalho T, Catenazzi A, De la Riva I, Fisher MC, Flechas SV, Foster CN, Frías-

Álvarez P, Garner TWJ, Gratwicke B, Guayasamin JM, Hirschfeld M, et al., Canessa S. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363(6434):1459–1463.

Comentado [MGG20]: DOI

Stegen G, Pasmans F, Schmidt BR, Rouffaer LO, Van Praet S, Schaub M, Canessa S, Laudelout A, Kinet T, Adriaensen C, Haesebrouck F, Bert W, Bossuyt F, Martel A. 2017. Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. *Nature* 544(7650):353–356. DOI: 10.1038/ nature22059.

Thomas V, Wang YU, Van Rooij P, Verbrugghe E, Baláž V, Bosch J, Cunningham AA, Fisher MC, Garner TWJ, Gilbert MJ, Grasselli E, Kinet T, Laudelout A, Lötters S, Loyau A, Miaud C, Salvidio S, Schmeller DS, Schmidt BR, et al., Pasmans F. 2019. Mitigating *Batrachochytrium salamandrivorans* in Europe. *Amphibia Reptilia* 40(3):265–290. DOI: 10.1163/15685 381-20191157.

Towe AE, Gray MJ, Carter ED, Wilber MQ, Ossiboff RJ, Ash K, et al., Miller DL. 2021. *Batrachochytrium salamandrivorans* can Devour more than Salamanders. *The Journal of Wildlife Diseases* 57(4):942-948. DOI: 10.7589/JWD-D-20-00214.

Waddle JH, Grear DA, Mosher BA, Grant EHC, Adams MJ, Backlin AR, et al., Winzeler ME. 2020. *Batrachochytrium salamandrivorans* (*Bsal*) not detected in an intensive survey of wild North American amphibians. *Scientific reports* 10(1):1-7. DOI: 10.1038/s41598-020-69486-x.

Yuan Z, Martel AN, Wu J, Van Praet S, Canessa S, Pasmans F. 2018. Widespread occurrence of an emerging fungal pathogen in heavily traded Chinese urodelan species. *Conservation Letters* 11:e12436, 1–8. DOI: 10.1111/conl.1243.