

# **Who, where and when? The complexity of tracking amphibian chytrid pathogens**

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The scientific work presented in this thesis was conducted from October 2014 to December 2021 at the Institute of Biology (FU) and the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, Germany. The work was supervised by Prof. Dr. Jonathan M. Jeschke, head of the Ecological Novelty group at FU and IGB. PD Dr. Mark-Oliver Rödel, head of the herpetological collection at the Museum für Naturkunde, was co-supervisor.

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- Disputation am: February 15, 2022
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## **Declaration of independence**

I declare, for all legal purposes, and according to the regulations from the Freie Universität Berlin and the Department of Biology, Chemistry, Pharmacy, that this cumulative thesis contains only original work of which I am author or co-author. No person or entity contributed to this work outside what is allowed by the aforementioned regulations.

## Summary

This cumulative thesis focuses on the study of amphibian chytrid pathogens (*Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans*). It includes contributions from three different manuscripts, two of which have been published, while the third one is in the review process. The research presents the results of an effort to collect, organize and analyze data from published reports of infection. The first chapter discusses a framework for cryptic species and points out many of the problems that complicate the study of chytrid pathogens and their hosts (e.g. cryptic lineages and pseudoindigenous lineages). The second and third chapters focus on *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans*, respectively. These sections constitute the core of this thesis. In this work, data from reports of chytrid infection was systematically collected from over 600 publications and their supplementary materials. The analysis of the data provided information that individual reports could not, and allowed to identify over 1000 host species, including possible vectors of disease and threatened amphibian species. Furthermore, the overlapping distribution of infected species revealed information on areas suitable for the pathogen. The database resulting from this work is also a useful tool to prioritize the areas that need to be intensively studied in the future and to implement conservation measures.

## **Zusammenfassung**

Diese kumulative Dissertation befasst sich mit Chytridiomyceten, die Krankheitserreger von Amphibien sind (*Batrachochytrium dendrobatidis* und *Batrachochytrium salamandrivorans*). Sie umfasst die Inhalte von drei verschiedenen Manuskripten, von denen zwei bereits veröffentlicht wurden, während sich das dritte in der Revisionsphase befindet. In der Studie werden die Ergebnisse einer Untersuchung vorgestellt, bei der Daten aus veröffentlichten Infektionsberichten gesammelt, organisiert und analysiert wurden. Im ersten Kapitel wird ein theoretischer Rahmen für kryptische Arten erörtert, der die Probleme hervorhebt, die die Untersuchung von Chytrid-Erregern und ihren Wirten erschweren (z. B. kryptische Abstammungslinien und pseudonative Abstammungslinien). Im zweiten und dritten Kapitel geht es jeweils um *Batrachochytrium dendrobatidis* bzw. *Batrachochytrium salamandrivorans*. Diese Abschnitte bilden den Kern der vorliegenden Dissertation. In dieser Arbeit wurden Daten zu Berichten über Chytrid-Infektionen aus mehr als 600 Veröffentlichungen und deren ergänzenden Materialien systematisch zusammengestellt. Die Analyse der Daten lieferte Informationen, die in den einzelnen Berichten nicht enthalten waren, und ermöglichte die Identifizierung von mehr als 1000 Wirtsarten, einschließlich potenzieller Infektionsüberträger und bedrohter Arten. Die Überschneidungen in der Verbreitung der infizierten Arten gaben zudem Aufschluss über Gebiete, die für den Erreger günstig sind. Die im Rahmen dieser Arbeit erstellte Datenbank ist auch ein nützliches Instrument für die Festlegung von Prioritäten für weitere Untersuchungen und für die Umsetzung von Naturschutzmaßnahmen.

## List of publications with author contribution

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The following publications derived from this thesis:

Jarić I, Heger T, Castro Monzon F, Jeschke JM, Kowarik I, McConkey KR, Pysek P, Sagouis A, Essl F (2019) Crypticity in biological invasions. *Trends in Ecology and Evolution* 34:291–302. DOI: <https://doi.org/10.1016/j.tree.2018.12.008>

IJ proposed the original idea and wrote a manuscript draft with ideas gathered in an InDyNet brainstorming session. This session was led by IJ and TH, FCM, JMJ, KI, KRM, PP, SA and FE participated. All authors jointly reviewed and revised the manuscript and contributed significant parts to it.

Castro Monzon F, Rödel MO, Jeschke JM (2020) Tracking *Batrachochytrium dendrobatidis* infection across the globe. *EcoHealth* 17:270–279. DOI: <https://doi.org/10.1007/s10393-020-01504-w>

FCM and MJM conceived the idea that led to this manuscript, FCM collected the data, conducted the analysis and wrote a first draft of the manuscript. This manuscript was reviewed and revised by MJM and MOR. The input of all authors was significant and greatly contributed to the final manuscript.

Castro Monzon F, Rödel MO, Ruland F, Parra Olea G, Jeschke JM (*under review*) Where and in which amphibian species is the pathogen *Bsal* found? Submitted to *EcoHealth*.

FCM and MJM conceived the idea that led to this manuscript, FCM collected the data and conducted the analysis, with input from FR. The manuscript was reviewed and revised by MJM, MOR, FR and GP. The input of all authors was significant and greatly contributed to the final manuscript.

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## **General Introduction**

### **Science in the age of Big Data**

In the age of technology, data is abundant and accessible but information is scarce; in a sea of data, we face a situation in which we cannot adequately process it, extract useful bits of information and, accordingly, take effective actions. The problem caused by the large amount of data available is not new. Already in 1966, Bowman et al. discussed the problem presented by the need to sift through a large number of scientific publications to find and access the desired information. This problem has only increased in severity over time, as most scientific fields are experimenting an exponential growth (Pautasso 2012; Bornmann & Mutz 2015). As the volume of data increases, we risk not using it efficiently and the gap between possible and realized knowledge might grow larger (Jeschke et al. 2019). This problem does not only represent a waste of resources and a missed opportunity; it also affects the quality of research.

By its very nature, the scientific endeavor requires a researcher to test, evaluate and work on previously published data. The failure to properly identify and evaluate the published material can lead a researcher to work and build upon flawed or incomplete work. This, in turn, might affect policies and decisions that we take in many important aspects of our lives. In fields relevant to this thesis, such as conservation ecology and epidemiology, information is essential in order to take appropriate measures regarding the protection of species, the protection of an area or to focus research where needed.

Naturally, tools have been developed to help researchers find relevant information. With the development of computers and the advent of the internet, search engines have been created that allow researchers to identify relevant papers linked by citation relationship. It is also now possible to search among millions of papers for keywords within the title or abstract. The development and use of tools to find and analyze valuable data was expected and might need to develop further if we are to cope with an increasingly large volume of publications that we have access to (Hawking 2001). Despite the increasing computational power available and the development of neural networks, our technology is still limited and cannot provide us with answers to some important questions, at least not without extensive work. We cannot, for example, use a search engine to understand in which countries infection by a pathogen have been reported.

### **Synthesizing research results: (systematic) reviews**

We rely on reviews produced by groups of experts to put data in context, to evaluate its quality and to draw conclusions. Reviews are able to provide a wide overview of a field, discuss particular questions and synthesize large volumes of information. On occasions (e.g. chapter one of this thesis), this can be used to discuss a problem, making a point while presenting selected cases in a narrative way. When one seeks not only to highlight a situation but also to present data (e.g. chapter two and three), the way that it is gathered is of concern. A problem is that researchers writing narrative reviews might have their own biases regarding which data is included in a review and how it is evaluated.

Researchers are supposed to be unattached and impartial, abandoning a theory when evidence no longer supports it. However, in practice, researchers often spouse theories that they once championed and are slow to admit conflicting ideas and accept change (Fox 2011, Lokatis & Jeschke 2018). Max Planck (1949) discussed the problem while observing that *“A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it.”* This somewhat pessimistic view ought not to be a call to abandon all hope. Reality might not be as somber as the image that Planck presents, but his observation draws attention to the existence of a bias that we should be aware of and a limitation in our tools.

To limit bias in reviews, a number of guidelines have been developed to gather and evaluate data in an explicit, rigorous and transparent way (e.g. Moher et al. 2010, Elliot et al. 2017). In these types of studies, data is collected in a process that is not all that dissimilar to the methods used by a researcher that samples organisms in the field. No net or trap is placed, but a query is designed to retrieve articles from a database. Relevant papers are subsequently identified using pre-established exclusion and inclusion criteria, and specific pieces of information are extracted. This is a systematic and methodical way to collect data, one that is superficially simple. However, the task has lots of nuances and involves long and grueling work.

The process of identifying relevant publications requires casting a wide net, a flexible and not too specific query that will return all relevant publications (along with hundreds if not thousands of irrelevant ones). Sifting through the captured results is then done methodically. Actions and time spent

pile up as they are repeated for each paper that the query returned. Scanning over thousands of abstracts takes weeks, downloading and indexing hundreds of papers a couple days and extracting pieces of information that might be dispersed in the publications and supplementary materials takes months of work. However, the data collected can be very valuable.

Information appears from the accumulation of data and allow systematic reviews to reveal information that cannot be drawn from individual studies. In this way, systematic reviews can help to evaluate the support that a hypothesis has (e.g. Lokatis & Jeschke 2018), to evaluate how effective a treatment is (e.g. Cano et al. 2021) or to estimate how widespread infection is (e.g. Olson et al. 2021). Systematic reviews have gained widespread acceptance in the medical field, where information regarding life-threatening conditions are vital. In conservation ecology, where the survival of threatened species is at stake, unbiased quantitative data from systematic reviews is no less important.

### **The earth's biodiversity crisis**

We need data to properly respond to challenges we face in a world that we are changing rapidly and dramatically. The magnitude of the changes cannot be overstated: all over the world swamps are being drained to give ground for farmland, rivers are redirected and, sometimes, whole valleys are flooded when dams are built (Guo et al., 2012; Venter et al., 2016). At higher scales, we have connected separate oceans with channels, we have acidified them, we have increased the amount of greenhouse gases in our atmosphere and increased the overall temperature of our planet (Gruber et al., 2011; Halim & Rizkalla 2011; IPCC 2021). Changing environments alter the area suitable for organisms. This often manifests as habitat reduction, fragmentation and degradation, and is a problem that threatens the survival of a large number of species (Hillers et al., 2008; Haddad et al., 2015; ). On the other hand, a few organisms thrive in these human-altered environments and some of them cause us trouble. Notoriously, some invasive species and pathogens that travel along humans, spread around the world and negatively affect us, our livestock, our crops and the native species that they find in their newfound habitats (Knapp 2005; Karraker & Dudgeon 2014; Pysek et al., 2020) .

We are seeing what has been denominated the sixth great extinction, an event where species are disappearing at an unprecedented pace all over the world. In the current situation about two vertebrate species go extinct every year (Ceballos et al. 2017). This is truly a tragedy. The extinction of a species is irreversible, it is the loss of something that took evolution millions of years to forge, a piece of our

world that our descendants won't be able to see or study. Species have historic and aesthetic value; their loss is similar to the loss of an art piece created by one of the great masters, something marvelous that is irreplaceable. In the academic sense, extinction also means losing a large amount of information. We won't be able to study a species and learn which were the strategies and mechanisms that it evolved to survive in its environment. We lose information on the niche that the species filled and information about the interactions it had with other organisms.

Besides the academic interest, there are also practical and utilitarian concerns as we reap benefits from the existence of species. We have identified, extracted and isolated a multitude of compounds with medical applications from plants and animal secretions and we might not get to study those that species that go extinct produce (Dhatrak & Kulkarni 2014; Rodriguez et al. 2016; Díaz et al., 2019). Our crops also benefit from the pollination services that many threatened wild bee species offer (Potts et al. 2016). When we maintain diversity, natural predators and competitors also keep in check organisms that can transmit human diseases (Ostfeld & Keesing 2000; Suzán et al. 2008).

### **Threatened amphibians and chytridiomycosis**

Ecological changes affect a large number of vertebrate taxa, but amphibians, the group that this thesis focuses on, is losing species at the greatest speed and has a large number of species with threatened conservation status (Skerratt et al. 2007; Bellard et al 2016). Amphibians face multiple threats including habitat degradation (Laufer & Gobel 2017), predation and competition by introduced species (Knapp 2005; Karraker & Dudgeon 2014) and infection by emerging infectious diseases (Miller et al. 2011; Scheele et al. 2019). These threats often interact with each other; introduced species often carry pathogens and individuals affected by habitat degradation, competition or predation might not be in a good condition to mount a successful immune response (Miaud et al. 2016, Martel et al. 2020).

While these threats interact, the presence of one alone can put species at risk. In the 1990s, amphibian populations in seemingly pristine areas were observed to decline (Blaustein et al. 1993). Later, many of these declines in amphibian populations were associated with the presence of infection by a chytrid fungus (Berger et al. 1998). This pathogen, *Batrachochytrium dendrobatidis*, was formally described in dendrobatid frogs kept in captivity (Longcore et al. 1999). The characteristic ulcers, skin sloughing, apparent ataxia and abnormal mouthparts that resulted from infection were quickly associated with symptoms previously observed in the wild (Fellers et al. 2001, Green & Sheerman 2001).

This pathogen belonged to a relatively unstudied basal taxon of fungi, known for infecting cyanobacteria algae and diatoms (Ibelings et al. 2004), but not so for infecting vertebrates or posing a serious threat to amphibians. As it happens in novel fields, the amount of information exploded and the type of data quickly changed over time. Early studies presented a wide battery of assays to detect the pathogen and understand how it affected its amphibian hosts. It was later realized that the pathogen affected the skin and, in doing so, interfered with the function of other organs (Voyles et al. 2007). Detection relied on the presence of ulcers in the skin of adult amphibians and the presence of lesions or deformities in the oral disk of tadpoles. However, even in early studies, presence of the pathogen was confirmed by means of histological analysis.

The development of molecular methods relying on qPCR (Boyle et al. 2004) allowed the use of swabs to detect the pathogen, thereby reducing the need to euthanize amphibians to confirm infection and speeding up tests. Moreover, the use of qPCR provided a measure of infection intensity, improving what was usually reported in terms of presence / absence. As testing for *Bd* became easier, the number of studies increased and reports of infection came from all over the world although, even before the development of qPCR, it had become apparent that the pathogen was widespread. Its presence was reported in Brazil, USA, Spain and South Africa (Fellers et al. 2001, Bosch et al 2001, Lane et al. 2003, Oliveira de Queiroz Carnaval et al. 2005). In light of these findings, the natural question that arose was where the pathogen originated from and how long it had been in different regions of the world, assuming that the pathogen was not endemic in the regions it was found.

This was an interesting question that had implications on the risk that *Batrachochytrium dendrobatidis* (henceforth abbreviated as *Bd*) posed in different regions of the world. Amphibians that evolved with the pathogen might have mechanisms to deal with infection, and their populations might not be at risk. Alternatively, if the pathogen was a cosmopolitan species, as it was once suggested, there was some event or condition (e.g. global warming) that caused it to become infectious in very different locations of the world at the same time (Pounds et al. 2006, Lips 2008).

Early on, evidence pointed to an origin in Africa as positives from this continent were reported in old preserved specimens (Weldon et al. 2004). Additionally, no reports of amphibian declines came from South Africa and at least one species (*Xenopus laevis*) appeared to be a resistant carrier. The hypothesis

that the pathogen originated in Africa amusingly postulated that the pathogen spread was associated with the use of pregnancy tests which, in the first half of the last century, were performed in laboratories often on African clawed frogs (Weldon et al. 2004, Vredenbug et al. 2013). Alas, this memorable hypothesis eventually lost support; old preserved specimens from Brazil and the USA tested positive for *Bd* (Rodriguez et al. 2014, Talley et al. 2015) and molecular studies pointed that the genetic diversity of the chytrid in Africa was low (James et al. 2009, Bataille et al. 2013).

Around the time that the project that led to this thesis was starting, the predominant hypothesis was that *Bd*, or at least one virulent strain of the pathogen, had spread from North America with the export and introduction of the American bullfrog (Fisher & Gartner 2007, Miaud et al. 2016). Preserved specimens had shown that the presence of *Bd* in several regions of the world was old, and molecular studies showed that *Bd* in different regions of the world differed significantly both in their genetic makeup and in virulence (Berger et al. 2005, Farrer et al. 2011, Refsnider et al 2015). At one point, it was even suggested that *Bd* might not be one species but several cryptic ones (Farrer et al. 2011). This was not an outlandish hypothesis; in 2014, a new chytrid pathogen of amphibians, *Batrachochytrium salamandrivorans*, was described (Martel et al. 2013).

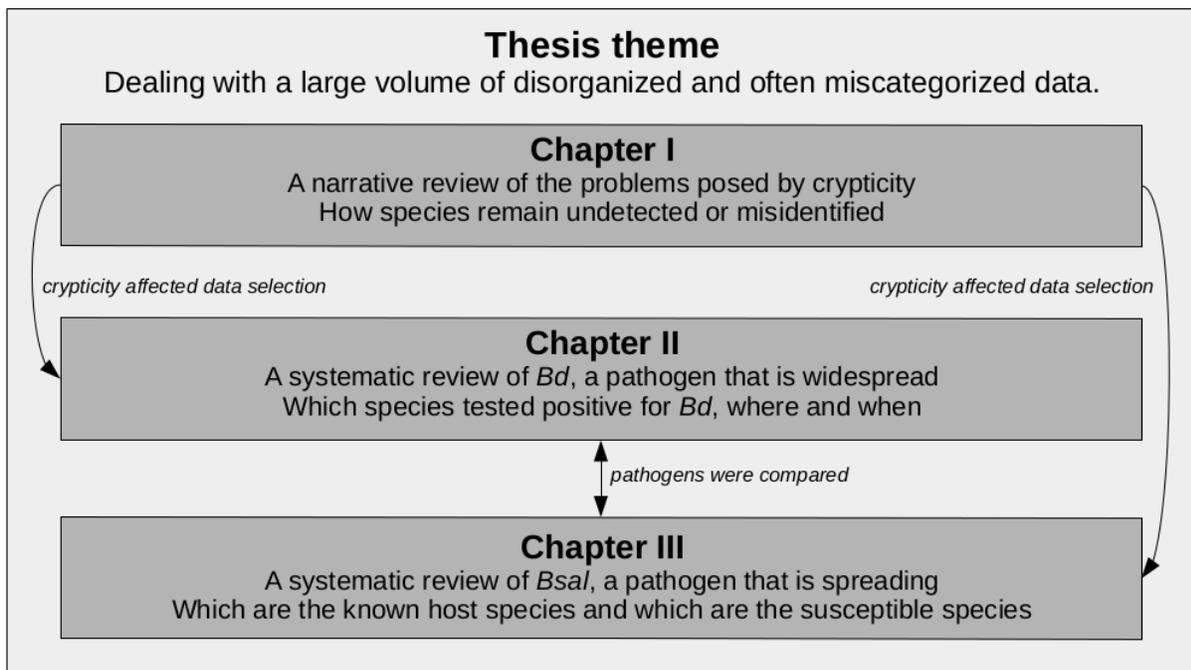
### **Central research questions**

This thesis is structured in three chapters, all of which work under a central theme. That is, the struggle to manage data that is often found in large volumes, unordered, incomplete or misclassified. Each chapter attempt to tackle different questions within this central theme. (1) The first chapter tried to solve the questions of why and how species (pathogens among them) can remain undetected or are misidentified and how large and frequent this problem is. (2) The second chapter focuses on a widely spread pathogen of which a large volume of data has been published. In this second chapter I answer the questions of where has infection been detected, when it was detected and in which species. Furthermore, I sought to find if there were observable temporal or spatial patterns of infection (e.g. high or low infection). (3) Lastly, in the third chapter, a pathogen whose distribution is relatively small was studied. This pathogen poses a risk to amphibian populations if it spreads. This third chapter seeks to answer the questions of which are the known host and vulnerable species.

### **First thesis chapter**

It was clear that amphibian chytrids were widespread, but their origins and the distribution were still not well known. The test to detect *Bd* strains differed from the one commonly used to detect the pathogen and was harder to conduct. It was also not well known if the pathogen had been affecting amphibians for long. In this situation, the author of this thesis contributed to the discussion that led to a co-authorship on a paper on cryptic species (Jarić et al. 2019). This paper constitutes the first chapter of this cumulative thesis and discusses how species remain unstudied as they remain undetected, as their presence is not recognized, as they are not properly identified or as the impact they have is not recognized. The author of this thesis contributed to the discussion and with the writing on this paper. The contribution can be recognized on the prominent role that *Batrachochytrium dendrobatidis* plays in a paper from a field (invasion ecology) where discussion of pathogens is often absent.

The paper presented in the first chapter was part of a collaboration in the Invasion Dynamics Network (<https://indynet.de>) and intended to reach a large audience. This means not focusing exclusively on emerging infectious diseases, but discussing them along other invasive alien species which are not infectious. This is a sensible option as the problems that make chytrid pathogens hard to study (pseudoindividual lineages, cryptic lineages, delayed effects and detection, cryptic affected species) are also present in the study of non-pathogenic invasive alien species. The study introduces the reader to some of the challenges that the author of this thesis faced when collecting and capturing data.



**Figure 1.** Flow chart of the chapters in this thesis. The arrows indicate the influence and relationship between chapters.

## **Second thesis chapter**

Chapters 2 and 3 form the core part of this thesis. I initiated a study to compile data from published reports of amphibian chytridiomycosis. This effort constitutes the overarching theme of this thesis. At the time when this project started, no up-to-date database of infections was available. Previously, there were projects to compile such data (Fisher et al. 2009, Olson et al. 2013 & 2014), but the online database that distributed it ([www.bd-maps.net](http://www.bd-maps.net)) was no longer functioning. Part of the work discussed in this thesis was undertaken as a systematic review of published data of *Bd* detections. This work constitutes the second chapter of this thesis; it is a published paper (Castro Monzon et al. 2020) that seeks to understand where, when and in which species *Bd* has been reported.

The data presented in this second chapter draws information that was systematically collected from over 450 papers where the presence of *Bd* was tested in the field. The analysis of the compiled data provides information that the separate studies do not. Specifically, an updated list of known *Bd* hosts, a list of the countries and amphibian families that have been studied. Conversely, data on amphibian-rich locations that have not been well studied is highlighted. This study also provides information on areas suitable or unsuitable for *Bd* by studying the overlapping distribution of species in which infection has been detected.

## **Third thesis chapter**

I also collected data from tests conducted to detect *Batrachochytrium salamandrivorans* (abbreviated as *Bsal*) in the field. However, it soon became apparent that *Bsal* required a different kind of study and data should be analyzed separately. *Bsal* and *Bd* are related, and both pathogens infect amphibians and cause chytridiomycosis; however, their distribution and hosts are quite different. *Bsal* has been found in a relatively small number of countries, and most prospective studies report negative findings. These studies are very important to ensure that the presence of the pathogen is detected in a timely manner, but provide little information regarding the susceptibility of amphibians to *Bsal*. This meant that to properly evaluate the susceptibility of different amphibian hosts, a study of *Bsal* needs to include data from experimental exposure experiments and reports of infection from animals kept in captivity.

The paper that constitutes the third chapter (Castro Monzon et al., in review) is a systematic review of studies reporting tests to detect *Bsal* in the field, in captivity or in laboratory exposure experiments.

This study seeks to understand in which countries *Bsal* was tested for, which are the known host or susceptible species and where these species are distributed. Information presented in this chapter discusses which species might be threatened and which might present a conservation threat as vectors of the pathogen. Additionally, areas where *Bsal* might pose a risk or that have not been well studied are highlighted. This systematic review deals with a pathogen that, seemingly, is expanding its range and poses a potential problem to caudate amphibian species, but that might still be contained. This contrasts well with the data presented in the preceding chapter.

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## Chapter 1: Crypticity in biological invasions

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### Abstract

Ecological effects of alien species can be dramatic, but management and prevention of negative impacts are often hindered by crypticity of the species or their ecological functions. Ecological functions can change dramatically over time, or manifest after long periods of an innocuous presence. Such cryptic processes may lead to an underestimation of long-term impacts and constrain management effectiveness. Here, we present a conceptual framework of crypticity in biological invasions. We identify the underlying mechanisms, provide evidence of their importance, and illustrate this phenomenon with case studies. This framework has potential to improve the recognition of the full risks and impacts of invasive species.

### Highlights

Crypticity of biological invasions may blur invasion impacts and reduce their predictability.

The impacts are often only detected in retrospect, and understood with delay, long after control measures would have been effective.

Crypticity of biological invasions can be driven by inherent crypticity of alien species and their ecological functions and by time lags, spatiotemporal variability, and anthropogenic impacts.

### Crypticity in Biological Invasions: A Conceptual Framework

**Invasive alien species** (IAS) (see [Glossary](#)) can have large-scale effects on the environment, including alterations to nutrient cycling, changes in disturbance regimes, and modifications to physical structures (Pyšek et al., 2012). However, **ecological functions**, and consequently the direction and magnitude of IAS impacts, can change dramatically over time (Sokol et al., 2017), or appear after long periods of an innocuous presence of the invader (Simberloff et al., 2013). As a result, IAS impacts are difficult to predict, and many interactions with other biota remain unnoticed or lead to unexpected consequences (Meyer et al., 2008). The full potential of an invader to affect native biota through different functions is

often only understood in retrospect, when the impacts have already become evident, and long after the control measures would have been effective.

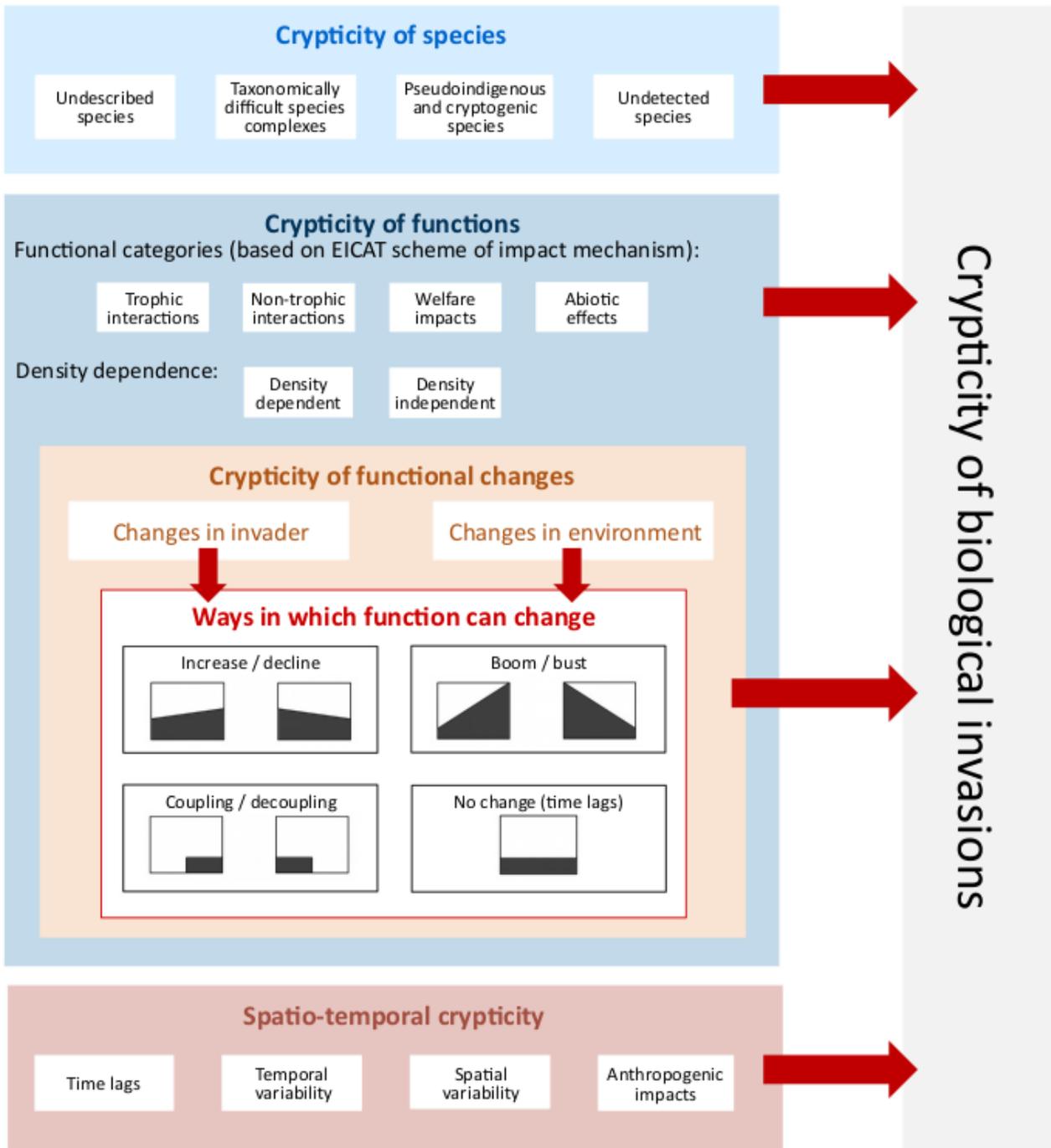
Whether we can detect **alien species** and the manifestations of their ecological functions or whether they remain cryptic depends on ecological properties of the alien species, its new environment, and the circumstances of its invasion. Crypticity in IAS may lead to underestimation and reduced predictability of longterm impacts and constrain timeliness and effectiveness of IAS management.

Similarly, a sudden partial or complete loss of an invader's critical ecological function can remain undetected. In this situation, the resulting reduction in negative impacts of managed IAS may not be recognized and may lead to unwarranted and costly management measures.

Here, we propose a conceptual framework to untangle different sources of crypticity of alien species and their functions (Figure 1 and Table 1). In particular, we highlight the role of three key issues potentially leading to crypticity in biological invasions: (i) **crypticity of species**, (ii) **crypticity of functions**, and (iii) spatio-temporal crypticity (Figure 1 and Table 1). An additional, overarching factor is the 'observer' problem, a lack of knowledge and understanding of relevant processes and mechanisms that constrains identification of key ecological functions (McGeoch et al., 2012; Essl et al., 2018).

### **Crypticity of Species**

Current and potential future impacts of alien species are hard to detect and predict if the alien species themselves are cryptic. This includes undescribed species, taxonomically difficult and unresolved species complexes, **pseudoindigenous** and **cryptogenic species**, and undetected species (Essl et al., 2018; Carlton et al., 1996). While crypticity of species is often represented by morphological crypticity, it can also be driven by other factors such as species rarity and occurrence in less accessible areas, or habitats. In invasion science, crypticity of species may lead to underestimation of introduction and invasion rates, as well as of resulting impacts (Collado et al., 2017).



**Figure 1. Conceptual Framework of Crypticity in Biological Invasions.** A lack of knowledge on the impact of an alien species can be driven by crypticity of species, crypticity of functions or functional changes, and spatio-temporal crypticity. Functional changes can be driven either by changes in the invader or its environment; black-and-white panels represent different modes of functional change, such as increase or decline in the ecological function, stronger changes that correspond to boom or bust dynamics, functional coupling or decoupling, and no apparent change that may occur due to time lags. EICAT, Environmental Impact Classification for Alien Taxa.

**Table 1.** Role of ecological properties in facilitating the occurrence of different categories of crypticity and useful methods for studying and identifying different categories of crypticity

Category and description	Facilitating ecological properties	Methods for reducing the lack of knowledge and increasing detectability	Refs
Crypticity of species: species that are not identified, or not detected in their non-native range, so their functions also remain unobserved	Small body size, less accessible habitat (e.g., aquatic or below-ground environments), endoparasitism, camouflage, systematically complex and/or poorly studied species group, interspecific morphological homogeneity, taxonomic instability	Environmental DNA, DNA barcoding, metagenomics, automated species identification, camera traps, bioacoustics, citizen science initiatives	Gallo et al., 2011 Gaston et al., 2004 Almeida et al., 2008 Davis et al., 2018 Linke et al., 20018 Robinson et al., 2018 Boelman et al., 2007
Crypticity of functions: functions that are not perceived, not expected, or not easily identified	Novelty of a species for the recipient community, novel behavioral patterns, pathogenicity, parasitism, or provision of novel substrates, habitats, carrion subsidies, resource pulses, novel pathogen or parasite vectors or reservoirs, phoresis or zoochory	Targeted field studies and experiments, continuous ecosystem monitoring	Novais et al., 2017 Myczko et al., 2017 Boelman et al., 2007
Crypticity of functional change: functions change in a way that is hard to observe or predict (i.e., nonlinearly), or the drivers or resulting effects are inconspicuous	Exposure to density-independent factors, fast life history, ability for rapid evolutionary and epigenetic changes, ability to hybridize, phenotypic plasticity, phenological and demographic susceptibility to environmental factors, climate change and anthropogenic disturbances	Field studies, experiments (i.e., microcosm and mesocosm), simulation models, improved risk assessment tools	Iacarella et al., 2018 Lopez et al., 2018 Eppinga et al., 2013 Molofsky et al., 2014 Johnson et al., 2009 Rasmussen et al., 2016
Spatio-temporal crypticity: delayed effects of functional changes, spatio-temporal variation and other factors that reduce detectability of species and their functions	Species potential to exhibit long-term accumulation or depletion of slow pools in an ecosystem, spatio-temporal variability, slow life history, function likely related to evolutionary processes or cascading effects, exposure to anthropogenic impacts	Improved use of available long-term data (historic records, herbaria, chronosequences), long-term ecosystem research projects, continuous ecosystem monitoring, surveys along environmental and anthropogenic disturbance gradients, microcosm experiments, models	Strayer et al., 2017 Essl et al., 2015b Strayer et al., 2006

For example, many pathogens now recognized as invasive were either unknown or scarcely known before their introduction (Roy et al., 2017, Ricciardi et al., 2016). A pathogen can be native at the species level, but one or more of its lineages can be invasive, as is the case for the chytrid *Batrachochytrium dendrobatidis* affecting amphibians (Box 1) (Farrer et al., 2011).

Crypticity is also an important issue for other types of novel organisms, such as synthetic and genetically modified organisms (Jeschke et al., 2013). Species can remain unobserved in their novel range until detected by chance or after targeted search efforts have been initiated. For example, the presence and dominance of the highly invasive freshwater snail *Physa acuta* in northern and central Chile, with multiple introductions of different lineages, was detected only after detailed morphological and genetic studies, partly because of its morphological similarity with native congeners (Collado et al., 2017).

Crypticity of alien species is also relevant in taxonomically difficult [cryptic species complexes](#) comprising both native and alien taxa, or alien taxa only, as well as their hybrids. Molecular analyses revealed that the moon-jellyfish (*Aurelia* sp.) is in fact a complex of 13 species, with one of them globally distributed by multiple introductions (Dawson et al., 2005). Common reed (*Phragmites australis*) populations in North America consist of both native and introduced European subspecies, with the latter being highly invasive and comprising three distinct lineages and hybrids, forming a complex and evolving patchwork of genotypes (Figure 2A) (Pysek et al., 2018). Such taxa can differ in their potential impacts on the environment, based on the specific functions they perform (Bickford et al., 2007).

Finally, alien species might remain undetected if they establish viable populations without attaining visible abundances (e.g., because they are camouflaged, or rare), occupy uncommon or undersampled habitats, or belong to understudied and overlooked species groups. Rare alien species can however still be functionally significant, with obvious examples that include pathogens, pathogen hosts, and toxic aliens.

### **Crypticity of Functions**

Some ecological functions are inherently difficult to observe and may remain unnoticed for long periods after IAS introduction and establishment. Crypticity of functions can be represented either by functions that are undetected or by those that are novel and thus hard to predict beforehand, although they might be highly detectable after their effects are manifested. The difference between these two modes of functional crypticity can have relevant management implications. Although unpredictable, novel functions may become apparent soon after the invasion if they lead to high impacts, while some undetected functions may never yield a high impact and remain cryptic.

The problem of crypticity of IAS functions could be more efficiently addressed through a proper classification system of ecological functions. While no such system has been proposed to date, the Environmental Impact Classification for Alien Taxa (Blackburn et al., 2014; Hawkins et al., 2015) represents a promising basis for this purpose. It comprises **environmental impact** mechanisms that can be grouped into the following four broader categories: trophic interactions (predation, parasitism, grazing, herbivory, and browsing), non-trophic interactions (competition, hybridization, disease transmission), abiotic effects (chemical, physical, or structural impacts on ecosystems), and welfare impacts (poisoning, toxicity, bio-fouling), which correspond well to functional roles alien species can fulfill.

The Mediterranean mussel (*Mytilus galloprovincialis*) invasion in South Africa is a prime example for a case in which an unknown ecological function performed by an alien species led to unexpected (and, according to the authors (Branch et al., 2004), completely unpredictable) effects. Although most impacts of this species were successfully predicted, mussel attachment to the eyestalks and mouthparts of the crab *Ovalipes trimaculatus* were not and led to massive mortalities in this crab species (Figure 2B) (Branch et al., 2004). The novel, initially cryptic effects of the Mediterranean mussel were ultimately detected, but only long after the impacts were manifested and the opportunity for timely management measures missed. Novel functions of IAS tend to be cryptic, because they are inherently hard to predict. Novelty of traits and/or taxa has been advocated as a predictor of high impacts (Ricciardi et al., 2004). However, while the presence of novelty may be used to predict high-risk IAS, clear understanding of the way impacts will unfold may be missing. Novel functions may be indirect or subtle, thus initially overlooked or ignored (Blackburn et al., 2014, Loo et al., 2009), as was the predation by a giant African slug (*Achatina fulica*) on a native veronicellid slug (*Veronicella cubensis*) in Hawaii that had gone undetected for more than 5 decades (Meyer et al., 2008). Since this species was previously known only as a herbivore and scavenger, its predatory feeding represented a novel, and consequently, a cryptic function (Meyer et al., 2008). Furthermore, some ecological functions are more likely to be inconspicuous; for example, specific novel weapons such as allelopathy, novel behavior, provision of transformed substrates and habitats by alien plants, carrion subsidies and resource pulses, novel vectors, and phoresis (Table 1) (Crooks et al., 2005; GiblinDavis et al., 2013; Guiden et al., 2017; Novais et al., 2017). Emergence of novel ecological functions brought about by alien species is especially likely on islands, characterized by endemic and unbalanced biota (Drake et al., 2009).

The effects of alien parasites and pathogens also tend to be cryptic (Roy et al., 2017). Pathogens are able to quickly evolve, adapt, shift to novel hosts, hybridize, and exchange genes with native species, which can even lead to the evolution of new pathogens (Roy et al., 2017; Ricciardi et al., 2017; Jeschke et al., 2013; Hulme et al., 2014; Frainer et al 2018). As a result, many alien pathogens with severe impacts were not recognized as threats before their establishment in new hosts (Roy et al., 2017). Novel interactions with native organisms may further increase their pathogenic potential, or they might have no effect on host fitness until the host establishes interactions with another species (Ricciardi et al., 2017; Frainer et al., 2018; Prenter et al., 2004).

Apparently innocuous alien species can have critical ecological functions as vectors of diseases or parasites, or through phoresis or zoochory (Ricciardi et al., 2015; GiblinDavis et al., 2013). Such ecological roles may emerge long after initial introduction and establishment, for example, when the parasites are transmitted later than the vectors (Torchin et al., 2003). Lagged parasite or pathogen transmission can be caused by critical population densities required for transmission, by multiple introduction events from different sources, or by a specific set of spatio-temporal conditions required for transmission (Prenter et al., 2004). Similarly to pathogen transmission, phoresis can remain largely unnoticed and result in cryptogenic invasions, due to the small size of carried species (GiblinDavis et al., 2013).

Indirect functions of aliens are especially unpredictable and likely to remain unobserved, often occurring through altered behavioral patterns of the invading species. Complexity of behavioral responses and interactions contributes to the crypticity of behavior-based functions (McConkey et al., 2015; Iacarella et al., 2018). Besides their direct role as parasite vectors, alien species can also act as reservoirs and thus support parasite species that could otherwise disappear from the invaded region (Prenter et al., 2004). Introduced pheasants (*Phasianus colchicus*) in the UK were identified as reservoirs for the caecal nematode *Heterakis gallinarum*. While the nematode did not affect pheasant populations, it caused population declines in the native grey partridge (*Perdix perdix*; Figure 2H) (Prenter et al., 2004).

## Glossary

**Alien species:** an organism occurring outside its natural range after crossing biogeographical barriers due to direct or indirect human agency (Essl et al., 2018).

**Crypticity of functions:** a function that is either not perceived, unexpected, or not easily identified. Novel functions are very likely to be cryptic, because they are inherently hard to predict.

Crypticity of functional change: undetected or unexpected post-introduction change in an ecological function. Crypticity of species: species are not identified, or not detected in their nonnative range, so their functions also remain unobserved. Cryptic species complexes: taxonomically difficult species complexes, with two or more distinct taxa erroneously classified (and hidden) under one species name (Bickford et al., 2007).

**Cryptogenic species:** taxa of unknown biogeographic origin, which results in an uncertain alien status (Carlton et al., 1996). Ecological function: a role that an alien species performs in habitats within its novel range.

**Environmental impact:** measurable change to the properties of an ecosystem caused by alien species (Blackburn et al., 2014).

**Invasive alien species (IAS):** established alien species that are rapidly extending their range in the new region (Richardson et al., 2000).

**Pseudoindivigenous species:** alien species erroneously considered to be native.

Similarly, alien species can represent exotic and alternative food resources that subsidize native consumers, with unpredictable and potentially large indirect impacts on native communities (Carlsson et al., 2009; Myczko et al., 2017). The red swamp crayfish (*Procambarus clarkii*) invasion in Doñana, southwestern Spain, resulted in novel and unforeseen dispersal pathways of native and alien plants and invertebrates through interaction with their waterfowl predators (Figure 2D) (LovasKiss et al., 2018).

Alien species can also indirectly alter interactions among native species and lead to a cascade of ecological effects (Loo et al., 2009). Introduced beech scale (*Cryptococcus fagisuga*; Hemiptera), for example, does not cause discernible effects on North American beech trees directly but makes them predisposed to attacks by the fungus *Neonectria faginata*, which damages the tree and causes cascade effects (Figure 2E) (Loo et al., 2009). Indirect functions are especially difficult to detect if they manifest over large distances. For example, pollen from the plantations of alien Monterey pine (*Pinus radiata*) in New Zealand was found in Southwest Pacific trenches at depths of up to 10 800 m, where it represents a novel food resource for deep-sea benthic organisms (Figure 2G) (Leduc et al., 2018).

**Box 1.** Multifaceted Crypticity of the Amphibian Chytrid Parasite *Batrachochytrium dendrobatidis*

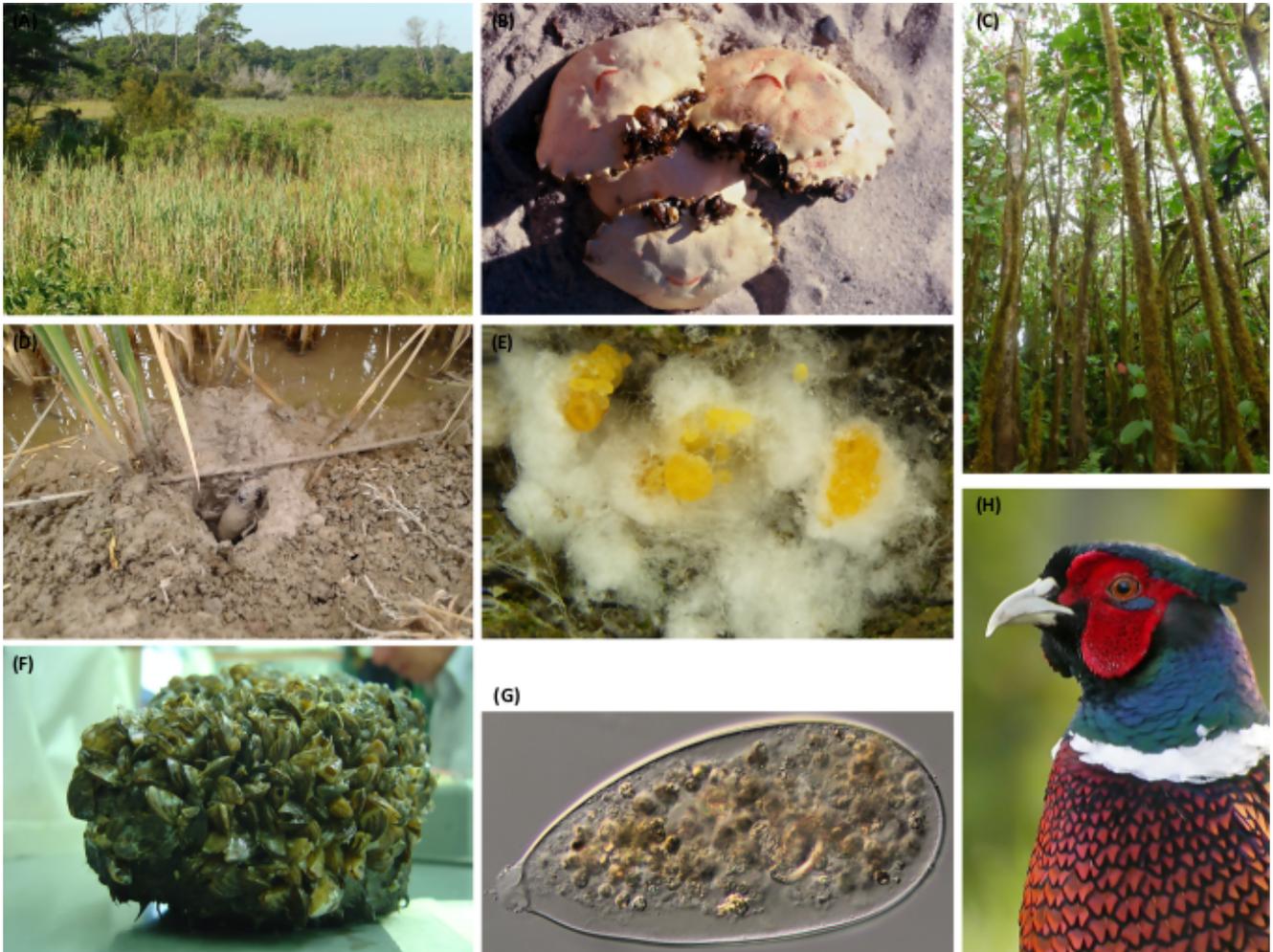
The link between infection by the chytrid parasite *B. dendrobatidis* and amphibian declines in Central

America and Australia was not detected until 1998, when the parasite had already spread throughout the world. Museum specimens and recent molecular analyses suggest that this parasite was present globally for decades before its detection (O’Hanlon et al., 2018). Another challenge is that *B. dendrobatidis* has several strains that vary in virulence, which has only recently been discovered (Farrer et al., 2011).

Crypticity in *B. dendrobatidis* invasion has additional layers, as it also includes amphibian hosts that are difficult to identify: the gray tree frog (*Hyla versicolor*) and Cope’s gray tree frog (*Hyla chrysoscelis*), for example, are virtually identical (Figure I). Even when infection has been detected, it is difficult to ascertain which species is being affected (Talley et al., 2015). The confusion on species identity goes further, as taxonomy of amphibian groups changes, sometimes splitting a species into two and thus making it challenging to determine the species to which previous work refers. Such is the case of the Rio Grande leopard frog (*Lithobates berlandieri*), an invasive organism whose taxonomy changed, dividing the species into two, *Rana berlandieri* and *Rana brownorum*. It is now difficult to ascertain which species was reported as invasive or susceptible to chytrid infection (e.g., (Kaiser et al., 2012)), and for that matter which was the strain of chytrid infecting the sampled amphibians.



**Figure 1.** Tree Frog Hosts of *Batrachochytrium dendrobatidis*. Gray tree frog (right) and Cope’s gray tree frog (left), two chytrid hosts indistinguishable based on external morphology (photo by James Harding).



**Figure 2.** Examples of Different Forms of Crypticity in Invasive Alien Species. **(A)** Common reed (*Phragmites australis*) populations in North America form a complex and evolving patchwork of native and introduced subspecies and their hybrids (Pyšek et al., 2019) (photo by Petr Pyšek). **(B)** Mediterranean mussel (*Mytilus galloprovincialis*) attachment to the eyestalks and mouthparts of the crab *Ovalipes trimaculatus* leads to massive mortalities in this crab species (Branch et al., 2004) (photo by George M. Branch). **(C)** red quinine tree (*Cinchona pubescens*) introduction on the Galápagos Islands resulting in unforeseen and changing characteristics and effects (Jäger et al., 2009) (photo by Carolina Carrion). **(D)** Red swamp crayfish (*Procambarus clarkii*) invasion in Doñana producing novel and unforeseen dispersal pathways of native and alien plants and invertebrates through interactions with their predators (Lovas-Kiss et al., 2018) (photo by Andy J. Green). **(E)** Introduced beech scale (*Cryptococcus fagisuga*) does not produce direct effects on North American beech trees, but it predisposes them to attack by the fungus *Neonectria faginata* (Loo et al., 2009) (photo by Chris Malumphy). **(F)** Decline of larger specimens in zebra mussel (*Dreissena polymorpha*) populations in the Hudson River alters its filter-feeding impact on zooplankton (Pace et al., 2010) (photo by Heather M. Malcom). **(G)** Pollen from the plantations of alien Monterey pine (*Pinus radiata*) in New Zealand was found in Southwest Pacific trenches, where it represents a novel food resource for deep-sea benthic organisms (Leduc et al., 2018); photomicrograph shows a gromiid specimen from the Tonga Trench (10 811-m water depth) with ingested *P. radiata* pollen (photo by Daniel Leduc, NIWA). **(H)** Introduced pheasants (*Phasianus colchicus*) in the UK acting as reservoirs for caecal nematodes *Heterakis gallinarum* that caused population declines in the native grey partridge (*Perdix perdix*) (Prenter et al., 2004) (photo by Lars Petersson). A, crypticity of species; B-H, crypticity of function and/or functional change.

## Crypticity of Functional Change

Ecological functions of IAS can be identified and well understood at one point in time, but they may subsequently change in quality or magnitude, resulting in considerable changes in their impact. Post introduction changes in functional roles performed by alien species differ in both direction and magnitude, depending on drivers and the type of ecological function. Functional changes can become apparent in divergent ways such as increasing or decreasing function, dramatic changes due to boom or bust dynamics (Strayer et al., 2017), and functional coupling or decoupling processes with novel functions being established, or existing functions being lost (Figure 1). However, certain types of functional changes tend to be cryptic, even though they may cause relevant changes in IAS impacts. The introduced red quinine tree (*Cinchona pubescens*) had strongly transformed ecosystems of formerly treeless highlands on the Galápagos Islands (Figure 2C) (Jager et al., 2009), but these effects are declining along with an unexpected decrease in canopy cover of the invader due to yet unknown reasons (Jager et al., 2018). While strong ecosystem transformation effects of red quinine tree invasion were expected, the unforeseen changes in invader characteristics will likely modify previous impact patterns.

Examples of **cryptic functional changes** include evolutionary and epigenetic changes; hybridization; shifts in demographic structure; and changes in interspecific interactions, environmental factors, and phenology (Ricciardi et al., 2017, Prenter et al., 2004, Lopez et al., 2018). Such mechanisms do not follow the standard relationship established by Parker et al. (Parker et al., 1999), where IAS impacts are based on per capita effects multiplied with species abundance; instead, such mechanisms have density-independent effects.

The introduction of an alien species to a novel environment can lead to its rapid evolution. While often highly unpredictable and mostly inconspicuous, evolutionary changes have had a role in some of the most damaging invasions worldwide, and they are expected to be further stimulated by habitat degradation, climate change, and genetic admixture due to multiple introductions (Ricciardi et al., 2017; Hufbauer et al., 2007; Ebeling et al., 2008). Rapid post-introduction evolutionary changes can lead to functional changes while simultaneously remaining undetected. Post-introduction admixtures of previously isolated lineages from different source populations might also induce rapid evolution and

lead to changes in genome size and structure, associated with stronger IAS impacts (Pysek et al., 2018; Eppinga et al., 2013; Molofsky et al., 2014).

The importance of epigenetic changes for biological invasions is poorly understood (Ricciardi et al., 2017), but recent findings indicate that they can affect invasion success more strongly than genetic changes (Perez et al., 2012). Epigenetic changes can alter gene regulation and modify physiological, morphological, life-history, and behavioral traits (Ricciardi et al., 2017). Their ability to produce strong and rapid adaptive shifts, without genetic changes and even at low levels of genetic variability, contributes to the crypticity of such processes (Ricciardi et al., 2017; Perez et al., 2012).

Hybridization is an important mechanism underlying biological invasions, creating new genotypes with often differing ecological functions (Crooks et al., 2005; Ellstrand et al., 2000), which makes it a likely driver of crypticity. An example of functional novelty in plants can be found in knotweeds (genus *Fallopia*), where the hybrid *Fallopia bohemica* resulting from crossing of two Asian parents in their invaded European range spreads comparatively faster, grows more vigorously, is more competitive, and is able to occupy a wider range of habitats (Pysek et al., 2003). Microbial taxa are especially prone to fast evolutionary changes due to natural selection or horizontal gene transfer, which may lead to the emergence of novel abilities or new pathogen strains (Ricciardi et al., 2017).

Demographic structure of alien species can undergo substantial and abrupt shifts even in stable populations, such as changes in age or stage class, or particular sex ratio that was essential for their function in the ecosystem. Such changes tend to be cryptic, as the resulting change in impact is not density dependent. For example, reduction in the abundance of larger specimens of the invasive zebra mussel (*Dreissena polymorpha*) in the Hudson River altered the filter-feeding impact of this species on zooplankton and allowed recovery of zooplankton community, even though the overall mussel population remained at the essentially same level of abundance (Figure 2F) (Pace et al., 2010).

Biological invasions bring together alien species from various biogeographical regions, habitats, climates, and life histories, resulting in novel, unpredictable, and often cryptic interactions among the introduced taxa (Hulme et al., 2014). Native species in the recipient community are also able to modify ecological functions of the alien species. Parasites are especially likely to produce complex alterations in the physiology and behavior of hosts, which will mediate other interspecific interactions and

functional roles of the host, such as competition and predation, in a way that can be highly cryptic (Frainer et al., 2018; Prenter et al., 2004). For example, infection of the amphipod *Gammarus pulex* by the acanthocephalan fish parasite *Echinorhynchus truttae* led both to reduced predation and change in target prey size classes (Fielding et al., 2003).

Functional responses in alien species will be most unpredictable and cryptic when they are mediated by multiple stressors with non-additive, synergistic, or antagonistic interactions (Lopez et al., 2018). Ecological functions can be affected and modified simultaneously by various environmental factors such as temperature, water salinity, CO<sub>2</sub>, and moisture as well as by climate change, ecosystem changes, and changes in habitat structure, resource distribution, and nonstructural conditions, such as noise and light levels (McConkey et al., 2015; Lopez et al., 2018).

Phenological shifts can produce cryptic functional changes in apparently stable populations when they disrupt temporal matching between species or processes, such as plants and nectarivores, or between the peak abundances or emergence of key life stages (e.g., egg laying, hatching, metamorphosis, end of dormancy) in a prey species and its predator (Jaric et al., 2015). Climate change is a widely documented driver of phenological shifts in alien species (Hulme et al., 2014; Walther et al., 2009; McClelland et al., 2018).

### **Spatio-temporal Crypticity**

Besides the mechanisms described above that may cause crypticity in species or their functions, crypticity may arise due to four mechanisms that we summarize under ‘spatio-temporal crypticity’: (i) time lags, (ii) temporal variability, (iii) spatial variability, and (iv) anthropogenic impacts.

Time lags in biological invasions are common and may occur at each stage of the invasion process, and IAS impacts consequently often become discernible or substantial after considerable delays (Blackburn et al., 2014; Crooks et al., 2005; Essl et al., 2015a). Mechanisms that mediate delayed responses to environmental factors such as changes in biotic interactions are manifold and poorly understood, and their effects therefore tend to be underestimated (Essl et al., 2015b). Time lags in plant invasions can last well over a century (Kowarik et al., 1995). They are expected to be longest and least predictable in cases of evolutionary change, either in alien or in related interacting native species (Crooks et al., 2005), as well as in cases of long-term accumulation or depletion of slow pools in an ecosystem (e.g.,

soils or sediments). Time lags in invasions have a strong effect on functional change crypticity by masking true lag mechanisms and key functions and their changes (Essl et al., 2015a).

Even if a species is detected in its alien range, and its ecological functions and their changes are not inherently cryptic, they can still remain undetected if the process is masked by temporal and spatial variability of the recipient community and anthropogenic impacts (e.g., seasonal, annual, or regional variation). Strong spatio-temporal variability in interspecific interactions will lead to both quantitatively and qualitatively different interactions between the alien and native species over space and time (Strayer et al., 2006). Ecosystem heterogeneity, characterized by complex environmental gradients that cause differing local conditions, will result in variable functional patterns and environmental impacts (Ricciardi et al., 2015; Iacarella et al., 2018; JanuchowskiHartley et al., 2018).

Functional changes and the emergence of novel ecological functions in alien species can be masked by anthropogenic impacts and other disturbances in the recipient community. For example, the role of invasive Japanese stiltgrass (*Microstegium vimineum*) in carbon and nitrogen cycling in forest ecosystems in Connecticut was masked by logging (Sokol et al., 2017). Such overlapping effects may fundamentally alter the observed direction and magnitude of interactions attributed to the alien species alone and can hamper our understanding of its ecological functions (Sokol et al., 2017).

### **Concluding Remarks, Consequences for Science and Management, and Ways Forward**

After decades of extensive research and despite impressive progress in invasion science, our understanding of how ecosystems may change over time is incomplete (Sokol et al., 2017; Strayer et al., 2006). Knowledge of life histories and ecological characteristics of alien species is often based on studies in their native range (Parker et al., 2018), which limits their potential use for predictions of novel interactions in the alien range (Meyer et al., 2008). Low predictability of potential ecological interactions of alien species may further result from incomplete understanding of the recipient community. Even with further improvement of our predictive capabilities, there will be unexpected consequences of introductions, making the adoption of the precautionary principle crucial (Branch et al., 2004; Jaric et al., 2012), especially given the ongoing high rate of emergence of new alien species (Seebens et al., 2017). Invasion scientists should also alert the public and policymakers about subtle or non-obvious effects of alien species (Simberloff et al., 2013).

Consideration of cryptic functional dynamics is particularly relevant when planning activities to monitor or manage invasions. It is necessary to improve our understanding of relevant mechanisms and dynamics during biological invasions and to develop appropriate sets of monitoring metrics that allow for capturing such processes. We present here a set of research methods and tools that can help to effectively tackle each of the crypticity categories (Table 1). Inclusion of promising novel approaches, such as environmental DNA combined with metabarcoding, automated species identification, and citizen science initiatives, would provide great benefits to the ongoing monitoring programs (Gallo et al., 2011; Borrell et al., 2017). Here, to build links between the fields of functional ecology and invasion biology, interdisciplinary collaboration will be fruitful. Long-term monitoring programs will also be necessary to overcome confounding effects such as time lags, fluctuations, and transient changes. Furthermore, existing policy measures should be improved by specifically accounting for the different sources of crypticity, outlined within the conceptual framework. Since the crypticity makes impacts of biological invasions unpredictable to a large extent, policy measures should focus primarily on prevention, risk assessment, and adaptive management. One promising strategy, already introduced in the European Union regulation on IAS to prevent their further introductions and spread, is to develop measures that address the most common transportation pathways, instead of a focus on single species. Such measures would simultaneously affect cryptic species, if they are expected to use same pathways as known species.

The framework presented here highlights crypticity in its various forms and illustrates how crypticity can make IAS impacts hard to detect. It is intended to raise awareness; improve understanding of crypticity in biological invasions, its complexity, and the risks it poses to IAS management; and guide future research efforts (see Outstanding Questions).

#### **Outstanding Questions**

- What is the proportion of cryptic invasive alien species (IAS), including undescribed and undetected species?
- What is the frequency of occurrence of cryptic functions and functional changes in IAS?
- Which taxonomic and functional species groups are most likely to exhibit crypticity?
- Which ecosystem conditions and population characteristics increase the likelihood of crypticity in biological invasions?
- Which traits are most suitable for screening IAS for risks of exhibiting cryptic functional

dynamics?

- How can invasion science and IAS management effectively tackle the problem of uncertainty and unpredictability in cryptic functional dynamics?
- Which tools and methods are most promising for early detection of novel functions in invasive alien species?
- Is it possible to develop an optimum set of policy measures that would adequately address the problem of crypticity in biological invasions?
- What is the actual magnitude of economic and environmental impacts caused by crypticity in biological invasions?

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## **Chapter 2: Tracking *Batrachochytrium dendrobatidis* infection across the globe.**

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### **Abstract**

Infection records of *Batrachochytrium dendrobatidis* (*Bd*), a pathogen that has devastated amphibian populations worldwide, have rapidly increased since the pathogen's discovery. Dealing with so many records makes it difficult to (a) know where, when and in which species infections have been detected, (b) understand how widespread and pervasive *Bd* is and (c) prioritize study and management areas. We conducted a systematic review of papers and compiled a database with *Bd* infection records. Our dataset covers 71 amphibian families and 119 countries. The data revealed how widespread and adaptable *Bd* is, being able to infect over 50% of all tested amphibian species, with over 1000 confirmed host species and being present in 86 countries. The distribution of infected species is uneven among and within countries. Areas where the distributions of many infected species overlap are readily visible; these are regions where *Bd* likely develops well. Conversely, areas where the distributions of species that tested negative overlap, such as the Atlantic Coast in the USA, suggest the presence of *Bd* refuges. Finally, we report how the number of tested and infected species has changed through time, and provide a list of oldest detection records per country.

### **Introduction**

The parasitic chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has been associated with amphibian declines across the world. *Bd* stands out from other emerging diseases by having driven several of its host species to extinction and gaining a notorious place as the disease with the greatest impact on vertebrate biodiversity (Skerratt et al. 2007; Scheele et al. 2019). The dramatic impact *Bd* has on amphibian communities has stimulated intense research and has driven a quest to understand which amphibian communities or species are susceptible to infection, where has infection been detected and since when is infection present in an area. Answering these questions “who, where and when” presents a great challenge.

In the past, efforts have been undertaken to compile and analyze published data on infection (Fisher et al. 2009; Olson et al. 2013; Olson and Ronnenberg 2014) and to maintain a database that kept track of new reports ([www.bd-maps.net](http://www.bd-maps.net)). Olson and Ronnenberg (2014) have shown *Bd* infections in 71

countries and 695 species, a number often cited to illustrate the scale of the pandemic. This compilation of data has produced valuable insight into how widespread and pervasive *Bd* is, revealing the distribution of infected species and showing how traits and environmental conditions are associated with infection. Unfortunately, the *Bd*-maps database is no longer functional.

Over time, new reports of infection have appeared from areas where this pathogen had not previously been detected (e.g., Conradie et al. 2016; Seimon et al. 2015; Bletz et al. 2015). These reports appear as we fill gaps in our knowledge, but also as *Bd* distribution changes over time. As environmental conditions change so does the severity of *Bd* infection, the species that it infects and the range that is suitable for infection to develop (Burrowes et al. 2004; Rohr and Raffel 2010; Bosch et al. 2018). Evolutionary and demographic processes such as migration, hybridization and adaptation can also bring previously unexposed hosts into contact with *Bd* or new and more virulent *Bd* lineages (Rosenblum et al. 2013; Savage and Zamudio 2016). With the involuntary aid of humans, *Bd* is known to move between continents and over areas where *Bd* cannot develop (Mazzoni et al. 2003; Weldon et al. 2004). *Bd* lineages, which are seemingly innocuous to amphibians within their known range, can be lethal for amphibians from regions that have not been in contact with that lineage and are evolutionarily naïve to it (Becker et al. 2017). The arrival of a *Bd* lineage to a new location where a different *Bd* lineage was already present brings about the possibility of hybridization. Two such hybrids have been found (Byrne et al. 2019), and there are observations that hybrids can be more virulent than the parental species (Greenspan et al. 2018). This last point is highly relevant in light of the recent discovery of *Bd* lineages in regions where they were not known to be present (Byrne et al. 2019).

As infection changes and new data appear, it becomes difficult to keep track of which species have been found with infection and when and where these species were sampled. New data become available but are not currently compiled in a database that allows producing and updating summary statistics. The number of infected species, for example, has not been updated since Olson and Ronnenberg's (2014) work. Other summary statistics, such as the number and percentage of infected species across all countries and amphibian families, can only be produced with data from several studies. In this study, we assembled a database where we compiled records of infection. This allowed us to produce summary statistics that reflect how widespread and adaptable *Bd* is. We particularly seek to understand in which countries, regions and species *Bd* has been detected, how infection distribution changed over time, and if there are clear patterns in the distribution of infected species.

## Methods

We conducted a systematic review of available literature to study spatial and temporal patterns of *Bd* infection in wild amphibian populations. We followed the PRISMA guidelines for systematic review of data (Moher et al. 2010) in order to improve study repeatability: future studies can follow the same procedure to obtain relevant sources and thus extend the dataset based on the same method. We are painfully aware that, despite the best intentions of researchers, online databases often become non-functional. We, therefore, offer our data in this paper (see Supplementary Table 1), as a comma separated values file (csv), a cross-platform standard that has been in use for almost 50 years and is likely to remain stable in years to come.

We restricted our sources to peer-reviewed publications indexed in the Web of Science (<http://isiknowledge.com>), with the exception of one non-indexed journal, Herpetological Review, because in one section of this journal many *Bd*-related reports are published (see data by Olson et al. 2013). Thus, our study does not include data from gray literature, unpublished accounts of infection, conference contributions and publications that are not indexed in the Web of Science (other than Herpetological Review). We intentionally excluded data from reviews, as we focus on original primary sources. Hence, no data were extracted from any review.

We searched the Web of Science on May 4, 2020, using the following string: (chytrid\* OR batrachochytrium OR fungal) AND (amphibian\* OR frog\* OR salamander\* OR anuran\* OR urodelan\* OR caecilian\*). We excluded news items, reviews, editorial material, letters, corrections, meeting abstracts and book chapters. Additionally, we looked for all Herpetological Review *Bd*-articles up to the first volume of this journal in 2020. We searched articles containing the words “batrachochytrium” or “chytrid.” We selected relevant papers from the Web of Science and Herpetological Review according to the following criteria: (1) amphibian hosts were tested for *Bd*; (2) *Bd* presence or absence was ascertained by means of histological or molecular methods, or by isolating the pathogen; (3) the conditions were not intentionally manipulated to alter infection and (4) the amphibian hosts were collected in the field.

As we focus on *Bd* here, data from *Bsal* and other pathogens are not included in our dataset. Further, we did not include papers that reported the presence of *Bd* on the basis of environmental DNA in non-

amphibian hosts. Similarly, we excluded papers that diagnosed *Bd* solely on the presence of lesions on amphibians. There are conflicting reports on the accuracy of visual inspection to diagnose *Bd* infection. Knapp and Morgan (2006) report an 86% accuracy, whereas Navarro-Lozano et al. (2018) report only 40%, and Padgett-Flohr and Goble (2007) report 36% accuracy.

We excluded papers that used amphibians raised in captivity, specimens that came from the amphibian trade or that were tested for infection after being in an experimental setup. If only part of the data met the selection criteria, only these data were included in the database. Studies that tested specimens preserved in ethanol or formalin were included if they fitted the inclusion criteria.

Based on these criteria, we retained 554 publications for data extraction. For every record, we tried to assess the species names, the country in which the species were collected, first and last year in which the study was conducted, first and last date in which each species was sampled, first and last date in which each species was found with infection, whether or not the samples came from a preserved collection and whether the test turned out to be positive for *Bd*. A test was taken as positive if respective authors identified *Bd* as the causal agent of infection by means of histological, molecular analysis or if the authors isolated and cultured the pathogen.

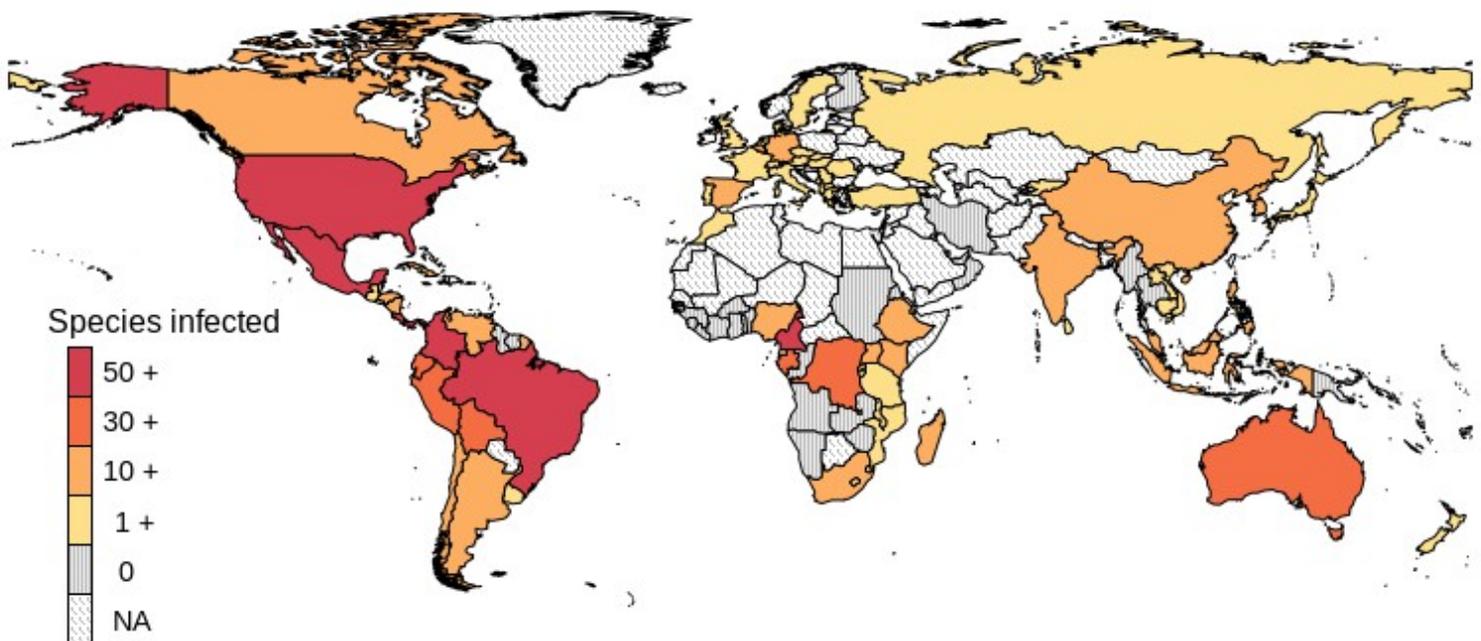
The species names, as reported, were matched with those of the Amphibian Species of the World database (Frost 2020), with the last update on May 6, 2020. Hybrids, kleptons, amphibians with unresolved taxonomic status and amphibians that could not be matched with a species as per Frost (2020) were recorded but not counted as species in the data analysis. We calculated the total number of species sampled and infected, and estimated the percentage of species that have been found with infection. Data on species conservation status and distribution were extracted from the IUCN Red List database (<https://www.iucnredlist.org>); these data were last checked on May 6, 2020.

## **Results**

Our literature search produced 554 relevant papers according to our selection criteria. We found data from all continents where amphibians exist, encompassing 119 countries. In 61 of these countries, 10 or more amphibian species were sampled and tested for *Bd*, a number that we consider adequate for analysis. Few species from countries like Laos and Tanzania are present in the data, making it difficult

to draw conclusions on how widespread and prevalent *Bd* is in those countries (see Supplementary Table 2 for details).

Variation in the number of sampled species among countries was expected given the latitudinal patterns of diversity. Not surprisingly, more species were sampled in countries with greater amphibian diversity. However, a non-proportionally large number of species came from North America (particularly the USA), whereas relatively few species came from the equatorial regions of South America and from Madagascar (Supplementary Table 2 for details). Similarly, large areas in continental Africa, India, Borneo and Papua New Guinea remain understudied both in terms of the number of untested species in those areas and in terms of the percentage of untested species (Supplementary Figure 1 for details). In Africa and Southeast Asia, few studies testing for *Bd* have been conducted and several years have passed since the last study in some of these areas (Supplementary Figure 2).



**Figure 1**

Spatial distribution of the number of *Bd*-infected amphibian species in each country. For specific data points see Supplementary Table 2.

*Bd*-infection was found in 86 of the 119 sampled countries (Fig. 1). Infection was also present in most countries where at least 10 species were sampled and in the 10 countries with the largest number of amphibian species in the world (Supplementary Table 2). Similarly, infection has been found in the 10

countries with the largest number of threatened amphibian species (IUCN status = critically endangered, endangered or vulnerable). The data included amphibians from 71 families. Infection was reported for 62 (87%) of these families. Within amphibian families, the number of sampled species varied, an expected result given the differences in family size. However, some moderately large amphibian families such as Ceratobatrachidae or Brachycephalidae were not well represented in the data. In 34 families, at least 10 species were sampled, and infection was reported in all these well-sampled families (Supplementary Table 3).

### **Number of infected species and prevalence of infection globally**

We used two summary statistics for evaluating the prevalence of infection: the number of infected species in a country and the percentage of sampled species that tested positive per country. In papers included in this study, a total of 1966 species were tested for *Bd* of which 1062 (54%) were found with infection.

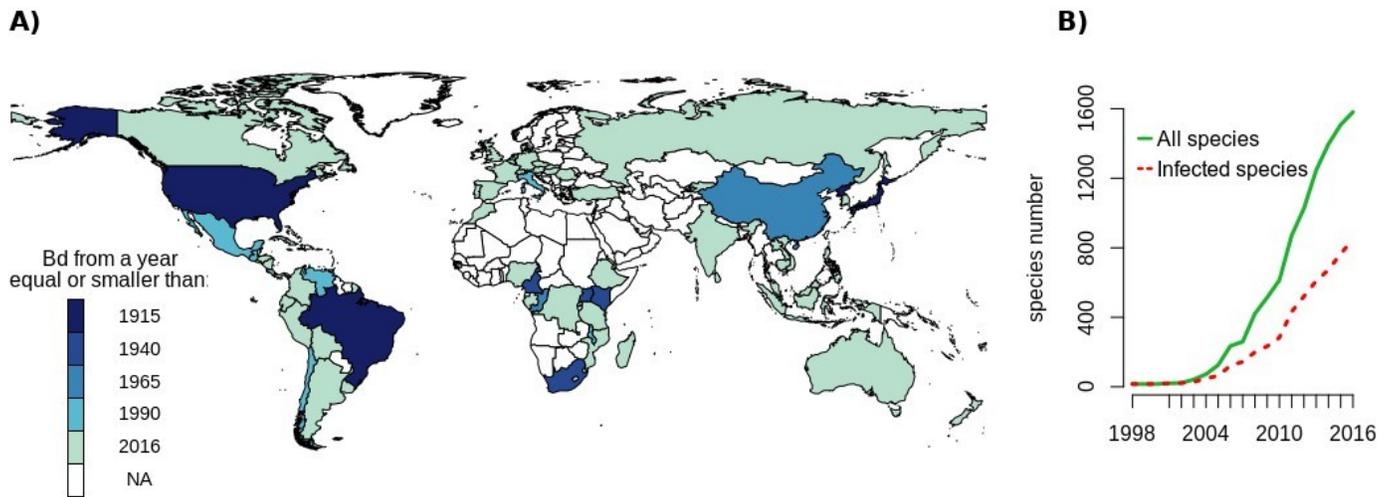
### **Tracking the infection over time**

We compiled a list of first detection records for all countries in which infection has been detected (Supplementary Table 4) including records of infection on dates prior to 1999 from 24 countries. The compiled list shows *Bd* records from North America, South America, Asia and Africa from more than 80 years ago. Records from preserved specimens show that by 1980 *Bd* was also present in Central America and Europe (Fig. 2A). The total number of tested species, infected and otherwise, has grown over time as new species are being tested (see Fig. 2B). Within tested species, the percentage of species that have been reported with infection was about half (54%) which is only slightly higher than the percentages reported by Olson et al. (2013) and Fisher et al. (2009): they reported 42% and 50%, respectively.

### **Geographical range of infected species**

Regions where the spatial distribution of *Bd*-infected species is different from the overall species distribution reveal interesting patterns (Fig. 3). In Australia, for example, species in which infection has been detected concentrate along the east coast in regions farther from the equator. In the USA, the distribution of species in which infection has been detected, roughly mirrored species richness patterns.

However, species that have not tested positive concentrate over an area that extends over the Atlantic Coast in the USA from Mississippi to Virginia (Fig. 3). The range of infected species that went across national borders also revealed areas or countries where infection might not have been detected or directly tested for, but where a large number of susceptible species reside. One such area can be seen



**Figure 2**

Temporal patterns in *Bd* infections. A) Earliest infection records. B) Cumulative count of sampled and infected amphibian species over the world reported in papers published since 1998. For specific data points see Supplementary Tables 1 & 4.

along the lowlands in Cameroon, producing a corridor that stretches eastward all the way to Uganda (Fig. 3). Differences in the distribution of infected and non-infected species can also be seen in other areas of the world. These include a concentration of host species over China and the Korean Peninsula, and a concentration of resistant species in southern Europe along the Italian peninsula, Greece and Turkey (Fig. 3).

#### Associations between *Bd* susceptibility of amphibian taxa and geographical distribution

We found marked differences in the percentage of species with infection among amphibian families. These differences are often associated with a high species infection prevalence in an area as many amphibian families have restricted ranges due to biogeographical processes. On one end of the spectrum, high species infection rates were reported in families from South America such as

Telmatobiidae (95%) and Hylodidae (85%). On the other end, low species infection rates were reported in families from Southeast Asia such as Megophryidae (7%) and Hynobiidae (17%); the latter only being sampled in South East Asia. Variation among larger families did not reach such extreme values and was closer to 50% (Supplementary Table 3). Examples of this include Plethodontidae (47%), Bufonidae (50%) Craugastoridae (50%), the Hylidae (70%) and Microhylidae (30%).

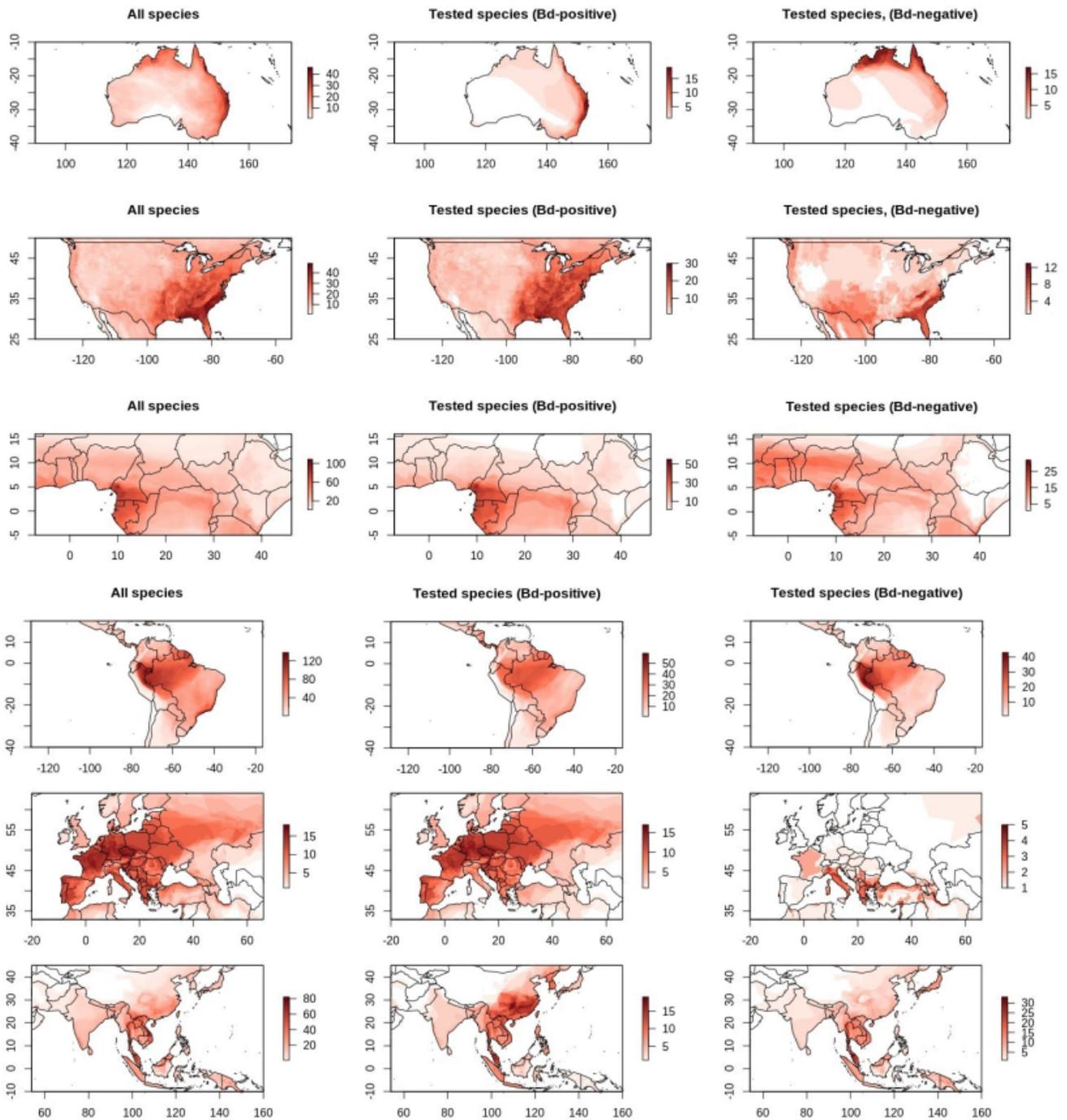
## **Discussion**

Our results illustrate the spread of *Bd* over time and provide an important update of previous work addressing where, when and in which species *Bd* infection has been found (Fisher et al. 2009; Olson et al. 2013). While Olson and Ronnenberg (2014) reported 695 species that have been infected with *Bd*, we found 1062 infected species. This does not necessarily indicate an increase in *Bd* prevalence or an increase in its distribution; the accumulation of data and the growth in the research field could also produce such an increase. Indeed, the percentage of species that have been found with infection does not show a clear temporal trend. However, the fact that the rate at which infected species accumulate does not appear to slow down suggests that we are still discovering *Bd* in previously untested locations and that we are yet to fully test most of the susceptible species and to fully understand the spatial distribution of *Bd*.

Our study shows records of infection in 86 out of 119 countries. Most of these records are relatively recent and date after 1999. It is hard to ascertain whether infection spread recently to an area or it was long present at undetectable levels; in peninsular Malaysia, for example, extensive search was conducted for years before the infection was detected (LeBlanc et al. 2014). However, early records from surrounding areas are revealing (Fig. 2). Records before 1999 were found for 24 countries, including data showing that *Bd* had spread around the world from more than a century ago. Positives have been found from 1888 in the USA, 1894 in Brazil, from 1910 in what today is North Korea and from the 1930s in Cameroon, South Africa, Kenya and Uganda (Supplementary Table 4). The presence of *Bd* positives in museum specimens across the world together with patterns of genetic diversity found previously (O'Hanlon et al. 2018) suggests there were multiple introduction events in the past. We know that *Bd* has spread with the unintentional aid of humans (Walker et al. 2008) and it probably did so in the past as well. This is particularly likely when considering the large distances that *Bd* crossed to spread across continents.

We highlight the extent of *Bd* spread up to this day and the variety of hosts that the pathogen is able to infect. This was reflected in the number and proportion of countries where infection has been detected ( $86/119 = 72\%$ ), the number and proportion of amphibian families with infection ( $62/71 = 87\%$ ) and the number and proportion of species with infection ( $1062/1966 = 54\%$ ). At a global scale, the number and proportion of infected species as well as its great spread showed how pervasive and adaptable *Bd* is.

When dealing with a lack of data, the distribution of species that are susceptible to *Bd* or that have not been found with infection can be useful. The distribution area of *Bd*-infected species is, in some areas, quite different from the overall distribution of species and reveals spatial patterns of infection (Fig. 3).



**Figure 3.** Amphibian species distribution in six regions of the world. The leftmost column shows all species, the middle column only species that tested positive for *Bd* and the rightmost column only species that have only tested negative. Color intensity correspond to the number of species in a region, scales vary between plots. Notice patterns in the distribution of species that have been found with and without infection

These patterns probably correspond to geographical and environmental conditions that affect infection. The pattern in Australia, for example, roughly follows the *Bd* suitability models by Murray et al. (2011), probably reflecting the influence of environmental conditions on infection. In Asia, a large

number of susceptible species concentrate in China and the Korean peninsula, which is in line with models on the suitability of *Bd* by Rahman et al. (2020). However, disagreeing with that work, we do not observe a high concentration of susceptible species in the Indian Western Ghats. *Bd* has been postulated to have its center of diversity around the Korean peninsula (O'Hanlon et al. 2018), and the pathogen is presumably well adapted to the conditions and hosts existing in the area where it evolved. Besides areas where susceptible species concentrate, the distribution of species that have not tested positive is also revealing. In the USA, for example, a pattern is formed by the distribution of non infected species over the Atlantic Coast of the USA from Mississippi to Virginia. This suggests again that the altitudinal profile and the environmental conditions prevalent in the region affect *Bd* infection. Another pattern, found in Central Africa, shows a high proportion of species that have been found without infection that are present in dry regions. Further, we observed a corridor-like area formed by the distribution of infected species. This area stretches from the Cameroonian lowlands all the way to the Democratic Republic of Congo. However, only small discontinuous regions within the corridor have been predicted to be suitable for *Bd* by models (Penner et al. 2013; Zimkus et al. 2020). If indeed *Bd* infection is absent or of low severity in that location, the corridor would provide a refuge for a large number of susceptible amphibian species. Equally interesting is the low concentration of susceptible species over the Amazonian region of Peru. Against our expectations, we did not observe a high number of infected species over the Atlantic forest as predicted by Becker et al. (2017).

The observed patterns should be interpreted with caution. For example, the low amphibian diversity in Europe gives great weight to individual species, and the presence or absence of infection in a single species could radically change observed patterns. We observed that species in which infection has not been detected concentrate in southern areas of Europe, mostly the Italian peninsula, Greece, the Balkans and northern Turkey. However, with the exception of the Italian peninsula, these regions are not well studied (Supplementary Figure 2), and the observed patterns might change as *Bd* is studied more intensively there as well.

*Bd* is widespread, although infection and prevalence are not necessarily high in all regions where it is found. Also, there are isolated regions like Papua New Guinea where this pathogen has not been detected (Bower et al. 2019). Constant monitoring and the establishment of proper biosecurity mechanisms can help to ensure that *Bd* does not expand its range and to react rapidly to its detection. These measures are equally important in regions where *Bd* is present in order to prevent and react to new and more virulent *Bd* strains or related pathogens such as *Batrachochytrium salamandrivorans*, as

well as to react to changes in infection dynamics. Monitoring programs and strict biosecurity measures are thus highly important. *Bd* has shown how a pathogen can maintain infection for decades across the globe, and have massive detrimental effects on a wide range of hosts. The appearance of an analogous pathogen that could infect another taxonomic group, domestic animals or even humans presents a worrying scenario.

## **Conclusions**

*Bd* is widespread and pervasive, being able to infect more than 50% of the studied species and having more than 1000 known amphibian hosts. As of this publication, amphibians in most countries and families have been tested. However, several of them have been barely and infrequently studied. We stress the need to prioritize efforts to study and evaluate the effect that *Bd* has on amphibian populations across the world. Our data highlight amphibian-rich regions and families where *Bd* has been detected, but where only few or no species have been tested. Countries such as Laos and Tanzania cannot be properly evaluated right now (see Table 2). Similarly, there are moderately large amphibian families, such as Ceratobatrachidae or Brachycephalidae, that have not been well studied (see Table 3).

We investigated the distribution of *Bd* over time, pointing out some temporal, spatial and taxonomic patterns of infection. We acknowledge the speed with which data accumulate and the need to maintain a commitment to update our dataset in the future. We extend a cordial invitation to researchers interested in using, updating or extending this dataset to join our effort to maintain data on *Bd* records.

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## **Data availability**

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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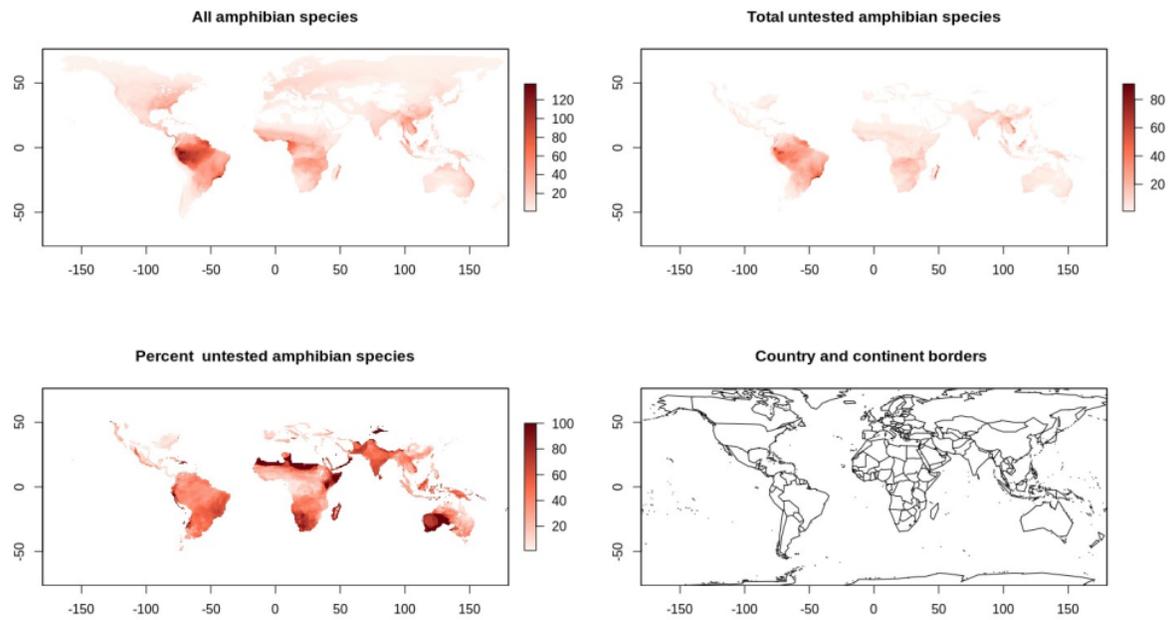
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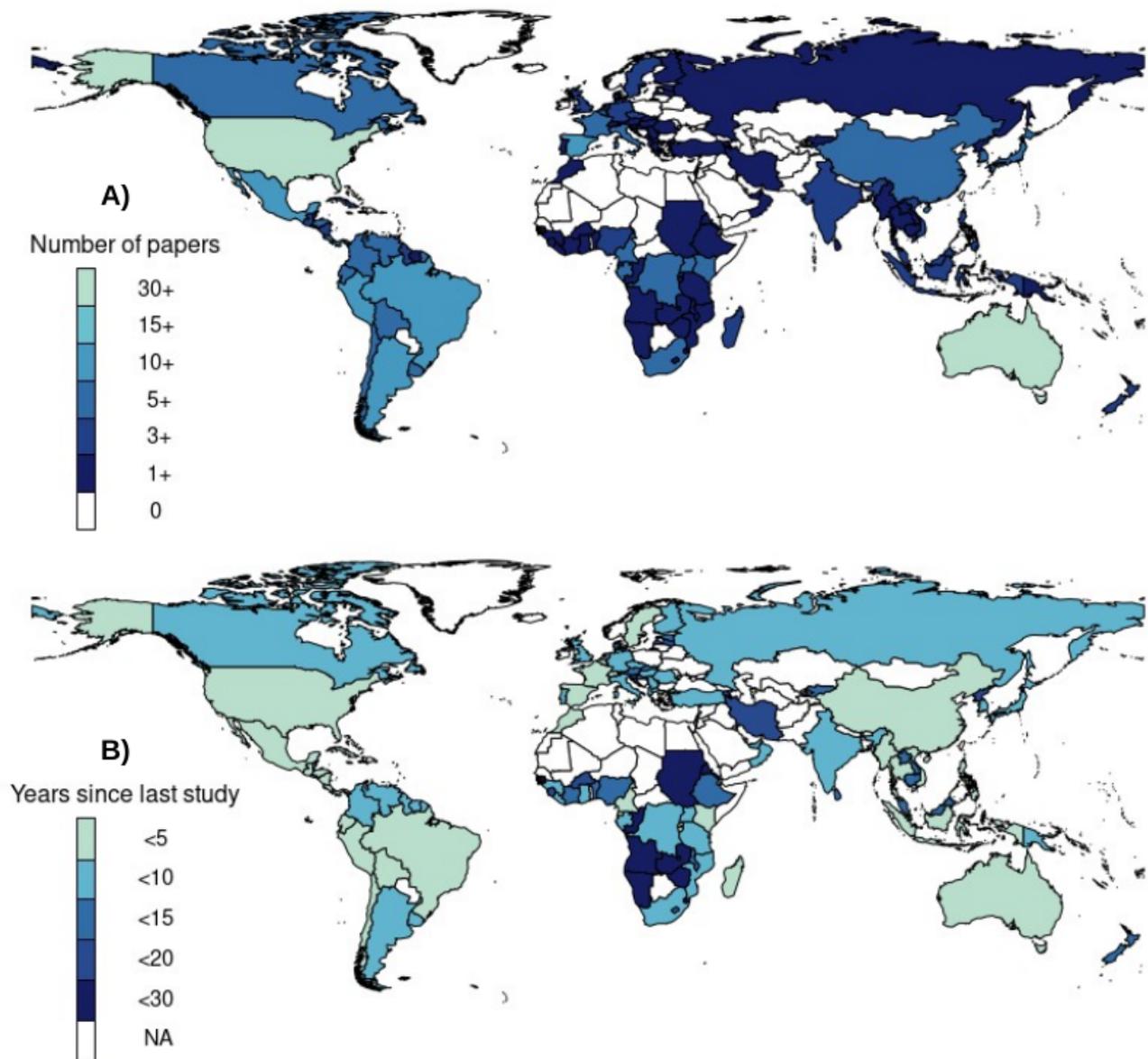
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## Supplementary materials



### Supplementary Figure 1

The top left plot displays the distribution of amphibians over the world. Notice a great concentration of amphibians species in the equatorial regions, particularly in South America, Central Africa and Southeast Asia. These regions were not equally sampled (top right plot). This is further highlighted by the percentage of untested amphibians (bottom left). Country and continent borders were not overlaid, as they might hinder visualization of coastal/border areas of interest (e.g. Madagascar and Central America). They are displayed for reference on the bottom right plot.



### Supplementary Figure 2

Countries that have not been studied by a large number of papers or that have not been studied in a long time are displayed in dark blue. Notice also countries in white do not appear in the 554 publications used in this study. **A)** Most countries have been explored by less than 10 papers while amphibians from the USA have been studied in 198 papers. **B)** Some countries are seldom studied, data from these countries might have been collected several years ago. New recent studies from these areas are much needed. For specific data points of all countries in the dataset see Supplementary Table 2.

### **Chapter 3: Castro Monzon F, Rödel MO, Ruland F, Parra Olea G, Jeschke JM. Where and in which amphibian species is the pathogen *Bsal* found?**

Submitted as an original contribution to the *EcoHealth* journal.

#### **Abstract**

*Batrachochytrium salamandrivorans* (*Bsal*), a species related to the pernicious pathogen *Batrachochytrium dendrobatidis*, was found and identified in Europe in 2011. Now, a decade later, a large amount of information is available. This includes data from studies in the field, reports of infection in captive amphibians, laboratory studies testing host susceptibility and data from prospective studies that test for *Bsal*'s presence in a location. We conducted a systematic review of the published literature and compiled a dataset of *Bsal* tests. We identified 66 species that have been reported positive for *Bsal*, 20 of which have a threatened conservation status. The distribution of the species that have been found with infection encompasses 69 countries, highlighting the potential problem that *Bsal* poses. We point out where tests to detect *Bsal* have taken place and also highlight areas that have not been well monitored. The large number of infected individuals belonging to the families Plethodontidae and Salamandridae suggests a taxonomic pattern of susceptibility. Our results allow to evaluate the risk posed by *Bsal*, to identify species that might be vulnerable and areas where more research is needed to fill existing knowledge gaps.

#### **Introduction**

The amphibian chytrid pathogen *Batrachochytrium salamandrivorans* (*Bsal*) presents a risk to amphibian communities across the world. The pathogen has been hypothesized to have originated in East Asia (Martel et al. 2014) and was first identified in 2010 in a region bordering Belgium and the Netherlands (Martel et al. 2013); however, museum records later showed that it was already present in

Germany by 2004 (Lötters et al. 2020a). *Bsal* has maintained infection over several years in the locations where it was first found, and has been associated with the local disappearance of the fire salamander (*Salamandra salamandra*). Since its discovery, the pathogen has also been found in the wild in Germany and in several Asian countries (Nguyen et al. 2017, Laking et al. 2017, Yuan 2017). Recently, it was also reported for Spain by two independent research groups in different regions of the country (Lastra González et al. 2019, Martel et al. 2020).

The pathogen has also been found in captive animals in several countries (Martel et al. 2018; Sabino Pinto et al. 2018). The presence of *Bsal* in captive animals has been pointed out as a possible source of infection in the wild (Martel et al. 2014). In addition, susceptible species that are distributed outside the current known range of the pathogen have been successfully infected in laboratory exposure experiments, and *Bsal*-related deaths have been observed in a number of them (Martel et al. 2014; Friday et al. 2020).

The potential threat that *Bsal* poses is highlighted by the damage that a related pathogen, *Batrachochytrium dendrobatidis* (*Bd*), has inflicted on amphibian populations. *Bd* has been found over the world, presumably also spreading from Asia, and has been linked to amphibian declines in many regions globally (Skerratt et al. 2007; Scheele et al. 2019). *Bd* has over 1000 known host species (Castro Monzon et al. 2020) and has been deemed as the disease with the greatest impact on vertebrate biodiversity (e.g. Bellard et al. 2016).

In face of the threat that *Bsal* could pose, research efforts focusing on this new pathogen have intensified, foremost in Europe. Respective studies have revealed which species are being affected by

*Bsal* and how it is spreading (e.g. Spitzen-van der Sluijs et al. 2016; Lastra González et al. 2019). This research is complemented by monitoring studies in locations where *Bsal* is yet to be detected (e.g. Muletz et al. 2014; Parrot et al. 2017). Both types of studies are crucial for timely responses to *Bsal* in the right locations.

Several studies have tested amphibians held in captivity (Fitzpatrick et al. 2018; Sabino-Pinto et al. 2018). In addition to provide information on a possible source of infection for wild amphibians, they also highlight which amphibian species are susceptible. Lastly, the susceptibility of amphibians of different taxonomic groups has been tested in exposure experiments (Martel et al. 2014; Friday et al. 2020). These experiments reveal in a controlled setup which species could possibly be carriers and which ones could be severely affected by *Bsal*.

While data on *Bsal* is becoming increasingly available, it is dispersed in a multitude of publications. Pieces of information, such as the number of known *Bsal* hosts or the species in which *Bsal*-related deaths have been reported, are thus not always straightforward to obtain. This can cause problems, as researchers might not be aware of all available information, and decision-makers might not be able to react on time. For example, the first field report of *Bsal* detection in Spain (Lastra González et al. 2019) was neither cited nor discussed when the pathogen was re-discovered in Spain later on (Martel et al. 2020).

Now, over ten years after *Bsal* was first reported, we believe it is useful to compile and review the available information. We therefore conducted a systematic review of published papers and compiled a database with records of *Bsal* tests performed on amphibians in the field, in captivity or experimental

setups. Similar efforts to compile records of infection have been undertaken in the past with the related pathogen *Bd* by Fisher et al. (2009), Castro Monzon et al. (2020) and Olson et al. (2011, 2014, 2021). Such efforts have helped to better understand *Bd* infection. For *Bsal*, there is a systematic review (Baláž et al. 2017) of published data up to 2016 focusing on Europe. That review gathered data from 30 publications and identified positive tests in 12 European species and three Asian ones. Since its publication, however, a large number of reports have appeared, for example in a special issue of the journal *Salamandra* in 2020 (e.g. Lötters et al. 2020a, b; Schulz et al. 2020). In our study, we included articles published up to 2021 and more than double the number of publications used for the last *Bsal* systematic review (Baláž et al. 2017).

With our study, we aim to properly evaluate the current situation of *Bsal* research in the world. Specifically, we seek to understand which species have been reported with positive *Bsal* tests, in which species *Bsal*-related deaths have been reported, and which are the families of infected species. We investigate if species of some families are more frequently infected than others. We also seek to understand how many studies have been conducted in different countries, how often they have been conducted and when the most recent study took place. Our contribution allows to better grasp the magnitude of the problem posed by *Bsal* and can allow to prioritize research in countries that are not being well monitored.

## **Methods**

We conducted our work following the PRISMA guidelines for systematic reviews (Moher et al. 2010) to increase repeatability and to ensure that this study and its accompanying dataset can be updated and extended as new data is published. For this purpose, we openly provide our dataset as supplementary material.

We searched the Web of Science for peer-reviewed articles on March 4, 2021, using the query: (chytrid\* OR batrachochytrium) AND (amphibian\* OR frog\* OR salamander\* OR anuran\* OR urodelan\* OR caudat\* OR caecilian\*). In addition, we also manually searched correspondence articles from the journal *Salamandra* up to the first volume of 2021, as these do not include abstracts and keywords and were thus not all captured by our Web of Science query. Similarly, we included articles from the journal *Herpetological Review* up to the first volume of 2021. We added publications from this non-indexed journal to our database, as it contains a section for papers related to amphibian chytridiomycosis. In both *Herpetological Review* and *Salamandra*, we looked for articles containing the words "batrachochytrium" or "chytrid".

Our query returned several articles where amphibians were tested for *Bd*, several of which also presented data on *Bsal*. We did not record data on *Bd* for this study, as that was recently done by Castro Monzon et al. (2020) and Olson et al (2021). We only selected articles where amphibian hosts were tested for *Bsal* either via qPCR or histological analysis. This included papers that reported tests in amphibians collected in the wild or kept in captivity, or in preserved amphibians from museums. Papers where amphibians were tested in an experimental setting, defined by the intentional exposure of amphibians to *Bsal*, were also included.

We included each relevant publication in our dataset and recorded the following data: names of the tested amphibians, the country where the amphibians were tested, the first and last year amphibians of a species was tested, the first and last year the species tested positive, the number of amphibians tested and how many *Bsal*-related deaths were reported. Species names as reported were associated with

species names in the Amphibian Species of the World Database (Frost 2021), if possible. Data from hybrids, kleptons and organisms with unresolved taxonomic status were recorded, but not included in the analysis.

## Results

*Bsal* positive tests have been reported in a total of 66 species in ten different amphibian families. *Bsal*-related deaths have been reported in 28 species, all of them caudates from the Salamandridae and Plethodontidae families. Twenty-one of the species that have been reported with positive tests are threatened with extinction (IUCN Red List status critically endangered, endangered or vulnerable). *Bsal*-related deaths have been reported in eight of these threatened species. Additionally, nine of the *Bsal*-positive species are known or suspected to be invasive (data from Frost 2021). Special attention should be taken to these species. Caudates invasive species might not be as infamous as their anuran counterparts (e.g. *Rhinella marina*, *Lithobates catesbeianus* and *Xenopus laevis*) however, infected caudates might bring the pathogen with them and can be transported long distances. For example, one of the species in the list, *Lissotriton vulgaris*, has often been translocated in Europe and has also established a population in Australia (Tingley et al. 2015, Dubey et al. 2018). Another species in the list, *Ichthyosaura alpestris*, has established populations in the UK, New Zealand, southern France, and Spain (Bell 2016, Frost 2021). Martel et al. (2020) argued that the presence of introduced species such as *I. alpestris* and *Triturus anatolicus* in Spain is associated with the introduction of *Bsal* there.

Our data comes from 64 papers that reported tests for *Bsal* in amphibians; 46 of these papers also reported tests for *Bd*, usually by means of duplex qPCR as per Blooi et al. (2013). Reports of negative tests abound, with 29 papers only reporting negative tests. Tests come from 31 countries, although 15 of these countries have only been studied once (Table 1).

Not surprisingly, a large number of studies come from Germany, Belgium and the Netherlands, countries in which *Bsal* was detected several years ago. However, the largest number of studies came from the United States. Data from Mexico and Central America is scarce and even completely absent for some countries (Fig. 1). We found reports of tests in the field from 30 countries but positives have only been reported in eight. Although in the wild *Bsal* has only been detected in eight countries (Fig. 1, Table 1), the native and non-native distribution of species that are known *Bsal* hosts encompasses 69 countries (Fig. 3). These countries hold ~75% of all existing caudate species. *Bsal* has been tested in the field in 16 European countries, but positives have so far only been found in the Netherlands, Belgium, Germany and Spain (Fig. 2). *Bsal* has been found in 26 European species (Table 2), but in the field, the pathogen has only been detected in eight. *Bsal*-related deaths have been reported in 14 European amphibian species (Table 2), all but one (the plethodontid *Speleomantes strinatii*) are salamandrids. Important for conservation purposes is the report of *Bsal*-deaths in five threatened caudates; fortunately infection in these species is yet to be found in the field (Table 2). Also relevant is the detection of *Bsal* in a museum specimen collected in Germany in 2004, that is the earliest known record of the pathogen in Europe (Lötters et al. 2020a).

**Table 1. The number of caudate species countries where tests have been conducted in the field.**

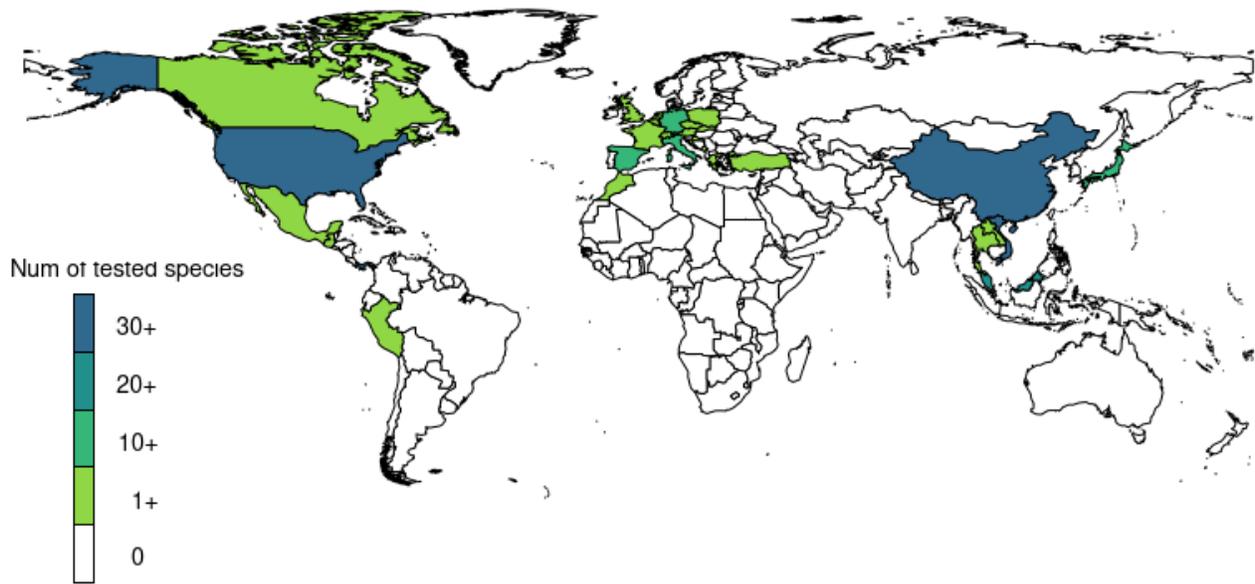
The number of caudate species in each country is shown (Frost 2021). Also presented is the percentage of caudate species with threatened conservation status (critically endangered, endangered or vulnerable). Additionally, the number of known *Bsal* susceptible host species that exist in a country is presented as well as the number of species that have tested positive in the field, the number of species tested and the number of studies that took place in that country (references for studies in each country in supplementary materials).

	# Species	% Threatened	# Known hosts	# Field infected	# Field tested	# Studies
USA	208	23%	15	0	94	18
Mexico	162	81%	8	0	7	3
China	84	66%	14	8	55	4
Guatemala	67	82%	0	0	6	1
Japan	48	43%	4	4	12	1
Panama	35	50%	0	0	61	1
Canada	22	0%	4	0	1	1
Turkey	21	60%	8	0	8	2

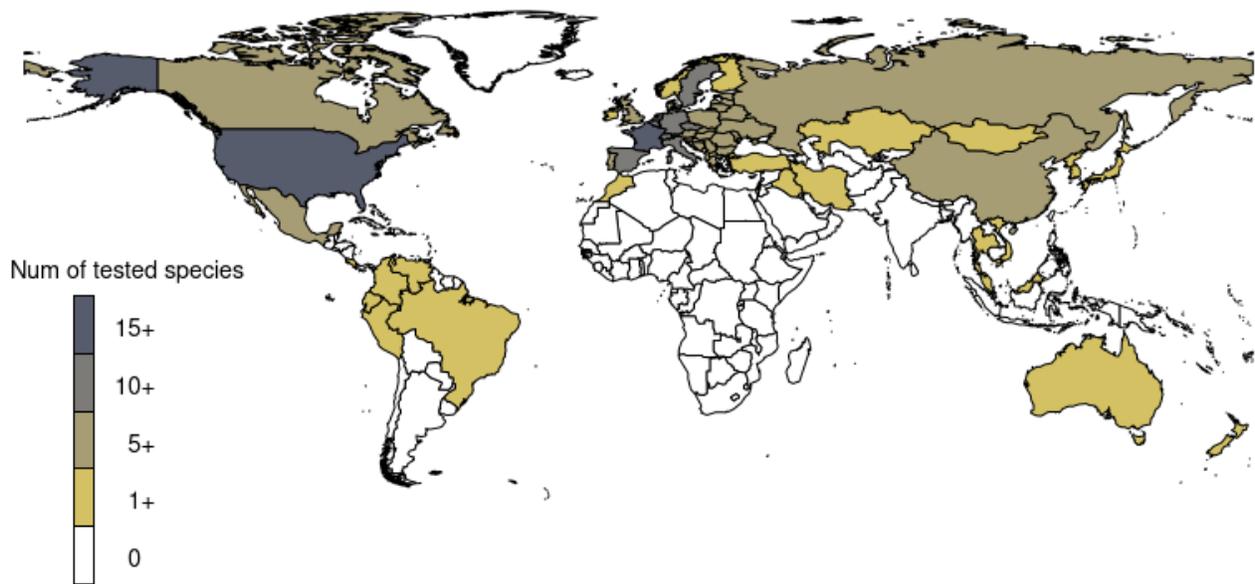
Italy	19	37%	9	0	13	2
France	14	15%	9	0	3	2
Spain	13	27%	9	4	16	5
Vietnam	9	50%	3	5	40	4
Croatia	8	14%	6	0	2	2
Czechia	8	0%	6	0	3	2
Germany	8	0%	6	6	11	13
Greece	8	28%	5	0	1	1
Austria	7	0%	6	0	2	2
Switzerland	7	0%	6	0	7	2
Montenegro	6	16%	5	0	2	1
Netherlands	6	0%	5	3	9	8
Slovakia	6	0%	5	0	1	1
Thailand	6	0%	2	1	3	1
Belgium	5	0%	5	2	7	5
Laos	5	40%	0	0	1	1
Poland	5	0%	4	0	1	1
UK	5	0%	4	0	4	4
Peru	4	0%	0	0	1	1
Morocco	2	50%	2	0	1	1
Brunei	0	0%	0	0	28	1
Malaysia	0	0%	0	0	25	1

Amphibian species from East Asia have been tested in seven countries: China, Japan, Thailand, Vietnam, Laos, Malaysia and Brunei (Fig. 1a). *Bsal* has been detected in 20 species from East and Southeast Asia (Table 2) and in the field in 17 of these species. One of the species that tested positive for *Bsal*, *Bombina microdeladigitora*, was an anuran, and there is a report of this anuran testing positive both in the field and in captivity. Also testing positive were two salamander species of the Hynobiidae family and one of the Cryptobranchidae. The remaining infected species were all members of the Salamandridae. There is also evidence suggesting that some Asian amphibians might be susceptible as *Bsal*-related deaths have been reported from laboratory exposure experiments in five species, two of which had a threatened conservation status (Table 2). Also interesting is the detection of *Bsal* in a preserved *Cynops ensicauda* specimen dating from 1861 (see Martel et al. 2014). At the time in which this manuscript is being written, this is the oldest known *Bsal* record. *Cynops ensicauda* is only distributed in Japan (Sparreboom 2014) and, presumably, the infected specimen originated there.

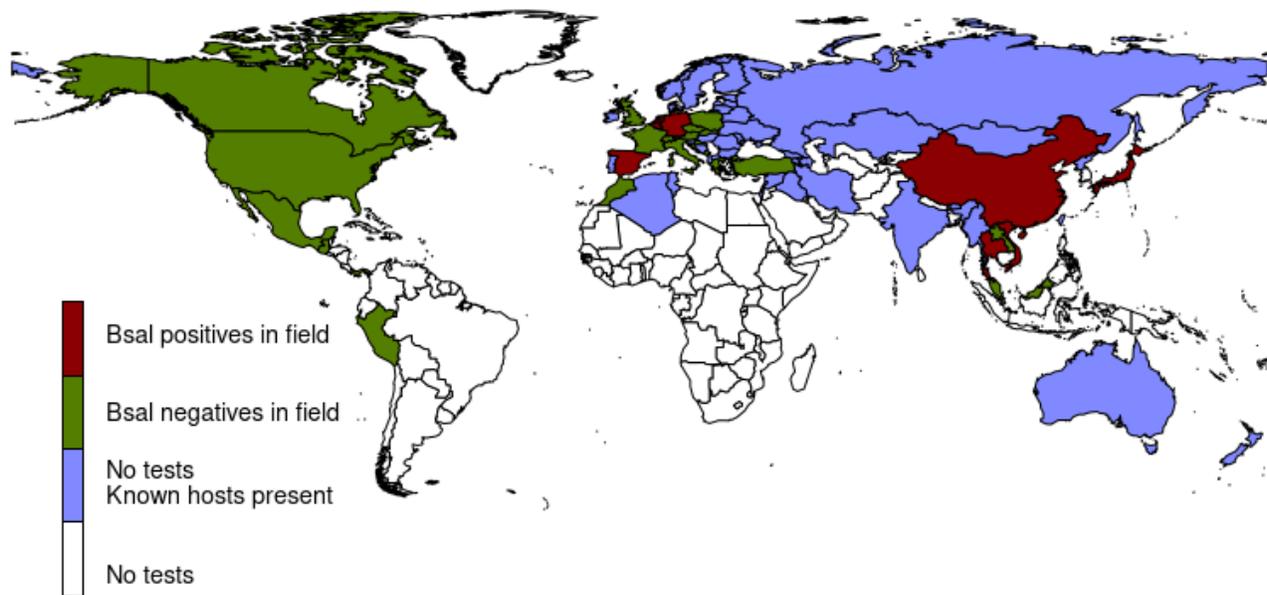
a) Field studies



b) Laboratory studies

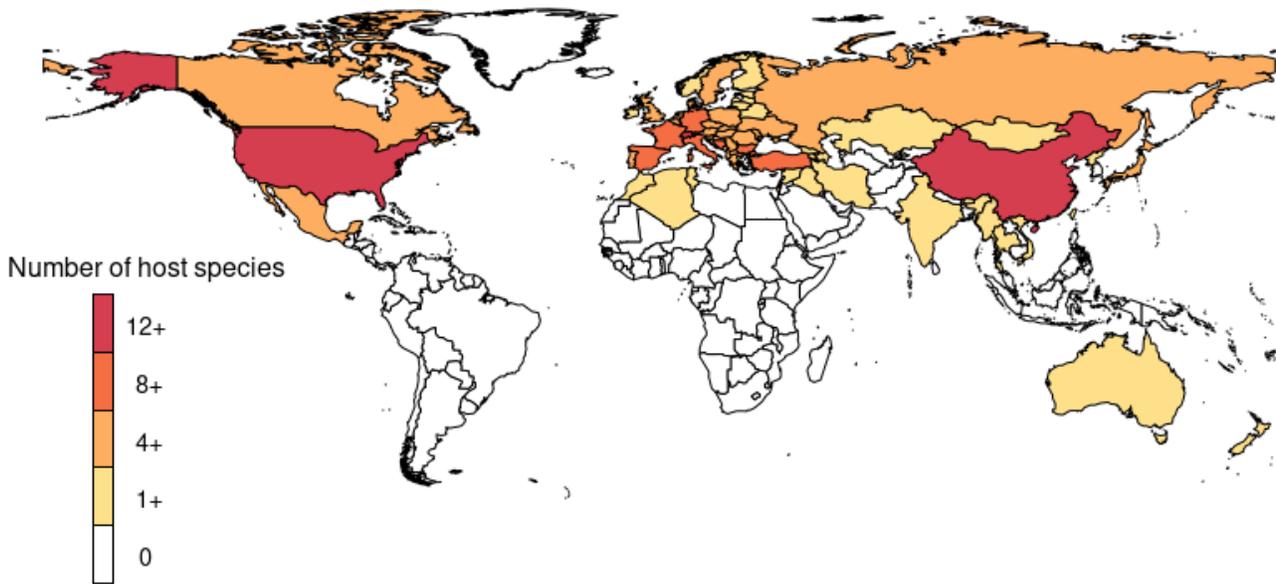


**Figure 1.** Number of amphibian species tested in each country in (a) the field and (b) the laboratory.



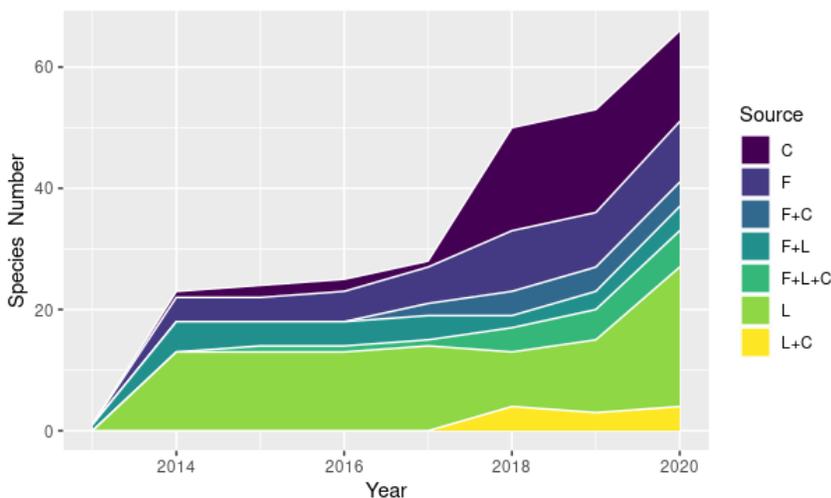
**Figure 2.** Countries in which *Bsal* has (i) been found in the field, (ii) has not been found or (iii) where no tests have been conducted, but where known hosts are distributed. Notice that both the native and non native distribution of host species is marked (e.g. in *Lissotriton Vulgaris* is found in Australia). Known *Bsal* host species exist in all colored countries except Peru, Venezuela, Lao, Brunei and Malaysia.

In the Americas, tests for *Bsal* have been conducted in the field in the USA, Canada, Mexico, Guatemala, Panama and Peru (Fig. 1a). *Bsal* is yet to be found in the Americas although laboratory exposure experiments and reports of infection in captivity show that at least 16 species native to the Americas are capable to host *Bsal* (Table 2). These include species from the Salamandridae, Ambystomatidae, Cryptobranchidae, Sirenidae and Plethodontidae families. Most importantly, *Bsal*-related deaths have been reported in seven species, four of which were plethodontids.



**Figure 3.** Countries are colored differently to highlight the number of known host species that are distributed there. Notice that both the native and non native distribution of host species is marked (e.g. in *Lissotriton Vulgaris* is found in Australia). Notice also that there are countries in Central America that have not been tested in the field (figure 1) and whose species have not been tested in laboratory (figure 3).

The number of species that have been detected with *Bsal* has grown since the pathogen was discovered (Fig. 4). This change is mostly driven by reports of species tested in the laboratory or in captivity. It reflects an accumulation of knowledge as new species are tested or as infection is tested in less susceptible species. The number of species found with *Bsal* in the field has also increased through time. Most of these species (15 out of 22) are distributed in East and Southeast Asia (Fig. 4).



**Figure 4.** The number of known hosts has increased over time. The graph disaggregates the data to highlight if infection was detected after experimental exposure in laboratory (L), in captivity (C) or in the field (F). A combination of those sources is also possible if, for example, infection has been found in a species in the field and in captivity (F+C).

**Table 2. All 66 species that have tested positive for *Bsal* in the field, in captivity or in laboratory.**

Species are ordered by region of origin. Species distribution is marked in the following way AF = North Africa, EU = Europe, CA = Central Asia, EA = East and Southeast Asia, AM = Americas. Suspected invasive species are marked with an asterisk. IUCN Red List status is indicated as follows: LC = least concern, NT = near threatened, VU = vulnerable, EN = endangered, CR = critically endangered; VU, EN and CR are highlighted in bold. *Bsal*-related deaths are highlighted in bold as well. When no data is available, NA is used.

Distribution	Family	IUCN	Species	Field	Captivity	Laboratory
AF	Salamandridae	<b>VU</b>	<i>Pleurodeles nebulosus</i>	NA	positive	NA
AF / EU	Salamandridae	NT	<i>Pleurodeles waltl</i>	negative	positive	deaths
EU	Alytidae	LC	<i>Alytes obstetricans</i> *	negative	negative	positive
EU	Salamandridae	LC	<i>Lissotriton boscai</i>	negative	positive	NA
EU	Proteidae	<b>VU</b>	<i>Proteus anguinus</i> *	NA	negative	positive
EU	Salamandridae	<b>CR</b>	<i>Calotriton arnoldi</i>	negative	NA	<b>deaths</b>
EU	Salamandridae	<b>EN</b>	<i>Euproctus platycephalus</i>	negative	negative	<b>deaths</b>
EU	Salamandridae	<b>VU</b>	<i>Lyciasalamandra helverseni</i>	negative	NA	<b>deaths</b>
EU	Salamandridae	<b>VU</b>	<i>Salamandra algira</i>	negative	<b>deaths</b>	NA
EU	Salamandridae	LC	<i>Triturus marmoratus</i>	positive	<b>deaths</b>	<b>deaths</b>
EU	Salamandridae	LC	<i>Salamandra salamandra</i>	<b>deaths</b>	<b>deaths</b>	<b>deaths</b>
EU	Salamandridae	LC	<i>Lissotriton helveticus</i>	positive	<b>deaths</b>	positive
EU	Salamandridae	LC	<i>Triturus cristatus</i>	positive	<b>deaths</b>	<b>deaths</b>
EU	Salamandridae	LC	<i>Ichthyosaura alpestris</i> *	<b>deaths</b>	positive	<b>deaths</b>
EU	Salamandridae	LC	<i>Lissotriton italicus</i>	negative	negative	<b>deaths</b>
EU	Salamandridae	LC	<i>Salamandrina perspicillata</i>	NA	negative	<b>deaths</b>
EU	Plethodontidae	NT	<i>Speleomantes strinatii</i> *	negative	negative	<b>deaths</b>
EU	Salamandridae	LC	<i>Salamandra atra</i>	negative	positive	NA
EU	Salamandridae	LC	<i>Salamandra corsica</i>	NA	positive	NA
EU / CA / EA	Hynobiidae	LC	<i>Salamandrella keyserlingii</i>	positive	negative	positive
EU / CA	Ranidae	LC	<i>Rana temporaria</i> *	positive	NA	negative
EU / CA	Salamandridae	LC	<i>Triturus karelinii</i>	NA	positive	NA
EU / CA	Salamandridae	NT	<i>Ommatotriton ophryticus</i> *	negative	positive	NA
EU / CA	Salamandridae	NT	<i>Triturus dobrogicus</i>	NA	positive	NA
EU / CA	Salamandridae	NA	<i>Triturus ivanbureschi</i>	NA	positive	NA
EU / CA	Salamandridae	LC	<i>Lissotriton vulgaris</i> *	positive	negative	<b>deaths</b>
EU / CA	Salamandridae	NA	<i>Triturus macedonicus</i>	NA	<b>deaths</b>	NA
EU / CA	Salamandridae	<b>VU</b>	<i>Neurergus crocatus</i>	NA	negative	<b>deaths</b>
EU / CA	Salamandridae	<b>VU</b>	<i>Neurergus strauchii</i>	NA	<b>deaths</b>	NA
EU / CA	Salamandridae	NT	<i>Salamandra infraimmaculata</i>	NA	positive	NA
EU / CA	Salamandridae	NA	<i>Triturus anaticus</i> *	positive	negative	positive
EA	Cryptobranchidae	<b>CR</b>	<i>Andrias davidianus</i>	NA	positive	NA
EA	Salamandridae	<b>VU</b>	<i>Paramesotriton fuzhongensis</i>	negative	positive	NA
EA	Salamandridae	<b>VU</b>	<i>Tylototriton zieglerei</i>	positive	negative	NA
EA	Bombinatoridae	<b>VU</b>	<i>Bombina microdeladigitora</i>	positive	positive	NA
EA	Salamandridae	<b>EN</b>	<i>Cynops ensicauda</i>	positive	positive	NA
EA	Salamandridae	<b>EN</b>	<i>Cynops orphicus</i>	positive	NA	NA
EA	Salamandridae	<b>EN</b>	<i>Pachytriton wuguanfui</i>	positive	NA	NA
EA	Salamandridae	<b>EN</b>	<i>Tylototriton vietnamensis</i>	positive	positive	NA
EA	Salamandridae	<b>VU</b>	<i>Paramesotriton aurantius</i>	positive	NA	NA
EA	Salamandridae	<b>VU</b>	<i>Tylototriton wenxianensis</i>	negative	negative	<b>deaths</b>

EA	Salamandridae	LC	<i>Cynops cyanurus</i>	positive	positive	<b>deaths</b>
EA	Salamandridae	LC	<i>Cynops pyrrhogaster</i>	positive	negative	<b>deaths</b>
EA	Salamandridae	LC	<i>Paramesotriton deloustali</i>	positive	positive	<b>deaths</b>
EA	Salamandridae	NT	<i>Paramesotriton hongkongensis</i>	positive	positive	NA
EA	Salamandridae	NT	<i>Tylototriton asperrimus</i>	positive	negative	NA
EA	Salamandridae	NA	<i>Tylototriton uyenoi</i>	positive	NA	NA
EA	Salamandridae	LC	<i>Tylototriton verrucosus</i>	positive	negative	NA
EA	Salamandridae	LC	<i>Cynops orientalis</i>	positive	negative	NA
EA	Hynobiidae	LC	<i>Hynobius nebulosus</i>	positive	NA	NA
AM	Ambystomatidae	LC	<i>Ambystoma opacum</i>	negative	positive	negative
AM	Ambystomatidae	LC	<i>Ambystoma maculatum</i>	negative	positive	positive
AM	Cryptobranchidae	<b>VU</b>	<i>Desmognathus apalachicola</i>	NA	NA	positive
AM	Salamandridae	<b>EN</b>	<i>Notophthalmus meridionalis</i>	NA	NA	<b>deaths</b>
AM	Salamandridae	LC	<i>Notophthalmus viridescens</i>	negative	<b>deaths</b>	<b>deaths</b>
AM	Salamandridae	LC	<i>Taricha granulosa</i>	negative	negative	<b>deaths</b>
AM	Plethodontidae	LC	<i>Desmognathus auriculatus</i>	negative	NA	<b>deaths</b>
AM	Plethodontidae	LC	<i>Eurycea cirrigera</i>	negative	negative	<b>deaths</b>
AM	Plethodontidae	LC	<i>Eurycea wilderae</i>	negative	NA	<b>deaths</b>
AM	Plethodontidae	LC	<i>Pseudotriton ruber</i>	negative	negative	<b>deaths</b>
AM	Plethodontidae	NT	<i>Aquiloerycea cephalica</i>	negative	negative	positive
AM	Plethodontidae	LC	<i>Ensatina eschscholtzii</i>	negative	negative	positive
AM	Plethodontidae	LC	<i>Eurycea guttolineata</i>	negative	NA	positive
AM	Plethodontidae	LC	<i>Eurycea lucifuga</i>	NA	NA	positive
AM	Cryptobranchidae	NA	<i>Desmognathus conanti</i>	NA	NA	positive
AM	Sirenidae	LC	<i>Siren intermedia</i>	negative	negative	positive

## Discussion

The data gathered and analyzed in this study supports Martel et al.'s (2014) observation of a higher susceptibility of caudate than anuran species for *Bsal*. We found reports of positive tests in amphibians from seven caudate families. In contrast, reports of positive tests from only three anuran families.

Moreover, most of the species that were reported with positive tests (63/66) were caudates. Reports of positive tests in anurans are few, such as the detection of *Bsal* in a single individual of *Rana temporaria* (Schulz et al. 2020). We found no reports of *Bsal*-related deaths among anurans, but the detection of *Bsal* in *Bombina microdeladigitata* specimens kept in a pet shop in Germany raises concerns about the possibility of anurans transmitting the pathogen (Nguyen et al. 2017).

In captivity, reports of infection appear more frequently in salamandrid than non salamandrid caudates (even when accounting for the number of individuals tested). Infection in plethodontid caudates is also

noteworthy; Plethodontidae is the only family, besides Salamandridae, in which *Bsal*-related deaths have been reported (Martel et al. 2018; Carter et al. 2019; Friday et al. 2020). This suggests that, while *Bsal* is able to infect a wide range of amphibian species, it affects predominantly salamandrid and plethodontid caudates.

The reports of *Bsal*-related deaths in 14 European salamanders show the potential risk that the pathogen poses. As of now, *Bsal* has been detected in the field in eight European species (Table 2). However, *Bsal*-related deaths in the field have only been reported in *Salamandra salamandra* and, more recently, in *Ichthyosaura alpestris* (Schmeller et al. 2020). Populations of *S. salamandra* have been reported to be affected or even to go extinct locally in regions of the Netherlands, Belgium and Germany (Martel et al. 2014, Schulz et al. 2020). A potential threat for other species is the expanding range of *Bsal* (Spitzen-van der Sluijs et al. 2016) or the translocation of infected individuals into a new area. Species with threatened conservation status and small distribution areas are particularly susceptible (see for example Martel et al. 2020). There are 12 European threatened caudate species, but we found no report of infection in the wild in any of them. All but three of these threatened species have been tested in the field, the exceptions being: *Chioglossa lusitanica*, *Proteus anguinus* and *Salamandra lanzai*. While infection has not been found in the field in any European threatened species, reports from individuals kept in captivity and exposed to the pathogen show that at least five can carry infection. These species are: *Calotriton arnoldi*, *Euproctus platycephalus*, *Lyciasalamandra helverseni*, *Salamandra algira* and *Proteus anguinus*. *Bsal*-related deaths have been reported in all these species except *P. anguinus* (Sabino-Pinto et al. 2015; Martel et al. 2018; Martel et al. 2020; Li et al. 2020). The recognition of a taxonomic susceptibility in plethodontid caudates also highlights the risk that European cave salamanders (*Speleomantes sp.*) are in.

The origin of *Bsal* has been postulated to be in East Asia (Martel et al. 2014). Species that evolved with the pathogen presumably have a greater resistance to infection. However, the historic distribution of *Bsal* is not known, and some species in Asia might be susceptible. Interestingly, Martel et al. (2018) report *Bsal*-related deaths from exposure experiments in four caudates from East Asia (Table 2). The affected species are *Cynops cyanurus*, *C. pyrrhogaster*, *Paramesotriton deloustali* and *Tylototriton wenxianensis*. These species have distributions that include Japan, central and southern China and north Vietnam. There are 89 threatened caudate species in East Asia and South East Asia, and only 40 have been tested, 25 of them in the field. We still know little about the susceptibility of these species to *Bsal*.

The Americas are incredibly important for caudate diversity, as 538 species of the 760 known caudate species appear in this region (Frost 2021). *Bsal* has not been detected in the field in the Americas, nor has it been found in captive animals there. However, most of the species in the Americas belong to the Plethodontidae family which, as previously discussed, has shown to be susceptible in exposure experiments. We found reports of infection in laboratory exposures from amphibians kept in captivity from 16 species distributed in the Americas (Table 2) and reports of *Bsal*-related deaths in seven of them (Table 2). These species are distributed in the USA, Canada and Mexico. Species distributed in Central America have not been tested in laboratory exposure experiments, which unfortunately leaves us with little information to evaluate the susceptibility of species in that region.

In the field, there have been 15 prospective studies in the USA, while only three in Mexico (Ellison et al. 2019; Olivares Miranda et al. 2020; Waddle et al. 2020) and two in Central America (Table 1). This difference is expected; research within each country is affected by economic, social and political circumstances, and spatial research biases are known for many other topics (e.g. Tydecks et al. 2018, Jeschke et al. 2019). This is nonetheless a critical problem, as the small number of prospective studies

in a region might not allow for timely detection of infection. Pathogens and their hosts are also not bound by political borders. Hence, the establishment of infection in a region places species in neighboring countries at greater risk. Mexico and Central America also hold more than half of the known caudate species, 74% of which are being threatened. An untimely detection of the pathogen is risky for many of these species.

The number of papers reporting *Bsal* tests had initially grown slowly. Less than five papers were published each year up to 2016, 10 papers each year thereafter and 24 in 2020. The publication peak in 2020 is extraordinary when compared with previous years, although it is partially explained by the simultaneous publication of a large number of papers in a special issue of the journal *Salamandra* dedicated to *Bsal*. The early development of a duplex method (Blooï et al. 2013), which allows to test for *Bd* and *Bsal* at once, helps prospective studies and has been widely adopted in the field. Most of the papers we found reporting tests for *Bsal* in the wild or in captivity also reported tests for *Bd*. The converse is not true, though; most of the papers that test for *Bd* do not conduct tests for *Bsal*. The number of studies testing for *Bd* in areas where caudates exist (e.g. the USA) is much larger than the number of studies we found testing for *Bsal* (Castro Monzón et al. 2020).

## **Conclusions**

The data presented in this study, regarding the locations and species that have been tested for *Bsal*, may help to plan future studies and protect vulnerable species. The 66 species in the list of known *Bsal* hosts should be carefully studied, and considerations should be taken to evaluate the risk that *Bsal* poses to these species. *Bsal*-related deaths have been reported in several threatened host species. This suggests a susceptibility in species that already face conservation challenges. Threatened species in Europe that are susceptible, such as *Calotriton arnoldi*, *Euproctus platycephalus*, *Lyciasalamandra helverseni* and

*Salamandra algira*, might be particularly vulnerable, as the pathogen has been found both in the field and in captivity in this region. Areas where threatened and susceptible European species are distributed should be constantly monitored. We assume that some species in Asia evolved with the pathogen and might be resistant, but the native range of *Bsal* is still unclear, and we found at least one report of *Bsal*-related deaths in Asian threatened species.

The data compiled here may also help to establish, evaluate and update measures to prevent the arrival of the pathogen in areas where it has not yet been detected. The USA, for example, implemented measures to restrict the import of a large number of amphibians on a list (50 CFR § 16.14). However, that list does not currently include 12 amphibian species of which we found infection reports. Of these species, six have been reported or are suspected to have colonized areas outside their native range in the past (Supplementary Materials Table 1). This is relevant for risk evaluation that species pose. In at least one case, *Bsal* spread has been associated with the presence of an invasive species (Martel et al. 2020). The list of infected species provided in this work may serve as a guiding tool for decision makers on which species are at risk.

Tests involving experimental exposure previously showed that some salamandrid and plethodontid species are susceptible. In this study, we showed that this susceptibility extends to a large number of species in those families, strongly suggesting the existence of a taxonomic pattern of susceptibility. The apparent existence of susceptibility in Plethodontidae highlights the risk that *Bsal* poses in North and Central America (Fig. 1b). Our study also highlights countries and amphibians from specific regions that need prospective studies, namely Mexico and Central America. Constant monitoring is key, as timely detection of infection is of utmost importance to protect vulnerable species. Extirpation of the pathogen becomes harder, if not impossible, once the pathogen has spread to a large area.

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## **General Discussion**

The overarching theme of this thesis is a struggle to process data to produce useful information. We face a challenge that requires to deal with a large volume of data that is often disorganized, incomplete, miscategorized or that presents different types of measurements. The thesis here presented tackled this challenge, firstly by examining one of the problems (crypticity) that we face when collecting and analyzing data. Then, data of two related pathogens was collected and analyzed. The information presented in the previous chapters has methodological, conceptual and practical implications for the study of *Bd* and *Bsal*. This work was not only presented here, it also resulted in three publications (Jarić et al., 2019; Castro Monzon et al., 2020; Castro Monzon et al., under review).

### **Crypticity, a problem to study pathogens and invasive species**

In the first chapter, problems that have led to the misidentification and incorrect evaluation of chytrid pathogens were discussed. These problems have long been recognized (Farrer et al., 2011; Bataille et al., 2013) although no framework had been established to present the mechanisms and causes of crypticity in amphibian chytrid pathogens and their hosts. More so, these problems had not been discussed in a context that included crypticity in host invasive species.

There is a disconnect between the field of epidemiology and invasion ecology. In the latter field, there is much discussion regarding what allows a species to move beyond its native range, to establish new populations, to spread and to impact native populations. This discussion rarely reaches the field of epidemiology. Conversely, pathogens often stay out of focus in the field of invasion ecology. Jeschke et al. (2013) discussed this apparent disconnect between the fields and argued that it is artificial and unproductive; both invasive species and their pathogens are tightly linked. Both ought to be studied together to fully account for the risk that they pose and to understand how they spread.

The discussion of how invasive species remain undetected or are misidentified is relevant in both invasion biology and epidemiology and bridges a gap between these fields. The data presented in the first chapter brings attention to the mechanisms underlying crypticity and, while doing so, generated a conceptual framework that is useful in the study of *Bd* and *Bsal*. Pathogens are only one part of the species addressed in the data but they are affected by several of the the mechanisms underlying

crypticity (chapter 1, table 1). In turn, several of the recommendations described to deal with crypticity also work to better identify and detect pathogens (chapter 1, table 1).

Measures, such as the promotion of citizen science, have proven useful in the past to detect signals of infection. For example, *Bsal* infection was detected in Germany by volunteers in an area that was not being monitored by any research project (Sandvoß et al., 2020) and there have been projects involving schools to detect *Bd* and *Bsal* (Wunder et al., 2012; Gimeno et al., 2015). Among other measures is the implementation of tests and surveys that account for the possible existence of cryptic species. Very specific molecular tests might not turn positives for divergent strains or related species. This has already been observed as tests that were designed to detect *Bd* do not return positives on *Bsal* infected individuals (Martel et al., 2013). In this way a pathogen can remain unidentified for a long period of time as its impact might not be immediately apparent (Jescke et al., 2013). The danger that new chytrid strains or species pose is one that looms over amphibians communities in the world. Now, more than twenty years after *Bd* was identified, the presence of a highly divergent amphibian chytrid pathogen was reported, although this is yet to be confirmed (Saare et al., 2021). It is not known if this represents a new species and, if so, how virulent it is. Regardless, for the future, it might be wise to follow another of the recommendations of the paper and casting a wide net (e.g. environmental DNA and metagenomics) to avoid false negatives that very specific tests might produce.

### ***Bd*, who is infected, where and when**

The information presented in the second chapter allowed to better understand how widespread and adaptable *Bd* is. The data helped to show a pathogen like no other in terms of the number of host species it has. A count of known host had been previously reported by Olson & Ronnenberg (2014). However, it increased from 695 to 1062 with the data presented in this work (also published as Castro Monzon et al., 2020). A few months after the publication of this data, it was used in a work that sought to complement it (Olson et al., 2021). This attests to the value and importance of the work presented in the second chapter.

The number of known host species is one of the most visible pieces of information presented, however, other pieces of information are equally valuable and interesting. Highlighted in the chapter were maps that showed the distribution of species that have been found with or without infection. These suggested the presence of areas suitable for the pathogen or the presence of refuges. The underlying factors that

affected infection were briefly discussed in the second chapter. It was shown that, in some cases, areas suitable for infection fitted predictions by models based on environmental factors (Murray et al., 2011), mostly humidity and temperature. This supports the value of the data but also raises questions regarding the presence of areas suitable for infection that do not fit previously known models.

The study here presented mostly addresses the questions of which species have been found with infection, where and when. The question of why the pathogen is observed in some areas is just tangentially explored, however, the groundwork for a study that addresses such a question was established. The species that have tested positive and areas of interest have already been identified and so have the studies that reported tests for *Bd*. A task that remains is that of identifying the support that different factors or conditions weight in the different studies. This also involves sorting specific and general hypothesis that have been proposed to explain why infection is observed in some locations.

An important goal of the work presented in the second chapter was to provide information that can help plan and prioritize future research and to help to better establish conservation plans. For that purpose, areas that have not been well studied or that have not been recently studied were highlighted. There was no emphasis in individual species as their numbers are quite large. However, the accompanying database allows those interested in certain taxon or region to retrieve data and identify when a species has been tested, whether or not they tested positive and which are the sources of the report.

Additionally, data was added that allows to filter by IUCN conservation status. In this way species that are already found in a precarious situation and that tested positive can be selected. This narrows the subject of interest, their population can then be studied by means of capture-recapture studies and the susceptibility can be tested in laboratory exposure experiments.

### **Bsal, who carries the pathogen and who is vulnerable**

In the third chapter a study that focused on *Batrachochytrium salamandrivorans* (*Bsal*) was presented. In many ways this is similar to the second chapter, which focuses on *Bd*, However, unlike *Bd*, *Bsal* is yet to spread all over the world. This affected the goals of the study and, consequently, the focus and impact this work sought to have. There was a greater emphasis on identifying susceptible, vulnerable and vector species. These tasks acquired importance and a sense of urgency now that recent reports suggest that the *Bsal* might be expanding its range and that it might affect amphibians in the Americas.

The list of known *Bsal* hosts is necessary for the establishment of effective controls and measures to prevent the spread of the pathogen. For this purpose, Baláž (2017) compiled a list of reports of infection up to 2016. However, since Baláž published his work a large number of new reports of infection have appeared. The work presented in the third chapter more than doubled the number of known hosts reported by Baláž.

Data collected included reports of *Bsal*-related deaths which provided a measurement of susceptibility and allowed to identify vulnerable species. This required moving beyond the work presented in the second chapter and collecting data from reports of infected amphibians in captivity and from exposure experiments. The final data was also supplemented and organized to highlight information of species such as their distribution, their IUCN conservation status and previous records that suggest that they are invasive. The study also pinpoints regions where few or no caudate amphibians have been studied in exposure experiments (e.g. Central America). The lack of exposure experiments in amphibians from these regions represent a knowledge gap that makes it difficult to evaluate the risk that *Bsal* poses there.

The study also shows how many monitoring studies have been undertaken in countries with caudate amphibians, while also pointing out the number of studied species, the amphibian diversity in the studied regions and the percent of threatened caudates in those regions. These indices are provided so that exploratory studies can be planned accordingly. The countries that have caudate species but where no monitoring studies have taken place have also been highlighted. Timely detection of infection is most important to protect and, in some cases, to contain the spread of infection.

## **General Conclusions**

This thesis worked over a consistent theme. The problem that large volume of disorganized information presents. It did so in within the fields of epidemiology, ecology and invasion biology. In the first chapter a conceptual framework that explained the underlying causes of crypticity was presented. The problem posed by crypticity was illustrated and a series of recommendations to lessen or take into account this problem were produced. In the second and third chapter the work that resulted in two databases was presented. These databases contained data from *Bd* and *Bsal* infection records. By compiling data, summary statistics could be produced and spatial and temporal patterns of infection became apparent.

The analysis of data revealed new and original information about the pathogens and their hosts; vulnerable species were identified as were gaps in our knowledge and areas that might need more research. The work presented in this thesis advanced our knowledge of pathogens, provided useful tools for their study, offered a database that has already been used by an independent work group, offered valuable information to plan new research and opened the door for further research projects.

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