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NOTE

Batrachochytrium dendrobatidis: requirement for further isolate collection and archiving

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ABSTRACT: The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) causes the disease chytridiomycosis, which is lethal to many species of amphibians worldwide. Many studies have investigated the epidemiology of chytridiomycosis in amphibian populations, but few have considered possible host–pathogen coevolution. More specifically, investigations focused on the evolution of *Bd,* and the link with *Bd* virulence, are needed. Such studies, which may be important for conservation management of amphibians, depend on access to *Bd* isolates. Here we provide a summary of known *Bd* isolates that have been collected and archived in various locations around the world. Of 257 *Bd* isolates, we found that 53% originate from ranids in the United States. In many cases, detailed information on isolate origin is unavailable, and it is unknown how many isolates are cryo-archived. We suggest the creation of a centralized database of isolate information, and we urge researchers and managers to isolate and archive *Bd* to facilitate future research on chytridiomycosis.

KEY WORDS: Amphibian declines · *Batrachochytrium dendrobatidis* · Chytridiomycosis · Pathogen preservation · Wildlife disease

INTRODUCTION

Chytridiomycosis is caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*; Berger et al. 1998, Longcore et al. 1999). In infected amphibians, *Bd* is found in the superficial layers of the epidermis and disrupts normal osmoregulatory functioning in the skin (Berger et al. 1998, Voyles et al. 2007, 2009a). Mass mortality events have coincided with the appearance of *Bd* in wild amphibian populations (Berger et al. 1998, Lips et al. 2006). The effect on some host species is extreme, leading to dramatic declines and possibly extinctions (Schloegel et al. 2006, Skerratt et al. 2007). Some populations that survive initial declines persist with various levels of infection (Retallick et al. 2004, Woodhams & Alford 2005), and *Bd* maintains at least moderate virulence in some species many years after introduction (Murray et al. 2009). These observations may be explained by factors such as variability in host resistance (Woodhams et al. 2007), host behavior (Rowley & Alford 2007), or environmental characteristics (James et al. 2009), but shifts in *Bd* virulence are also plausible.

Some evidence supports the possibility of differential virulence among *Bd* isolates. Laboratory experiments suggest that *Bd* virulence differs among isolates when introduced to a single susceptible amphibian species (Berger et al. 2005, Retallick & Miera 2007). In addition, phenotypic differences among isolates in proteomic signatures, morphological characteristics, and zoospore production (Fisher et al. 2009) could be associated with differences in virulence, although the mol-

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ecular basis for any differences in isolate virulence has not yet been identified. There seems to be relatively low genetic variability among isolates collected from globally widespread sources (Morehouse et al. 2003, James et al. 2009), but a recent study reported differential virulence among *Bd* strains that were either endemic to Japanese native amphibians or associated with introduced species (Goka et al. 2009). The possibility of differential virulence among distinct isolates highlights the requirement for ongoing surveillance, continued development of diagnostic assays for *Bd*, and further virulence research. However, advances in chytridiomycosis research will require access to *Bd* isolates. The methods for isolating and purifying *Bd* were first established in 1999 (Longcore et al. 1999). Some *Bd* isolates have been cryo-archived for future research (Boyle et al. 2003), and many are actively passaged under different nutrient and temperature conditions. Here we review available information on known *Bd* isolates.

MATERIALS AND METHODS

Isolate records were gathered from personal lists (L. Berger, James Cook University; A. Hyatt, CSIRO Australian Animal Health Laboratories; J. Longcore, University of Maine) and peer-reviewed papers (Morehouse et al. 2003, Berger et al. 2005, Morgan et al. 2007, Rosenblum et al. 2008, Symonds et al. 2008, Fisher et al. 2009, James et al. 2009). The database is comprised of information for 257 isolates, which probably represent a subset of all existing isolates (i.e. information on additional isolates may not be currently available in the published literature). We collected as much information as possible on isolate origin (host species, life stage, location, and disease status), isolate storage history (passage history, location, current storage conditions), and contact information for researchers working with isolates.

RESULTS

The majority of *Bd* isolates, approximately 53%, originate from ranids in the USA. However, a disproportionate number of originate from *Rana muscosa* (44 isolates) and *R. sierrae* (57 isolates) that were collected for a population genetics study in California (Morgan et al. 2007). Only 2 isolates come from caudates (Fig. 1). Most of the isolates (156) are from amphibian populations in the United States, which again is a reflection of the large number of isolates collected from *R. muscosa* and *R. sierrae* in California (Morgan et al. 2007).

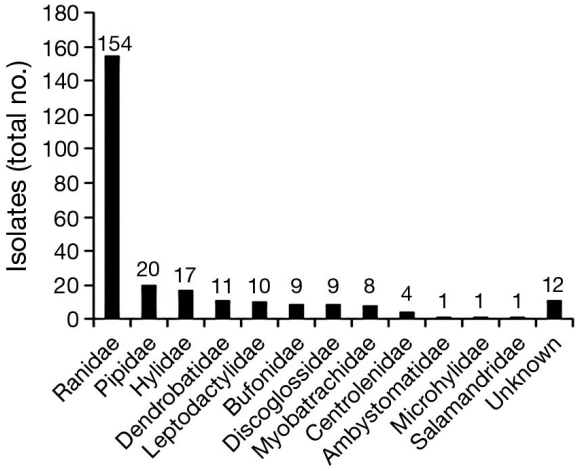

Fig. 1. *Batrachochytrium dendrobatidis*. Number of isolates per amphibian family collected and maintained in laboratory collections

Additional information on these isolates such as isolate origin (host species, date, life stage, location, disease status) and isolate storage history (passage history, location, current storage conditions) was collected whenever possible. Detailed information was difficult to obtain from the published literature, and in some cases no details were available. Of the identified isolates, little to no information was available about which isolates have been cryo-archived and are thus available for future research. Additionally, our analysis of available *Bd* isolates suggests that very few isolates are being collected and archived from important regions where *Bd*-associated amphibian declines have occurred, or may be currently taking place (e.g. Central America: Republic of Panama, Woodhams et al. 2008; Montserrat, G. Garcia pers. comm.; Southeast Asia: Indonesia, Kusrini et al. 2008; Philippines, R. Brown pers. comm.).

DISCUSSION

Confronting disease-related declines requires addressing a specific set of problems: identifying the etiological agent and possible point of origin, developing diagnostic assays and sampling protocols, and understanding the mechanisms of pathogenesis, transmission, and evolution of host–pathogen dynamics. Many of these challenges have been successfully addressed (e.g. Berger et al. 1998, Boyle et al. 2003, Hyatt et al. 2007, James et al. 2009, Voyles et al. 2009a), but others are still being investigated. To facilitate projects that will meet these challenges, we recommend that additional *Bd* isolates be collected and cryo-archived.

Table 1. Summary of *Batrachochytrium dendrobatidis* (*Bd*) isolates. Isolate records were collected from published literature and personal lists. Host species refers to the amphibian species from which *Bd* was originally isolated

Host	Total
Canada	**12**
Rana catesbeiana	12
USA	**156**
Ambystoma tigrinum	1
Bufo americanus	1
Bufo boreas	4
Bufo haematiticus	1
Dendrobates auratus	1
Dendrobates azureus	5
Dyscophus guineti	1
Hyla arenicolor	1
Litoria caerulea	1
Rana aurora draytonii	1
Rana catesbeiana	7
Rana clamitans	4
Rana muscosa	44
Rana palustris	1
Rana pipiens	9
Rana sierrae	57
Rana sylvatica	1
Rana yavapaiensis	12
Xenopus tropicalis	1
Unknown species	3
Panama	**25**
Atelopus zeteki	2
Bufo haematiticus	1
Centrolenella ilex	1
Cochranella euknemos	1
Colostethus inguinalis	3
Colostethus nubicola	1
Eleutherodactylus bufoniformis	1
Eleutherodactylus caryophyllaceus	2
Eleutherodactylus museosus	2
Eleutherodactylus podi-noblei	1
Eleutherodactylus talamancae	2
Hyla pameri	1
Phyllomedusa lemur	1
Smilisca phaeota	4
Smilisca sila	2

Host	Total
Brazil	**1**
Rana catesbeiana	1
Peru	**1**
Unknown species	1
Venezuela	**2**
Rana catesbeiana	2
South America (country unknown)	**3**
Cochranella albomaculata	1
Colostethus flotator	1
Hyalinobatrachium colymbiphylum	1
Puerto Rico	**1**
Eleutherodactylus coqui	1
Ghana	**3**
Xenopus tropicalis	3
South Africa	**7**
Afrana fuscigula	3
Xenopus laevis	4
Africa (country unknown)	**12**
Xenopus laevis	12
Japan	**1**
Ceratophrys cranwelli	1
Australia	**15**
Limnodynastes dumerilii	1
Limnodynastes peronii	1
Litoria booroolongensis	1
Litoria caerulea	2
Litoria lesueuri	1
Litoria peronii	1
Litoria rheocola	2
Mixophyes fasciolatus	3
Mixophyes fleayi	2
Nyctimystes dayi	1
Spain	**9**
Alytes muletensis	6
Alytes obstetricans	3
UK	**1**
Triturus vulgaris	1
Unknown country	**8**
Unknown species	8
GRAND TOTAL	**257**

Priority targets for *Bd* collection and cryo-preservation have been suggested (Voyles et al. 2009b), and recording detailed information on any additional isolates will be important for basic disease research.

Although protocols for isolating (Longcore et al. 1999) and cryo-archiving (Boyle et al. 2003) *Bd* are readily available, some of the required skills are not standard for medical microbiology labs. Obtaining isolates can be difficult, requiring some technical skills and persistence. A tutorial on basic techniques is available in multiple languages (see www.bdbank.org) and can be used in conjunction with the published literature, regional training courses, and consultation with a World Organization for Animal Health (OIE) reference laboratory for chytridiomycosis (e.g. the Australian Animal Health Laboratory, Geelong). The Bdbank website provides an online forum to facilitate virulence research on *Bd*. To that end, we hope to collate and standardize data on global isolates into a single database where information can be shared and accessed by chytridiomycosis researchers. Although isolate information can be cataloged on this website, there is, as yet, no formal arrangement for physical storage of isolates. Isolates can be archived at any laboratory, but for long-term storage and access, isolates should also be cryo-archived at the Australian Animal Health Laboratory, Geelong, Victoria.

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