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The North American bullfrog as a reservoir for the spread of *Batrachochytrium dendrobatidis* in Brazil

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Abstract

Global trade in the North American bullfrog *Rana catesbeiana* has been implicated in the introduction and spread of the amphibian pathogen *Batrachochytrium dendrobatidis*. Large-scale production of the North American bullfrog for human consumption has been ongoing since the early part of the 20th century, particularly in Brazil, a pioneer in bullfrog farming following its introduction in the 1930s. In order to determine whether bullfrogs introduced to Brazil and farmed for the food trade serve as reservoirs for amphibian disease, we tested individuals located in the Brazilian states of São Paulo and Pará to determine the prevalence of *B. dendrobatidis* infections. We were able to confirm *B. dendrobatidis* on five farms (78.5% infection prevalence overall) by PCR. Isolates were obtained from three of the five farms and genotyped at 17 loci using multilocus sequence typing. Five isolates from the Brazilian farms were genotypically similar to each other as well as to isolates from Central and South America. Isolates from farmed bullfrogs were more similar to isolates from introduced populations in Venezuela than those from the native range of the bullfrog in eastern North America. These results could have important implications for the origin and spread of *B. dendrobatidis* in Brazil, its neighboring regions and the conservation of native amphibian fauna. They may also suggest a potential for prior recombination within *B. dendrobatidis* in bullfrogs, which may have implications for the recent emergence of this pathogen.

Introduction

Researchers have speculated that the international trade in live North American bullfrogs *Rana catesbeiana*, among others, may have served to spread the amphibian fungal pathogen, *Batrachochytrium dendrobatidis*, into new geographic regions (Weldon *et al*., 2004; Fisher & Garner, 2007; Schloegel *et al*., 2009). Owing to its ability to withstand the onset of disease when infected with *B. dendrobatidis*, its expansion into new locales (including Europe, South America and Asia) and increasing positive detection of the fungal pathogen in introduced and captive populations throughout the world, the North American bullfrog appears to be a likely reservoir and vector for amphibian disease (Mazzoni *et al*., 2003; Daszak *et al*., 2004; Hanselmann *et al*., 2004; Garner *et al*., 2006; Schloegel *et al*., 2009).

Brazil was a pioneer in the commercialization and mass production of the North American bullfrog for human consumption. The bullfrog was chosen for cultivation because of its rapid growth, high fecundity and ability to adapt to a range of climatic regimes with relative ease. Three hundred bullfrog pairs were imported into Brazil from Canada in 1935 (Ferreira, Pimenta & Paiva Neto, 2002). A second importation of bullfrog pairs from North America occurred in the early 1970s (Mathias & Scott, 2004 report the importation of 60 pairs, while personal communications with the importer, Dr Luiz Dino Vizotto, indicate the importation of only 20 pairs). By 1988, 2000 frog farms

operated in Brazil. Over the following decade, the number of farms decreased to just 280 in 1997. Frog production, however, increased from 80 000 kg in 1988 to 270 000 kg in 1997 (Silva, 1997). This was due, in part, to advances in technical and scientific research, which enhanced the efficiency of frog breeding and cultivation.

The first reports of enigmatic amphibian declines in Brazil came from the Atlantic Rainforest in the south-east in the late 1970s and the early 1980s (Heyer *et al.*, 1988; Young *et al.*, 2001). Although a number of factors have been implicated in the cause of these declines (e.g. unusual climatic events, species invasions and UV-B radiation, among others), some researchers suggest disease as a likely contributor (Lips *et al.*, 1998; Young *et al.*, 2001). Studies have since detected *B. dendrobatidis* in various amphibian populations throughout South America (Mazzoni *et al.*, 2003; Hanselmann *et al.*, 2004; Toledo *et al.*, 2006). Analyses of patterns of spread of *B. dendrobatidis* in Latin America indicate that the pathogen was introduced relatively recently, with three proposed introduction events at different locales, including Ecuador, Venezuela and Brazil (Lips *et al.*, 2008). Introduced populations of the North American bullfrog can be found in each of these countries, but *B. dendrobatidis* infection of North American bullfrogs has only been confirmed in Venezuelan populations thus far (Hanselmann *et al.*, 2004). A previous survey for *B. dendrobatidis* in the North American bullfrog in Brazil failed to reveal any positive cases (Toledo *et al.*, 2006). The survey, however, involved only eight individuals (six farmed and two wild), and a larger sampling is required to confirm, with confidence, the presence or absence of *B. dendrobatidis* infection.

Our current study examines the potential for North American bullfrogs to serve as a conduit for the spread of *B. dendrobatidis* in Brazil. Samples from bullfrog farms in Brazil were assayed for the presence of *B. dendrobatidis* using molecular diagnostics. *Batrachochytrium dendrobatidis* was readily detected, and several axenic cultures of the fungus were isolated and compared with a global sample of isolates, including those from North American bullfrogs in the US, using a population genetic approach. The data presented shed light on the historical and current distribution of *B. dendrobatidis* in South America. Our findings have important implications for the management of the North American bullfrog in Brazil, with the intent of minimizing the risks associated with the introduction of amphibian disease to captive populations. An understanding of the persistence and spread of *B. dendrobatidis* in Latin America is crucial to the conservation of native amphibian fauna.

Methods

Sample collection

In June 2006, 288 adult *R. catesbeiana* were swabbed from four farms located within 90–120 miles north-east of São Paulo city, Brazil (Farms A, B, C and D) (Table 1). Each individual frog was swabbed along the ventral surface and on the webbing between the digits using Medical Wire and Equipment Company's MW100 dry swab. Toe clips were taken from a subset of individuals (40 in total; 10/farm) and placed in 70% ethanol. A fresh pair of gloves was used for each individual frog and equipment was sterilized with ethanol and subsequently flamed between specimens. The swabs were analyzed using quantitative PCR to detect *B. dendrobatidis* infection (Boyle *et al.*, 2004). Samples were run in triplicate and those that returned a low number of zoospore equivalents in only one or two wells were defined as indeterminate and were excluded from our calculations of infection prevalence. Toe clips were later fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for histological analysis of *B. dendrobatidis*. To standardize our observations, we examined 25 fields of view containing keratinized skin for each frog (using a $\times 40$ objective lens). We also received and swabbed a shipment of three live bullfrog tadpoles from a farm located in the Amazon region of Brazil in the state of Pará (Farm E) in September 2008. In January 2009, 40 additional *R. catesbeiana* specimens (15 tadpole and 25 adult) were swabbed on a repeat visit to Farm C. Farms A, C, D and E are all commercial farms, raising frogs for the food trade. Farm B supplies animals for research and for distribution to commercial farms located in Brazil.

Table 1 PCR detection of *Batrachochytrium dendrobatidis* from swabs of farmed, *Rana catesbeiana* in the states of São Paulo and Pará, Brazil

Date	Site	Life stage	*Batrachochytrium dendrobatidis* % infected by PCR (*n*)
June 06	Farm A	Adult	100% (86)
	Farm B	Adult	87% (30)
	Farm C	Adult	0% (100)
	Farm D	Adult	93% (82)
August 08	Farm E	Tadpole	100% (3)
January 09	Farm C	Tadpole	93% (15)
	Farm C	Adult	0% (25)

In August 2008, we revisited three of the four farms (Farms A, B and C) sampled in 2006 (the fourth, Farm D, no longer being in operation). We collected 13 tadpoles, with a bias for those with missing mouthparts, from our 2006 *B. dendrobatidis* positive farms (Farms A and B). We also observed missing mouthparts in Farm C tadpoles (previously shown to be free of *B. dendrobatidis* infection according to our 2006 data) and collected three individuals for further investigation. Tadpoles were bagged separately by site and transported live back to the laboratory. Three live, bullfrog tadpoles were shipped to São Paulo from Farm E (swabbed before dissection). An additional 15 tadpoles from Farm C were collected in January 2009 for isolation purposes.

Culture isolation of *B. dendrobatidis*

Tadpoles were euthanized in accordance with the procedures set forth by the Biological Institute, São Paulo, Brazil.

Animals were placed on ice for *c*. 10 min for desensitization before decapitation and pithing. Fresh tissue from the keratinized region of the tadpole mouthparts was then excised, placed on a slide with distilled water and cover slipped and examined under a microscope using a $\times 40$ objective lens to determine whether *B. dendrobatidis* zoosporangia were present (characterized by clusters of small spherical bodies with a cell wall, some with a visible septum). Fresh skin sections positive for infection were immediately processed for isolation following the protocol of Longcore (2000).

Population genetic analysis

We generated genotypes for eight new isolates and compared them with an existing global dataset (Table 2). The eight isolates were as follows: two isolates were from the Savannah River Site, SC, USA (SRS810 and SRS812; isolated from North American bullfrog tadpoles and grown in culture since August 2006), one isolate was from an introduced population of *R. catesbeiana* in the Andean region of Venezuela (JEL428) and five isolates were from captive bullfrogs isolated in Brazil during the current study (LMS902, LMS925, LMS929, LMS930 and LMS931). Genotyping of 17 nuclear loci by DNA sequencing was accomplished following the protocols in James *et al*. (2009). A network depicting genetic relatedness among individuals was calculated from the genotype data by statistical parsimony using the software TCS 1.21 (Clement, Posada & Crandall, 2000), after estimating genetic distances between isolates as described in James *et al*. (2009). A dendrogram of *R. catesbeiana* isolates and additional isolates from South and Central America was also reconstructed using UPGMA as implemented in PAUP v4.0b10 (Swofford, 2002). The significance of branches was assessed using 100 bootstrap pseudo-replicates.

Results

Identification of *B. dendrobatidis* in farmed Brazilian *R. catesbeiana*

Three of the four farms (Farms A, B and D) sampled in 2006 tested positive for *B. dendrobatidis* by PCR [86/86 (100%), 26/30 (86.7%) and 76/82 (92.7%), respectively] (Table 1). The number of swabs defined as indeterminate, and therefore not included in our total count of individuals, included 14 for Farm A, 20 for Farms B and 18 for Farm C. PCR analyses of the swabs from Farm E (2008) and Farm C (2009) revealed infection in 3/3 individuals and in 5/40 individuals, respectively (all positives were tadpoles). Histological sections of adult bullfrog toe clips revealed one small focal area of infection in 1/40 individuals. Microscopic examination of tadpole mouthparts from the 2008 and 2009 specimens revealed zoosporangia consistent with *B. dendrobatidis* in 1/5 tadpoles from Farm A, 8/8 tadpoles from Farm B, 17/18 tadpoles from Farm C and 3/3 from Farm E.

Culture isolation of *B.dendrobatidis*

We obtained six *B. dendrobatidis* isolates from six individual bullfrog tadpoles representing three farms in Brazil (Farms B, C and E). Two isolates were from Farm B (LMS902 and LMS925, September 2008), three from Farm C (LMS930, LMS931 and LMS932, January 2009) and one from Farm E (LMS929, September 2008). While both LMS902 and LMS925 were from Farm B, they were taken from different stocks and enclosures. All isolates from Farm C were from tadpoles that were a part of the same stock and enclosure. Owing to their common origin, we analyzed only two of the isolates from Farm C by DNA sequencing (LMS930 and LMS931).

Population genetics of *R. catesbeiana* isolates

Rana catesbeiana isolates comprise eight genotypes that roughly group into four sites in the phylogenetic network (Fig. 1). The isolates from Brazilian *R. catesbeiana* farms were of three similar genotypes that clustered with other neotropical *B. dendrobatidis* isolates, including the two isolates from an introduced *R. catesbeiana* population in Venezuela (Fig. 1). Farms C and E shared a genotype (LMS929, LMS930 and LMS931) that was identical to two isolates from Panama obtained in 2001 (PM-005, PM-007). The two isolates from Farm B (LMS901 and LMS925), taken from separate enclosures, each had unique genotypes that were distinct from those of Farms C and E. Isolates from the Savannah River Site (SRS810 and SRS812) were most similar to an *R. catesbeiana* isolate from Quebec (JEL262; Fig. 1).

One geographic pattern in the global population structure shows a statistically significant difference between isolates from continental North America and those from Central and South America (James *et al*., 2009). Three of the marker loci (BdC24, 9893X2 and R6046) have alleles that have only been found in isolates obtained from continental North America and Europe while all isolates from Brazilian farms lacked these alleles (Fig. 2).

Observed heterozygosity (per cent heterozygous loci) of the Brazilian farm isolates ranged from 47 to 82% compared with the global mean of 55% (James *et al*., 2009). Some of the *R. catesbeiana* isolates showed patterns indicative of loss of heterozygosity without meiotic recombination. For example, isolate LMS902 differed in genotype from Farm C isolates by loss of heterozygosity of three loci (8392X, mb-b13 and CTSYN1), but retained heterozygosity at the remaining 11 loci (Fig. 2).

In 2006, Farm C was a *B. dendrobatidis*-negative farm. When we returned in 2008 and 2009, however, infection was present (validated by microscopic examination and PCR). Our analyses showed that the Farm C isolates (LMS 930 and LMS 931) were identical to the Farm E isolate (LMS 929). We received verbal confirmation from Farm C that they imported 10 frogs from Farm E in June 2008, which were used as breeders during the January 2009 collections.

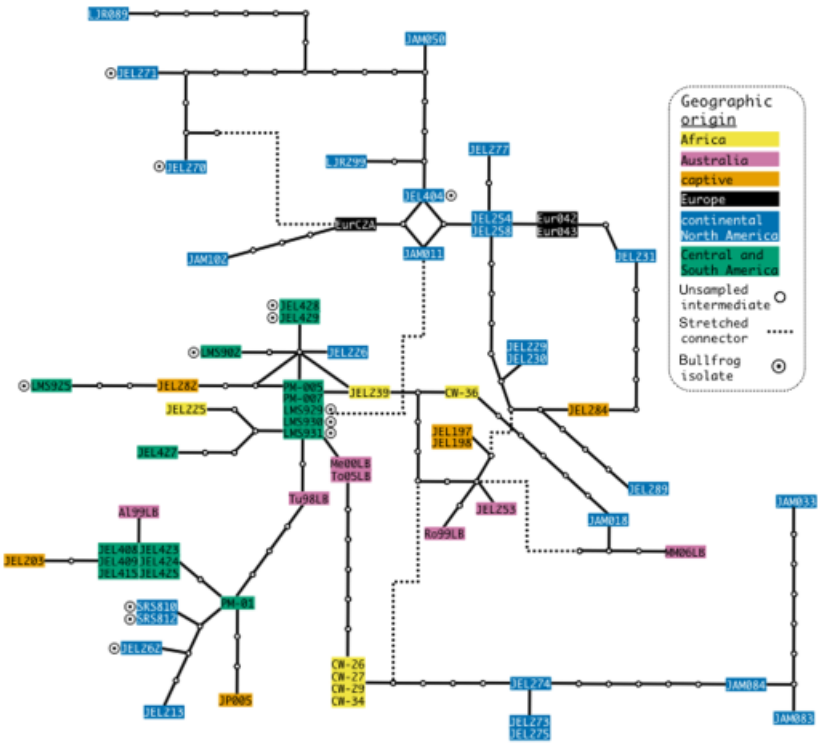

Figure 1 Phylogenetic network comparing Brazilian farm isolates with a global sample of *Batrachochytrium dendrobatidis* isolates (modified from James *et al.*, 2009). Isolates are colored according to geographic origin. Each line indicates a single mutational step, with small circles indicating intermediate genotypes not occurring in the observed data. Dashed lines also indicate a single mutational step.

They also reported that they imported 16 breeding, adult pairs and 20000 tadpoles from Farm B in August of 2007 and that they periodically re-introduce wild caught bullfrog tadpoles into their farm stocks (presumably established from escaped individuals). Additionally, tadpole enclosures on the farms often provide breeding pools for native amphibians (pers. obs.).

Discussion

Our study has shown that *B. dendrobatidis* is prevalent in farmed North American bullfrogs in two Brazilian states (São Paulo and Pará). No morbidity was observed as a result of infection. While a large proportion of the frogs on each farm were infected (78.5% mean prevalence for Farms A, B and D in 2006 and Farms C and E in 2008), histological examinations of a subset of animals revealed focal infection in only one individual. These data suggest that, when *B. dendrobatidis* is present on a farm, it occurs at a high prevalence but a low intensity, supporting earlier findings from captive and wild bullfrog populations (Mazzoni *et al.*, 2003; Hanselmann *et al.*, 2004; Schloegel *et al.*, 2009). It also supports the broader suggestion that bullfrogs are efficient carriers of this pathogen (Daszak *et al.*, 2004).

Analyses of the five isolates from captive Brazilian bullfrogs indicated that they group closely with each other and with isolates from other sites in Latin America, including Venezuela and Panama. Genotypes of isolates from Farms C and E (LMS930, LMS931 and LMS929) were identical to those of two Panamanian isolates (PM-005 and PM-007), isolated from native, wild populations. The striking similarity in captive bullfrog, wild bullfrog and wild, native frog isolates from Latin America suggests that there is, or has been, transmission among these populations and/or that the infections stem from a common source population.

According to James *et al.* (2009), the highest observed heterozygosity among the global isolates of *B. dendrobatidis* was found in isolates from *R. catesbeiana*. They proposed a model in which the fungal reproduction is largely clonal and genotypic variation results from loss of heterozygosity during mitosis. Under this model, the higher observed heterozygosity recorded among *R. catesbeiana* isolates could be indicative of a source population. For Brazilian farm isolates, the heterozygosity spanned a wide range, with isolates from Farms C and E possessing highly heterozygous genotypes (Fig. 2). The lower observed heterozygosity in Farm B could relate to the time the infection has been present at the farm, with loss of heterozygosity caused by mitotic recombination or perhaps rare sexual reproduction. Furthermore, the absence of alleles specific to North America and Europe in the Brazilian bullfrog isolates suggests that the current gene flow between these regions is limited or non-existent. A number of bull-

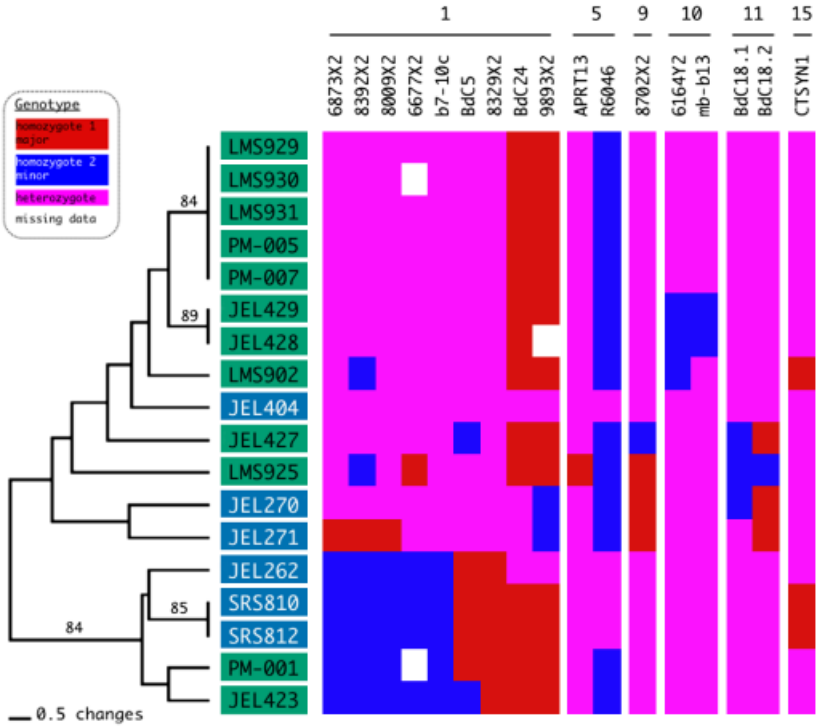

Figure 2 Genotype data for the North American bullfrog *Rana catesbeiana* isolates and related strains from Central and South America. The tree was constructed using UPGMA analysis of genotypic data, and numbers above nodes indicate bootstrap support using 100 pseudo-replicates. Isolates in green names are from Central or South America, and isolates with blue names are from continental North America. For each isolate, the genotype at each locus is indicated: purple for heterozygous genotypes, red for the higher frequency homozygous genotype, blue for the minority homozygous genotype and white for missing data. Majority and minority types are defined based on the previous global survey (James *et al.*, 2009). Locus names are shown above the genotypes and are grouped and ordered into physically linked blocks according to their location in the *B. dendrobatidis* genome (supercontig numbers shown above the locus names) from the assembly of strain JEL423 (http://www.broad.mit.edu/annotation/genome/batrachochytrium_dendrobatidis). For precise genomic locations, see James *et al.* (2009).

frog isolates collected at North American sites were also homozygous at one or more of these loci; therefore, it is not possible to deduce the direction of transfer of chytridiomycosis between the native range of *R. catesbeiana* and Brazil. The geographic specific alleles could have evolved after the bullfrogs were exported to Brazil, or the allelic diversity in the Brazilian isolates could be explained by a loss of heterozygosity resulting from a bottleneck event following introduction of *B. dendrobatidis* into these farms.

The pathogen *B. dendrobatidis* has been shown to be present in regions of Brazil where bullfrog populations are absent (Carnaval *et al.*, 2006), suggesting an alternate source for the fungus. A previous study (James *et al.*, 2009) found evidence of a genetic bottleneck in panglobal isolates of *B. dendrobatidis*, suggesting recent range expansions of the pathogen. Furthermore, Lips *et al.* (2008) proposed a possible scenario in which *B. dendrobatidis* was introduced into South America at isolated locales, and then radiated from these initial sites to nearby regions through riparian corridors. It is feasible that the bullfrog farms of Brazil could have served as a point of introduction from which the pathogen was able to spread. Alternatively, bullfrogs could have been infected by *B. dendrobatidis* from native Brazilian frogs. Additional data are needed to test these hypotheses adequately, including detailed analysis of historical data on patterns of bullfrog farming, sampling of archived specimens and sequencing of a larger, worldwide representation of isolates from wild and captive amphibians, including native frog populations near the Brazilian farms.

Anecdotal evidence suggests that there were two main importation events of the North American bullfrog into Brazil for the food trade. The earliest report of dramatic declines in native Brazilian frogs that fits the pattern of disease spread was in 1979 (Heyer *et al.*, 1988; Lips, 1998; Lips *et al.*, 2005), with the earliest known infection with *B. dendrobatidis* dating back to 1981 (Carnaval *et al.*, 2006). Given the proposed rate at which the fungus appears to spread through populations (282 km year^{-1} in South America; Lips *et al.*, 2008), frogs imported in the 1930s may have been free from infection, and it may not have been until the second importation (in the 1970s) that the fungus was introduced to Brazil (all farms in our study consist of frogs

from both importation events). This could be likely if the fungus did not originate in North America, but another continent (e.g. Africa as suggested by Weldon *et al.*, 2004). Finally, it is possible that the first shipment of animals did carry infection, but the strain was not pathogenic, or has been replaced by more virulent strains.

The tropical Latin American isolates group closely with a number of North American, African and Australian isolates. Various authors have discovered that recombination, and possibly sex, had occurred during the history of the current epidemic of *B. dendrobatidis* (Morgan *et al.*, 2007; James *et al.*, 2009). Recombination may be important for the pathogen as it adapts to local environments and host species, and recent studies of *B. dendrobatidis* indicate that various isolates exhibit differing degrees of pathogenicity (Berger *et al.*, 2005; Fisher *et al.*, 2009). With the dramatic escalation of globalization and trade in the last century, increased opportunities for hybridization between genetically divergent strains may have occurred, resulting in a highly virulent strain, which was later dispersed intercontinentally through the trade in live amphibians. These data are consistent with the high level of heterozygosity observed among *B. dendrobatidis* isolates, if heterozygosity is linked to virulence.

In our study, we found infection only in tadpoles (by PCR) in one farm (Farm C), which indicates that the fungus may have been introduced along with this stage, and the adults may not yet have been infected (accounting for the developmental time frame from a tadpole to a fully grown adult). Further sampling of different age groups and enclosures is required to verify this hypothesis. It is also possible that the tadpoles picked up the infection from native frogs entering the enclosures. Genotyping of farm isolates revealed that Farm C infections are identical to infections from Farm E. Farm E is a large farm in the city of Belém, Pará, and is known for distributing tadpoles to smaller farms throughout the country (pers. comm.). Farm C has indicated that they imported 10 frogs from Farm E that they use as breeders. It is therefore possible that the infection isolated from Farm C could have originated in Farm E. Given the varying sources from which Farm C stocks were obtained (i.e. Farm E, Farm B and wild sources), a possibly greater within-farm genetic variability might be observed if we isolated the fungus from individuals housed in another enclosure on the same farm (as was the case with Farm B). Given that isolates can vary in virulence, this mixing of populations could have profound consequences for the evolutionary future of the pathogen.

At present, the bullfrog aquaculture industry in Brazil has no mandated, or known, voluntary practices to minimize disease transmission among captive and/or wild populations. That *B. dendrobatidis* has been proven to be present in the North American bullfrog in Brazil should serve to increase awareness of the value of implementing bio-security measures to limit potential disease spread and preserve the health of native amphibians. However, large-scale eradication of this fungal disease on farms is unlikely to be logistically feasible at this time. Several treatments, such as heat and itraconazole baths, have been proposed to clear *B. dendrobatidis* infection from amphibians (Nichols & Lamirande, 2000; Woodhams, Alford & Marantelli, 2008). However, the effectiveness of these measures is arguable. At present, they are expensive, not applicable to large-scale populations and not proven safe for bullfrogs or for human consumption.

We propose that particular consideration should be given to the start-up of new farms. Enclosures should be built to prevent bullfrogs from escaping, but also to discourage native species from breeding in farm ponds, where they can introduce infection or acquire it and carry it to the wild. The origin and health of start-up stocks should also be taken into account. An investigation of die-offs of bullfrogs on a farm in Uruguay led to the discovery of *B. dendrobatidis* infection (Mazzoni *et al.*, 2003). Animals used to establish this farm were imported from Brazil and, considering the prevalence of *B. dendrobatidis* on Brazilian farms, it is plausible that the fungus was imported into Uruguay. Quarantine procedures, including testing and treatment of breeder adults that are not intended for consumption, could be used to produce disease-free stocks for new farm start-ups, thus preventing cross-farm contamination.

Water used by the farms in our study comes from nearby streams or rivers and is recycled back into these water bodies after undergoing filtration. Four of the five farms sampled (Farms A, B, C and D) purify the water with 2% chlorine 10ppm^{-1} held for 24 h, during which time the chlorine evaporates, before the water is released back into the environment. The fifth farm (Farm E) uses a system of sand filters to cleanse the water before expulsion. Studies indicate that a low concentration of chlorine in water is sufficient to kill *B. dendrobatidis*, therefore minimizing inadvertent spread through water wastes (Johnson *et al.*, 2003). Additional studies should be undertaken to examine the methods used by the farms, particularly the sand filtration system, to determine whether these methods are able to neutralize fungal contaminants.

We have demonstrated the presence of *B. dendrobatidis* in captive bullfrogs reared for the national and international food trades in Brazil. Because of the low power to resolve genetic differences among strains of *B. dendrobatidis*, we cannot conclude from our data whether the fungus was introduced to Brazil via the importation of the North American bullfrog, or whether infection on the farms originated from local sources. Additional data are required to identify the origin of *B. dendrobatidis* infections in both wild and captive populations. Based on our study, however, it does appear that bullfrog farms in Brazil can act as reservoirs for the persistence of the pathogen, allowing for spread between captive and wild populations. Researching the effects of trade on amphibian disease dynamics is vital towards increasing awareness of the risks associated with the national and international transport of infected animals. Trade and farming practices must be changed to minimize future, inadvertent introductions of *B. dendrobatidis*, and other

Table 2 Strains of *Batrachochytrium dendrobatidis* used for multilocus sequence analysis (modified from James *et al.*, 2009)

Strain	Geographic origin	Host
Al99LB	Alstonville, NSW, Australia	*Litoria caerulea*
CW-026	Namaqualand, South Africa	*Amietia fuscigula*
CW-027	Namaqualand, South Africa	*Am. fuscigula*
CW-029	Namaqualand, South Africa	*Xenopus laevis*
CW-034	Namaqualand, South Africa	*X. laevis*
CW-036	Port Elizabeth, South Africa	*Am. fuscigula*
EUR042	Pyrenees, Spain	*Alytes obstetricans*
EUR043	Pyrenees, Spain	*Al. obstetricans*
EURC2A	Sierra de Guadarrama, Spain	*Al. obstetricans*
JAM011	Mono Pass, CA, USA	*Rana muscosa*
JAM018	Mono Pass, CA, USA	*R. muscosa*
JAM033	Summit Meadow, CA, USA	*R. muscosa*
JAM050	Hitchcock Lakes, CA USA	*R. muscosa*
JAM083	Little Indian Valley, CA, USA	*R. muscosa*
JAM084	Little Indian Valley, CA, USA	*R. muscosa*
JAM102	Woods Lake, CA, USA	*R. muscosa*
JEL197	National Zoological Park, DC, USA	*Dendrobates azureus*
JEL198	National Zoological Park, DC, USA	*Dendrobates auratus*
JEL203	Bronx Zoo, New York, NY, USA	*Dyscophus guineti*
JEL213	Mono Co., CA, USA	*R. muscosa*
JEL225	Africa (from captive population in WI, USA)	*Silurana tropicalis*
JEL226	Yavapai Co., AZ, USA	*Rana yavapaiensis*
JEL229	Montrose Canyon, AZ, USA	*Hyla arenicolor*
JEL230	Montrose Canyon, AZ, USA	*R. yavapaiensis*
JEL231	Mesquite Wash, AZ, USA	*R. yavapaiensis*
JEL239	Ghana (imported)	*Si. tropicalis*
JEL253	Melbourne, Vic., Australia (captive)	*Limnodynastes dumerilii*
JEL254	Orono, ME, USA	*Rana pipiens*
JEL258	Orono, ME, USA	*Rana sylvatica*
JEL262	Quebec, Canada	*Rana catesbeiana*
JEL270	Point Reyes, CA, USA	*R. catesbeiana*
JEL271	Point Reyes, CA, USA	*R. catesbeiana*
JEL273	Clear Creek Co., CO, USA	*Bufo boreas*
JEL274	Clear Creek Co., CO, USA	*B. boreas*
JEL275	Clear Creek Co., CO, USA	*B. boreas*
JEL277	AZ, USA	*Ambystoma tigrinum*
JEL282	Toledo Zoo, OH, USA	*Bufo americana*
JEL284	WI, USA (captive)	*R. pipiens*
JEL289	Milford, ME, USA	*R. pipiens*
JEL404	Crocker Pond, Oxford County, ME, USA	*R. catesbeiana*
JEL408	El Cope, Panama	*Colostethus inguinalis*
JEL409	Silenciosa, Panama	*Eleutherodactylus talamancae*
JEL415	Between Loop and Silenciosa, Panama	*Eleutherodactylus podinoblei*
JEL423	Guabal, Panama	*Phyllomedusa lemur*
JEL424	Loop trail, Panama	*Cochranella euknemos*
JEL425	El Cope, Panama	*Bufo haematiticus*
JEL427	Puerto Rico	*Eleutherodactylus coqui*
JEL429	Venezuela	*R. catesbeiana*
JP005	Berkeley, CA, USA	*X. laevis*
LJR089	Laurel Creek, CA, USA	*R. muscosa*
LJR299	Point Reyes, CA, USA	*Rana aurora draytonii*
LMS 902	Pindamonhangaba, São Paulo, Brazil	*R. catesbeiana*
LMS 925	Pindamonhangaba, São Paulo, Brazil	*R. catesbeiana*
LMS 929	Belém, Pará, Brazil	*R. catesbeiana*
LMS 930	Tremembé, São Paulo, Brazil	*R. catesbeiana*
LMS 931	Tremembé, São Paulo, Brazil	*R. catesbeiana*
Me00LB	Melbourne, Vic., Australia (captive)	*Litoria lesueuri*
MM06LB	Mt Misery, Qld, Australia	*Litoria rheocola*

Table 2. Continued.

Strain	Geographic origin	Host
PM-01	Panama	*Eleuthodactylus caryophyllaceum*
PM-05	Panama	*Smilisca phaeota*
PM-07	Panama	*Sm. phaeota*
Ro99LB	Rockhampton, Qld, Australia	*L. caerulea*
SRS 810	Savannah River Site, SC, USA	*R. catesbeiana*
SRS 812	Savannah River Site, SC, USA	*R. catesbeiana*
To05LB	James Cook University, Qld, Australia (captive)	*L. caerulea*
Tu98LB	Tully, Qld, Australia	*Nyctimystes dayi*

Al99LB, Alstonville-Lcaerulea-99-LB-1; Me00LB, Melbourne-Llesueuri-00-LB-1; MM06LB, MtMisery-Lrheocola-06-LB-1; Ro99LB, Rockhampton-Lcaerulea-99-LB-1; To05LB, Townsville-Lcaerulea-05-LB-1; Tu98LB, Tully-Ndayi-98-LB-1.

pathogenic organisms, that could devastate amphibian populations worldwide.

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