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Batrachochytrium dendrobatidis prevalence and haplotypes in domestic and imported pet amphibians in Japan

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ABSTRACT: The international trade in amphibians is believed to have increased the spread of *Batrachochytrium dendrobatidis* (*Bd*), the fungal pathogen responsible for chytridiomycosis, which has caused a rapid decline in amphibian populations worldwide. We surveyed amphibians imported into Japan and those held in captivity for a long period or bred in Japan to clarify the *Bd* infection status. Samples were taken from 820 individuals of 109 amphibian species between 2008 and 2011 and were analyzed by a nested-PCR assay. *Bd* prevalence in imported amphibians was 10.3% (58/561), while it was 6.9% (18/259) in those in private collections and commercially bred amphibians in Japan. We identified the genotypes of this fungus using partial DNA sequences of the internal transcribed spacer (ITS) region. Sequencing of PCR products of all 76 *Bd*-positive samples revealed 11 haplotypes of the *Bd* ITS region. Haplotype A (DNA Data Bank of Japan accession number AB435211) was found in 90% (52/58) of imported amphibians. The results show that *Bd* is currently entering Japan via the international trade in exotic amphibians as pets, suggesting that the trade has indeed played a major role in the spread of *Bd*.

KEY WORDS: Chytrid · Amphibian disease · *Batrachochytrium dendrobatidis* · Chytridiomycosis · Pet trade · ITS · Japan

INTRODUCTION

As a consequence of economic globalization and the changes in transportation in recent years, goods are now being distributed rapidly on a massive scale over vast areas. This has been accompanied by an increase in the number of animals being traded internationally. Many wild animals, in particular reptiles and amphibians, are sold commercially as pets, for food, or for traditional medicines (Mazzoni et al. 2003, Schlaepfer et al. 2005, Fisher & Garner 2007, Garner et al. 2009, Smith et al. 2009). At the same time, emerging pathogens that threaten wild amphibian populations have been identified. The World Organisation for Animal Health (OIE) has listed *Batrachochytrium dendrobatidis* (*Bd*) and ranavirus as notifiable diseases under the Aquatic Animal Health Code and is working to control the spread of these pathogens (Schloegel et al. 2010, OIE 2012).

Bd, the fungal pathogen that causes chytridiomycosis, is responsible for a worldwide decline in amphibian populations. This pathogen has decimated amphibian populations in the tropical rain forests of Panama and Australia, and has been detected in wild

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and captive populations of amphibians in Africa, South America, Central America, North America, Europe, and Asia (Berger et al. 1998, 1999, Lips 1999, Mutschmann et al. 2000, Bosch et al. 2001, Weldon 2002, Bradley et al. 2002, Une et al. 2008).

There have been a number of reports on the origin and spread of *Bd*, and one hypothesis is that it originated in the African clawed frog *Xenopus laevis*. This frog was used in human pregnancy testing in the late 1930s and as a consequence it was exported from its native South Africa to countries around the world (Weldon et al. 2004). *Xenopus* spp. were further distributed for use in various fields of research (Weldon et al. 2007). The wide distribution of this species supports the hypothesis that it has caused the spread of *Bd*. Likewise, the cane toad *Bufo marinus* can asymptomatically harbor *Bd* infection (Berger et al. 1998, 1999), and the intentional introduction of this species as a biological pest control into different regions around the world is another likely cause of the spread of *Bd* (Daszak et al. 1999). Moreover, the American bullfrog *Lithobates catesbeianus*, commonly used for human consumption, is raised throughout the world and traded internationally. This species is not susceptible to *Bd* but is a carrier, and is now equal to the African clawed frog in terms of the spread of *Bd* (Mazzoni et al. 2003, Garner et al. 2006, Schloegel et al. 2009, 2012).

As well as these amphibians which have high-value uses in the fields of medicine and agriculture, the tiger salamander *Ambystoma tigrinum*, which is used as live bait for fishing, may also have contributed to the spread of *Bd* and ranavirus (Picco & Collins 2008, Picco et al. 2010). In addition, many other amphibians are traded around the world as pets and for zoological and aquarium collections (Schlaepfer et al. 2005, Andreone et al. 2006, Forzán et al. 2008, Nijman & Shepherd 2011, Spitzen-van der Sluijs et al. 2011).

This international trade in amphibians has facilitated the spread of *Bd*, providing new habitats and hosts for *Bd*, and is the major cause of the pandemic (Daszak et al. 2000, Skerratt et al. 2007, Kriger & Hero 2009). The first outbreak of *Bd* in Asia occurred in 2006 in Japan, where it caused a significant number of exotic pet amphibian deaths (Une et al. 2008). At that time the distribution pathways in Japan, from breeding facilities to the homes of hobbyists, allowed for disease spread and many epidemics were seen (Y. Une et al. unpubl. data).

Japan is home to 66 native species of amphibians (39 anurans and 27 urodeles), 60 of which are endemic to Japan. Of these, 42 species (67%) are listed as endangered and near-threatened on the Red List compiled by the Ministry of the Environment Government of Japan (2012). There is therefore a need to assess the effect on Japan's native fauna of pathogens that are unintentionally introduced by imported amphibians.

Bd has a broad host range, is highly infectious, and has a high case fatality rate. *Bd* zoospores in infected water can cause the infection to spread rapidly over a wide area (Johnson & Speare 2003). Animals that have been artificially raised are sometimes abandoned as unwanted pets (Karesh et al. 2005, Holsbeek et al. 2008). Consequently, any international amphibian trade is potentially linked to the spread of *Bd* and should be considered a significant risk.

We therefore surveyed imported and captive exotic amphibians in Japan in order to understand the prevalence of *Bd* and its haplotypes with the aim of determining the extent to which the international trade has exacerbated the spread of *Bd*.

MATERIALS AND METHODS

Sample collection

Samples were obtained from 820 adult individuals of 109 amphibian species that had been imported or raised and sold as pets during the 4 yr period from 2008 to 2011. Of these, 561 individuals of 93 species were imported and 259 individuals of 47 species were captive amphibians.

Amphibians were imported by 4 animal import companies (2 in Tokyo, 1 in Osaka, and 1 in Saitama) from 21 countries. Swab samples for *Bd* detection were either taken immediately or within 7 d of arrival in Japan. The species, country of export, date of import, and date of swab sample were all recorded.

Captive amphibians which had been kept for a long period of time were either from private household collections and were examined at veterinary clinics or had been bred in Japan as pets.

The swab samples for *Bd* detection were collected with great care to avoid contamination. Amphibians were sampled individually, using fresh rubber gloves for each animal, by swabbing the animal's ventral surface, inner thigh, and toe pads with cotton swabs (Men-tip 1P1501, Nihon-Menbo). A nested-PCR assay was used to detect *Bd* infection. All samples were treated according to the same protocol, and were kept in microtubes at −28°C until DNA analysis. PCR assays were performed within 15 d of sampling.

DNA extraction and nested-PCR assay

Each swab was placed in a microtube containing 200 µl of lysis buffer (1 mg ml^{-1} Proteinase K, 0.01 M NaCl, 0.1 M EDTA, 0.01 M Tris-HCl pH 8.0, 0.5% Nonidet P-40). The microtube was then vortexed at 15°C for 1 min. After removing the swab, the tube containing the extract was incubated at 50°C for 120 min and then at 95°C for 20 min. After incubation, the extract was diluted to 10% of its original concentration in TE buffer (0.001 M EDTA, 0.01 M Tris-HCl, pH 8.0) and used as the DNA template in the PCR assay.

We followed the method of Goka et al. (2009) for the nested-PCR assay. The forward primer for the first amplification was *Bd*18SF1 (5′-TTT GTA CAC ACC GCC CGT CGC-3′), which is located at the end of the fungal 18S rRNA gene, registered in the DNA Data Bank of Japan (accession number AF164302). The reverse primer was *Bd*28SR1 (5′-ATA TGC TTA AGT TCA GCG GG-3′), which is located at the start of the fungal 28S rRNA gene (accession number AY546693). In the second-round amplification, PCR products were amplified using the 2 primers *Bd*1a (5′-CAG TGT GCC ATA TGT CAC G-3′) and *Bd*2a (5′-CAT GGT TCA TAT CTG TCC AG-3′), which amplify fragments from the *Bd*-specific internal transcribed spacer 1 (ITS1) region and fragments up to the *Bd*-specific ITS2 region (Annis et al. 2004).

PCR assays were prepared with 2 µl of template DNA in a total reaction volume of 50 µl as described by Goka et al. (2001). The PCR mix contained 0.2 mM of each dNTP, 2 mM $MgCl_2$, 1.25 units of AmpliTaq Gold DNA polymerase, and 0.5 mM of each primer. All PCR reagents were purchased from Perkin-Elmer Applied Biosystems. The conditions for the first round of amplification were an initial denaturation for 9 min at 95°C; 30 cycles of 30 s at 94°C, 30 s at 50°C, and 2 min at 72°C; and a final extension for 7 min at 72°C. The conditions for the second amplification were an initial denaturation for 9 min at 95°C; 30 cycles of 30 s at 94°C, 30 s at 65°C, and 30 s at 72°C; and a final extension for 7 min at 72°C. Each sample was tested in duplicate. For each assay, we included a positive control using DNA extracted from a swab taken from *Ceratophrys ornata* in our previous study (Une et al. 2008) and a negative control using TE buffer without any template DNA. PCR products were separated on 6% polyacrylamide gels, and DNA fragment bands were made visible by ethidium bromide staining under UV light. Second-round amplification products were subcloned into a vector plasmid using a pT7 Blue Perfectly Blunt Cloning Kit (Novagen, EMD Bioscience) and transformed into *Escherichia coli* in accordance with the manufacturer's protocol. Three positive cloned fragments for each nested PCR product were sequenced using an ABI3730 Sequencer (Applied Biosystems) and T7 promoter and U19 reverse primers.

RESULTS

In total, 76 samples (9.3%) from 820 individual amphibians were identified as *Bd*-positive (Table 1). The *Bd*-positive samples were as follows: Anura, 8.9% (64/722 individuals); Caudata, 12.5% (12/96); Gymnophiona, 0% (none detected; Table 2). *Bd* was detected in 27 amphibian species in 13 families, as follows: Anura, 22 species in 8 families (Pipidae, Bufonidae, Leptodactylidae, Dendrobatidae, Hylidae, Hyperoliidae, Rhacophoridae, Microhylidae); and Caudata, 6 species in 5 families (Sirenidae, Proteidae, Ambystomatidae, Plethodontidae, Salamandridae). The prevalence was 100% in the families Sirenidae (2/2), Proteidae (1/1), and Plethodontidae (4/4) and was also high in the families Ambystomatidae, 28.6% (4/14); Rhacophoridae, 26.7% (31/116); and Pipidae, 20.6% (7/34). By origin, 10.3% (56/561) of imported amphibians and 6.9% (18/259) of captive amphibians were *Bd*-positive. *Bd* was detected in 9 of the 21 countries of origin: in order of prevalence, Bulgaria, Thailand, Peru, USA, Vietnam, Japan, Madagascar, Tanzania, and Germany (Table 2). Differences in infection prevalence among countries of origin could not be tested for statistical significance because the number of species and individuals differed greatly due to the opportunistic nature of the sampling regime.

The *Bd*-positive species originating from Germany were dendrobatid frogs and the Iberian ribbed newt *Pleurodeles waltl*, both of which were captive bred. Those originating from the other 7 countries were all wild-caught specimens. Those of Japanese origin were domestically bred exotic amphibians from the subfamily Ceratophryinae and *Xenopus laevis*.

PCR products from 76 positive samples obtained from 28 amphibian species were sequenced and *Bd* haplotypes were identified using the method described by Goka et al. (2009). The 11 haplotypes with their DNA Data Bank of Japan accession numbers were Haplotypes A (AB435211), C (AB435213), E (AB435214), L (AB435222), Q (AB435226), V (AB435231), Bd28 (AB723964), Bd29 (AB723965), Bd38 (AB723974), Bd41 (AB723977), and Bd43 (AB723979) (Table 3).

The *Bd* haplotypes and countries of origin were as follows: Bulgaria, Germany, Peru, and Tanzania

Table 1. Amphibian species held in captivity and from the international pet trade sampled for *Batrachochytrium dendrobatidis* (*Bd*) in Japan from 2008 to 2011. **Boldface** indicates species for which *Bd*-positive individuals were found

Order Family Species	n	*Bd* +ve	Origin (n or **+ve/n**)
ANURA			
Bufonidae			
Bufo alvarius	**1**	**1**	**USA (1/1)**
Bufo debilis	2	0	USA (2)
Bufo japonicus formosus	11	0	Japan (11)
Bufo terrestris	1	0	USA (1)
Bufo torrenticola	1	0	Japan (1)
Bufo viridis	5	0	Ukraine (5)
Dendrobatidae			
Dendrobates auratus	33	0	Canada (15), Germany (16), Japan (2)
Dendrobates azureus	21	0	Denmark (1), Germany (20),
Dendrobates benedicta	**1**	**1**	**Germany (1/1)**
Dendrobates fantasticus	7	0	Germany (7)
Dendrobates favovittatus	1	0	Germany (1)
Dendrobates imitator	**6**	**1**	**Germany (1/6)**
Dendrobates lamasi	5	0	Germany (5)
Dendrobates leucomelas	9	0	Germany (9)
Dendrobates lugubris	2	0	Germany (2)
Dendrobates mysteriosus	1	0	Germany (1)
Dendrobates pumilio	5	0	Germany (5)
Dendrobates reticulatus	**3**	**1**	**Germany (1/3)**
Dendrobates tinctorius	14	0	Germany (14)
Dendrobates truncatus	1	0	Germany (1)
Dendrobates uacari	1	0	Germany (1)
Dendrobates vanzolini	**6**	**1**	**Germany (1/6)**
Dendrobates variavilis	4	0	Germany (4)
Dendrobates ventrimaculatus	5	0	Germany (5)
Epipedobates tricolor	3	0	Germany (3)
Hyloxalus azureiventris	4	0	Germany (4)
Phyllobates bicolor	2	0	Germany (2)
Phyllobates lugubris	1	0	Germany (1)
Phyllobates terribilis	6	0	Germany (6)
Discoglossidae			
Bombina orientalis	2	0	China (2)
Hylidae			
Agalychnis callidryas	15	0	Germany (3), Nicaragua (8), USA (4)
Hyla arborea	**5**	**4**	**Bulgaria (4/5)**
Hyla cinerea	4	0	USA (4)
Hyla crepitans	4	0	Suriname (4)
Hyla gratiosa	2	0	USA (2)
Hyla japonica	6	0	Japan (6)
Hyla leucophyllata	1	0	Japan (1)
Hyla vasta	6	0	Haiti (6)
Litoria caerulea	52	0	Indonesia (52)
Litoria infrafrenata	15	0	Indonesia (15)
Pachymedusa dacnicolor	4	0	USA (4)
Phrynohyas resinifictrix	**9**	**1**	**Germany (1/7)**, Japan (2)
Phyllomedusa bicolor	4	0	Suriname (4)
Phyllomedusa sauvagii	18	0	Paraguay (11), USA (7)
Hyperoliidae			
Afrixalus fornasinii	1	0	Tanzania (1)
Hyperolius argus	2	0	Tanzania (2)
Leptopelis uluguruensis	**41**	**3**	**Tanzania (3/41)**
Leptopelis vermiculatus	14	0	Tanzania (14)
Leptodactylidae			
Ceratophrys cornuta	**8**	**1**	**Japan (1/1)**, Suriname (7)
Ceratophrys cranwelli	**48**	**3**	Germany (3), **Japan (3/44)**, USA (1)
Ceratophrys ornata	**70**	**3**	**Japan (3/69)**, USA (1)
***Ceratophrys* sp.**	**11**	**1**	**Japan (1/11)**
Chacophrys pierotti	**7**	**1**	**Japan (1/7)**
Lepidobatrachus laevis	**10**	**2**	Canada (2), **Japan (2/8)**
Odontophrynus sp.	3	0	Paraguay (3)
***Telmatobius* sp.**	**1**	**1**	**Peru (1/1)**
Mantellidae			
Mantella aurantiaca	1	0	Japan (1)
Mantidactylus pulcher	1	0	Madagascar (1)
Microhylidae			
Breviceps adspersus	4	0	South Africa (1), Tanzania (3)
Calluella guttulata	2	0	Thailand (2)
Dyscophus guineti	7	0	Germany (1), Madagascar (6)
Dyscophus insularis	2	0	Madagascar (2)
Kaloula pulchra	1	0	Thailand (1)
Phrynomantis bifasciatus	2	0	South Africa (2)
Phrynomantis microps	2	0	South Africa (2)
Plethodontohyla tuberata	2	0	Madagascar (2)
Scaphiophryne boribory	**3**	**1**	**Madagascar (1/3)**
Scaphiophryne gottlebei	2	0	Madagascar (2)
Myobatrachidae			
Limnodynastes salmini	1	0	Germany (1)
Pelobatidae			
Brachytarsophrys calinensis	1	0	China (1)
Megophrys nasuta	1	0	Malaysia (1)
Scaphiopus couchii	2	0	USA (2)
Pipidae			
Hymenochirus boettgeri	2	0	Indonesia (2)
Pipa pipa	6	0	Suriname (6)
Xenopus laevis	**26**	**7**	**Japan (7/26)**
Ranidae			
Occidozyga lima	10	0	Indonesia (10)
Pyxicephalus adspersus	1	0	Tanzania (1)
Pyxicephalus edulis	2	0	Tanzania (2)
Rhacophoridae			
Kurixalus verrucosus	**3**	**1**	**Vietnam (1/3)**
Nyctixalus pictus	**12**	**7**	**Thailand (7/12)**
Polypedates otilophus	30	0	Malaysia (30)
Rhacophorus arboreus	24	0	Japan (24)
Rhacophorus maximus	3	0	Vietnam (3)

Table 1 (continued)

Order Family Species	n	*Bd* +ve	Origin (n or **+ve/n**)
Rhacophorus reinwardti	3	0	Thailand (3)
Rhacophorus schlegelii	2	0	Japan (2)
Rhacophorus viridis amamiensis	1	0	Japan (1)
Theloderma asperum	**14**	**10**	**Thailand (10/14)**
Theloderma bicolor	**14**	**13**	**Thailand (13/14)**
Theloderma corticale	8	0	Thailand (8)
Theloderma horridum	2	0	Thailand (2)
CAUDATA			
Ambystomatidae			
Ambystoma mexicanum	10	0	Japan (10)
Ambystoma opacum	**6**	**3**	**USA (3/6)**
Ambystoma tigrinum	**4**	**1**	**USA (1/4)**
Amphiumidae			
Amphiuma tridactylum	3	0	USA (3)
Hynobiidae			
Hynobius kimurae	4	0	Japan (4)
Hynobius tokyoensis	1	0	Japan (1)
Plethodontidae			
Desmognathus fuscus	**4**	**4**	**USA (4/4)**
Proteidae			
Necturus maculosus	**1**	**1**	**USA (1/1)**
Salamandridae			
Cynops ensicauda popei	15	0	Japan (15)
Cynops orientalis	5	0	China (5)
Cynops pyrrhogaster	14	0	Japan (14)
Paramesotriton chinensis	5	0	China (5)
Pleurodeles waltl	**1**	**1**	**Germany (1/1)**
Salamandra salamandra	18	0	Ukraine (18)
Tylototriton kweichowensis	2	0	China (2)
Tylototriton shanjing	1	0	China (1)
Sirenidae			
Siren lacertina	**2**	**2**	**USA (2/2)**
GYMNOPHIONA			
Caeciliidae			
Potomotyphlus kaupii	1	0	Peru (1)
Typhlonectidae			
Typhlonectes sp.	1	0	Columbia (1)
TOTAL	820	76	

were Haplotype A; Madagascar was Bd43; Vietnam was Bd41; 2 haplotypes (A and Bd29) were detected from the USA; and 4 haplotypes (A, E, L, and Bd38) were detected from Thailand (Table 3). Haplotype A accounted for 90% (52/58) of positive samples imported from overseas (Fig. 1).

Five *Bd* haplotypes (A, C, Q, V, and Bd28) were detected in Japanese captive amphibians. Haplotype C, which was not found in amphibians from other countries, accounted for 44% (8/18) of infected individuals from Japan. In addition, the relative proportion of haplotypes detected in Japan also differed markedly from that observed in other countries (Fig. 1).

Table 2. *Batrachochytrium dendrobatidis* (*Bd*) prevalence by family and country of origin

Criteria	n	*Bd* +ve	*Bd* prev. (%) [95% CI]
Anura			
Bufonidae	21	1	4.8 [1.2–24]
Dendrobatidae	141	4	2.8 [0.8–7]
Discoglossidae	2	0	0 [0–84]
Hylidae	145	5	3.4 [0.1–8]
Hyperoliidae	58	3	5.2 [1.1–1.4]
Leptodactylidae	158	12	7.6 [3–10]
Mantellidae	2	0	0 [0–84]
Microhylidae	27	1	3.7 [0.1–19]
Myobatrachidae	1	0	0 [0–98]
Pelobatidae	4	0	0 [0–60]
Pipidae	34	7	20.6 [0.9–38]
Ranidae	13	0	0 [0–25]
Rhacophoridae	116	31	26.7 [19–36]
Caudata			
Ambystomatidae	20	4	20 [5.7–44]
Amphiumidae	3	0	0 [0–70]
Hynobiidae	5	0	0 [0–52]
Plethodontidae	4	4	100 [10–100]
Proteidae	1	1	100 [2.5–100]
Salamandridae	61	1	1.6 [0.4–11]
Sirenidae	2	2	100 [16–100]
Gymnophiona			
Caeciliidae	1	0	0 [0–98]
Typhlonectidae	1	0	0 [0–98]
Country of origin			
Bulgaria	5	4	80 [28–99]
Canada	17	0	0 [0–20]
China	16	0	0 [0–21]
Colombia	1	0	0 [0–98]
Denmark	1	0	0 [0–98]
Germany	139	6	4.3 [1.6–9.2]
Haiti	6	0	0 [0–46]
Indonesia	79	0	0 [0–5]
Japan	261	18	6.9 [4.6–11]
Madagascar	16	1	6.3 [1.6–30]
Malaysia	31	0	0 [0–11]
Nicaragua	8	0	0 [0–37]
Paraguay	14	0	0 [0–23]
Peru	2	1	50 [1.3–99]
South Africa	5	0	0 [0–52]
Suriname	21	0	0 [0–16]
Tanzania	64	3	4.7 [1–13]
Thailand	56	30	53.6 [40–67]
Ukraine	23	0	0 [0–15]
USA	49	12	24.5 [13–39]
Vietnam	6	1	16.7 [0.4–64]

Clinical signs of chytridiomycosis such as excessive shedding of skin, listlessness, and constricted pupils were found in horned frogs (*Ceratophrys* spp.) and Budgett's frogs *Lepidobatrachus laevis* kept in captivity in Japan. These amphibians were infected with Haplotype A. Among imported wild-caught amphibians, excessive shedding of skin was seen in a water

Table 3. *Batrachochytrium dendrobatidis* (*Bd*)-positive amphibians in captivity and from the international pet trade sampled in Japan between 2008 and 2011. WC: wild caught; CB: captive bred

Origin	Species	CB/ WC	*Bd* haplotypes (n +ve)
Bulgaria	*Hyla arborea*	WC	A (4)
Germany	*Dendrobates benedicta*	CB	A (1)
	Dendrobates imitator	CB	A (1)
	Dendrobates reticulatus	CB	A (1)
	Dendrobates vanzolini	CB	A (1)
	Phrynohyas resinifictrix	CB	A (1)
	Pleurodeles waltl	CB	A (1)
Japan	*Ceratophrys cornuta*	CB	C (1)
	Ceratophrys cranwelli	CB	A (1), C (1), Bd28 (1)
	Ceratophrys ornata	CB	A (1), C (2)
	Ceratophrys sp.	CB	C (1)
	Chacophrys pierotti	CB	C (1)
	Lepidobatrachus laevis	CB	A (1), C (1)
	Xenopus laevis	CB	A (2), C (1), Q (1), V (3)
Madagascar	*Scaphiophryne boribory*	WC	Bd 43 (1)
Peru	*Telmatobius* sp.	WC	A (1)
Tanzania	*Leptopelis uluguruensis*	WC	A (3)
Thailand	*Nyctixalus pictus*	WC	A (7)
	Theloderma asperum	WC	A (9), E (1)
	Theloderma bicolor	WC	A (11), L (1), Bd38(1)
USA	*Ambystoma opacum*	WC	A (2), Bd29 (1)
	Ambystoma tigrinum	WC	A (1)
	Bufo alvarius	WC	A (1)
	Desmognathus fuscus	WC	A (4)
	Necturus maculosus	WC	A (1)
	Siren lacertina	WC	A (2)
Vietnam	*Kurixalus verrucosus*	WC	Bd41 (1)

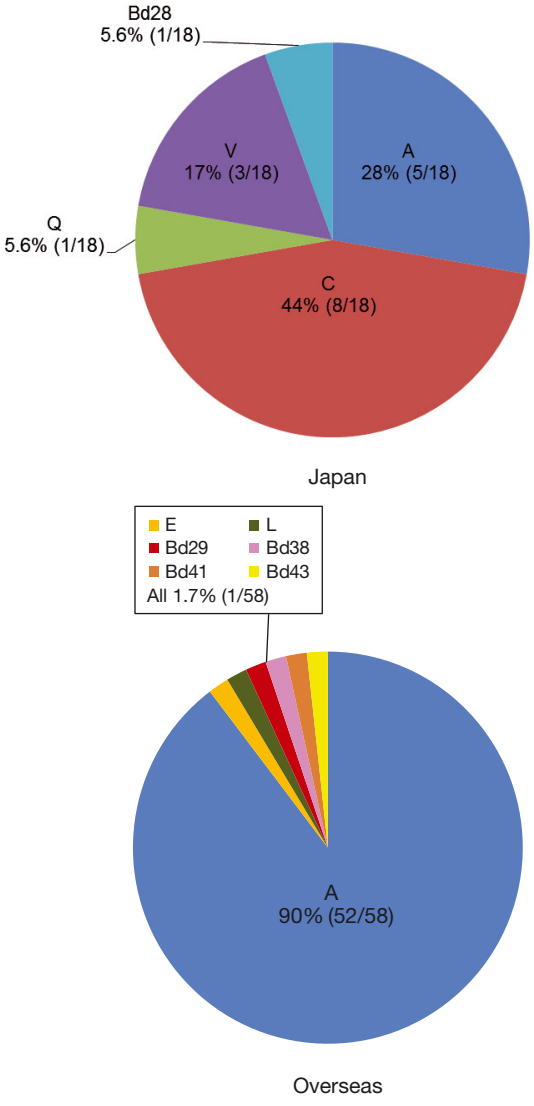

Fig. 1. *Batrachochytrium dendrobatidis*. Percentages of each fungal haplotype detected in amphibians in captivity in Japan contrasted with amphibians imported from Bulgaria, Germany, Madagascar, Peru, Tanzania, Vietnam, Thailand, and the USA

toad (*Telmatobius* sp.) originating from Peru infected with Haplotype A. All other *Bd*-positive individuals of other species appeared to be healthy and showed no signs of chytridiomycosis.

DISCUSSION

The trade of animals including wildlife has aided in pathogen dispersal and has frequently been the cause of pandemics such as SARS, H5N1 avian influenza, and chytridiomycosis (Karesh et al. 2005, Skerratt et al. 2007). There are many potential reasons for the decline in amphibian populations, but in its Global Amphibian Assessment, the International Union for Conservation of Nature (IUCN) cited infectious disease as a factor related to the extinction of amphibians (IUCN 2004).

We found that 10.3% (58/561) of exotic amphibians imported as pets into Japan were infected with *Bd*, which suggests that *Bd* has entered Japan through this route. In a similar study conducted in Hong Kong, which has a flourishing trade in amphibians, *Bd* was not detected (Rowley et al. 2007). Also, studies that surveyed pet fairs in the Netherlands (Spitzen-van der Sluijs et al. 2011) and the pet trade in the UK (Peel et al. 2012) reported low prevalence of *Bd* infection: 2.9% (26/897) and 3.2% (4/109), respectively. Possible reasons for the differences in detection rates include the fact that most amphibians imported into Japan are wild-caught specimens, which are highly susceptible

to stresses including the long transport time from Europe and the Americas, the difference in the climates of Japan and Southeast Asia, and the high sensitivity of the nested-PCR used in the present study.

Bd infects both anurans and caudates and has a high prevalence in Japanese giant salamanders *Andrias japonicus* and sword-tail newts *Cynops ensicauda* in Japan (Goka et al. 2009). The prevalence was also higher in caudates than anurans in our study. In particular, 11 of the 12 *Bd*-positive samples from the USA were from caudates. However, a relatively low *Bd* prevalence has been reported in wild caudates in the USA (Gaertner et al. 2009, Keitzer et al. 2011, Chatfield et al. 2012). Also, *Bd* prevalence among wild-caught amphibians imported from Asian countries (Indonesia, Malaysia, Vietnam, China, and Thailand) was 15.9% (31/189), which is much higher than the prevalence estimated from previous field studies in these areas (Kusrini et al. 2008, McLeod et al. 2008, Bai et al. 2010, Savage et al. 2011, Swei et al. 2011, Bataille et al. 2013). Furthermore, the *Bd* prevalence among *Xenopus laevis* bred in captivity in Japan was 26.9% (7/26), whereas the prevalence among wild *X. laevis* in South Africa was 2.7% (1.7–4.2%, 95% CI) (Weldon et al. 2004).

Bd prevalence thus differs between wild specimens in their natural habitat and captive-bred specimens in the pet trade. This difference is possibly due to the increased opportunity for the spread of infection caused by the high density of individuals in closed environments in the distribution process and especially in breeding facilities, as well as reduced immunity as a result of the stress of living in an environment different from their natural habitats. A high *Bd* prevalence in breeding colonies has been found among *Ambystoma mexicanum* and *Lithobates catesbeiana* (Mazzoni et al. 2003, Hanselmann et al. 2004, Garner et al. 2006, Frías-Alvarez et al. 2008, Schloegel et al. 2009). It is therefore clear that the *Bd* prevalence in amphibians distributed for trade does not necessarily reflect the pathogen prevalence in the wild.

Adequate measures to deal with pathogens in the artificial environment of breeding facilities and the distribution process are essential. Negligence in this area not only allows the transport of pathogens, but also amplifies population prevalence and the intensity of infection. Our results support the need for the establishment of health standards in the international trade of amphibians which is currently being evaluated by the OIE (OIE 2012).

Two hypotheses for the present global epidemic of chytridiomycosis were initially proposed: the novel or spreading pathogen hypothesis (Laurance et al. 1996, Berger et al. 1998, Skerratt et al. 2007) and the endemic pathogen hypothesis (Berger et al. 1998, Morehouse et al. 2003, Weldon et al. 2004, Morgan et al. 2007, Skerratt et al. 2007, James et al. 2009). There is substantial accumulating evidence for the spreading pathogen hypothesis as the cause of chytridiomycosis-driven declines (Berger et al. 1999, Skerratt et al. 2007, Lips et al. 2008, Murray et al. 2009, Vredenburg et al. 2010).

Bd may be typed into a number of haplotypes based on sequence differences in the ITS region (Goka et al. 2009, Bai et al. 2012). However, caution must be exercised when using ITS sequences to assess *Bd* diversity or determine phylogenetic relationships among strains (Schloegel et al. 2012, Bataille et al. 2013). On the other hand, the ITS region has served as a useful marker to detect and genotype *Bd* because of its high copy number and short length, which allows amplification even from low-quality DNA samples (Bataille et al. 2013). We obtained *Bd* ITS sequences from skin swab samples, a method useful for identifying individuals or populations harboring potentially novel *Bd* genotypes (Schloegel et al. 2012, Bataille et al. 2013). Haplotype A was detected in 93% (25/27) of *Bd*-positive samples from regions outside Asia in this study. This finding indicates that the genetic diversity of the *Bd* strains from regions other than Asia is remarkably low (Fig. 2). Meanwhile, 4 *Bd* haplotypes (A, E, L, and Bd38) were detected in amphibians originating from Thailand, 5 (A, C, V, Q, and Bd44) in those from Japan, and 1 (Bd41) in those from Vietnam. This is consistent with previous reports that Asian amphibians have unique *Bd* strains (Goka et al. 2009, Swei et al. 2011, Bai et al. 2012, Bataille et al. 2013).

We only sequenced a short single locus and 3 subclones per sample, all of which were identical to the original haplotype. Recent studies have shown that many more ITS copies and haplotypes are observed per single zoospore. As ITS sequences are not strain specific, other markers must be used to clarify molecular variation between strains (Schloegel et al. 2012, Longo et al. 2013). Although this limits the effectiveness of ITS sequencing as a primary identification tool for *Bd* strains (Bataille et al. 2013), it is worth noting that Asian countries have higher *Bd* haplotype diversity compared to other countries (Fig. 2). It is unclear whether the diversity is specific to Asian haplotypes or whether it extends to haplotypes from amphibians imported from areas outside Asia. Further studies are needed to investigate the genetic diversity and genealogical relationships of native and traded amphibians around the world.

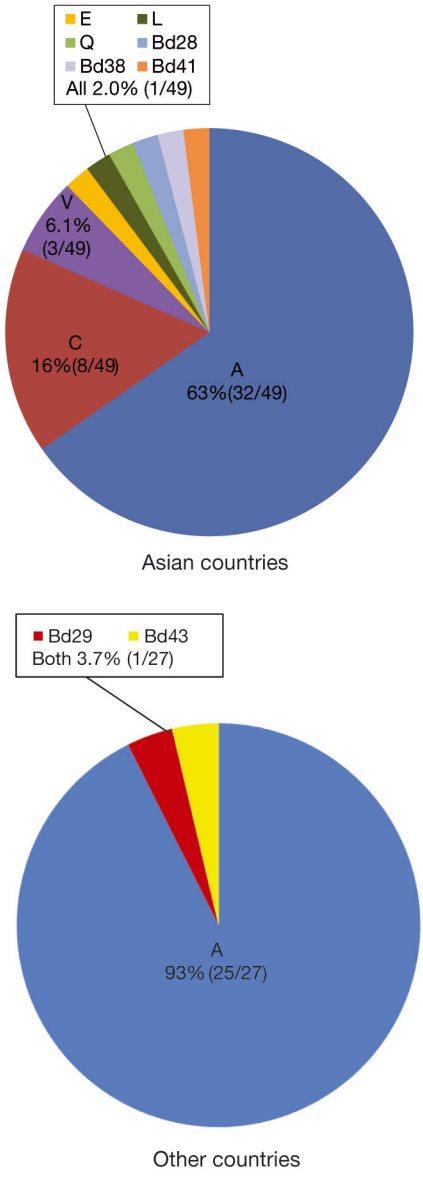

Fig. 2. *Batrachochytrium dendrobatidis*. Percentages of each haplotype detected in amphibians from Asia (Japan, Vietnam, and Thailand) contrasted with Bulgaria, Germany, Madagascar, Peru, Tanzania, and the USA

Haplotype A, which is a hypervirulent strain of the global panzootic lineage (*Bd*-GPL), was detected in amphibians which exhibited signs of chytridiomycosis in this study. To date, we have only discovered Haplotype A in exotic amphibians that died from spontaneous chytridiomycosis (Tamukai et al. 2011). As this haplotype has been shown to induce chytridiomycosis in experimental infections (K. Tamukai et al. unpubl. data), its pathogenicity in certain species is clear. However, although 27 of 30 *Bd*-positive samples taken from wild frogs imported from Thailand were found to be infected with Haplotype A, none of these individuals exhibited clinical signs of disease. Conversely, *Telmatobius* sp. imported from South America was found to be infected with Haplotype A, exhibited sings of chytridiomycosis, and the entire imported batch of approximately 30 individuals died after testing. Even in parts of Asia where *Bd* infection has been detected, no mass mortalities due to chytridiomycosis have been reported (Goka et al. 2009, Savage et al. 2011, Swei et al. 2011, Bai et al. 2012, Vörös et al. 2012, Bataille et al. 2013). While it appears that numerous factors contribute to the pathogenicity of chytridiomycosis including host immunity, bacterial epibiotic symbionts, *Bd* strain, and environmental variables (Rollins-Smith et al. 2002, Berger et al. 2004, Harris et al. 2009, Farrer et al. 2011, Voyles et al. 2011), our results support the hypothesis that either *Bd* is a potentially benign pathogen of Asian amphibians or it is endemic in Asia (Bataille et al. 2013).

The proportion of *Bd* haplotypes found in samples from Japan differed from those from other countries. Moreover, our results show evidence of a reduced prevalence and increased *Bd* ITS haplotype diversity in amphibians kept in captivity for a long period of time compared to wild-caught imported amphibians (Table 3, Fig. 1). We detected 50 haplotypes in Japanese native amphibians (K. Goka et al. unpubl. data). The exotic amphibians, which were kept in captivity for a long period of time, would most likely have come into contact with native amphibians during transport and at rearing facilities, allowing the formation of a variety of haplotype phases. This is based on the fact that with the exception of Haplotype A, all of the haplotypes found have only been detected in Japanese native amphibians. Recent genomic studies suggest that it is possible that *Bd* reproduces sexually (James et al. 2009, Farrer et al. 2011, Schloegel et al. 2012). It may be that the combination of stress that wild-caught specimens endure and the high potential for co-infection results in favorable conditions for hypervirulent strains to emerge (Farrer et al. 2011, Phillips & Puschendorf 2013). In captive populations, there is greater opportunity for niche differentiation and but less selection for hypervirulent strains.

The species infected with the greatest variety of haplotypes was the Japanese-bred *Xenopus laevis*, in which Haplotypes A, C, Q, and V were detected. This finding supports the theory that the *X. laevis* is a key *Bd* host species.

Three species (*Lithobates catesbeiana*, *Bufo marinus*, and *Xenopus laevis*) are considered important vectors in the worldwide spread of *Bd* (Daszak et al.

1999, Fisher & Garner 2007, Schloegel et al. 2012). Movement within Japan and international trade in *L. catesbeiana* and *B. marinus* has been restricted since 2005 by the Invasive Alien Species Act (Ministry of the Environment Government of Japan 2004). In addition, the demand for laboratory *X. laevis* in Japan is currently being met by domestic breeding facilities. Recent data suggest that global movement of amphibians contributes to *Bd* gene pools (James et al. 2009, Farrer et al. 2011, Schloegel et al. 2012). In conclusion, amphibians from the pet trade surveyed in the present study included a significant number that were healthy *Bd* carriers, which indicates that the distribution of exotic amphibians as pets plays a major role in the spread of *Bd*.

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LITERATURE CITED

Andreone F, Mercurio V, Mattioli F (2006) Between environmental degradation and international pet trade: conservation strategies for the threatened amphibians of Madagascar. Natura (Milano) 95:81–96

Annis SL, Dastoor FP, Ziel H, Daszak P, Longcore JE (2004) A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. J Wildl Dis 40:420–428

Bai C, Garner TWJ, Li Y (2010) First evidence of *Batrachochytrium dendrobatidis* in China: discovery of chytridiomycosis in introduced American bullfrogs and native amphibians in the Yunnan Province, China. EcoHealth 7: 127–134

Bai C, Liu X, Fisher MC, Garner TWJ, Li Y (2012) Global and endemic Asian lineages of the emerging pathogenic fungus *Batrachochytrium dendrobatidis* widely infect amphibians in China. Divers Distrib 18:307–318

Bataille A, Fong JJ, Cha M, Wogan GO and others (2013) Genetic evidence for a high diversity and wide distribution of endemic strains of the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* in wild Asian amphibians. Mol Ecol 22:4196–4209

Berger L, Speare R, Daszak P, Green DE and others (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proc Natl Acad Sci USA 95: 9031–9036

Berger L, Speare R, Hyatt AD (1999) Chytrid fungi and amphibian declines: overview, implications and future directions. In: Campbell A (ed) Declines and disappearances of Australian frogs. Environment Australia, Canberra, p 23–33

Berger L, Speare R, Hines HB, Marantelli G and others (2004) Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Aust Vet J 82: 434–439

Bosch J, Martínez-Solano I, García-París M (2001) Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. Biol Conserv 97:331–337

Bradley GA, Rosen PC, Sredl MJ, Jones TR, Longcore JE (2002) Chytridiomycosis in native Arizona frogs. J Wildl Dis 38:206–212

Chatfield MW, Moler P, Richards-Zawacki CL (2012) The amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, in fully aquatic salamanders from Southeastern North America. PLoS ONE 7:e44821

Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R (1999) Emerging infectious diseases and amphibian population declines. Emerg Infect Dis 5: 735–748

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife — threats to biodiversity and human health. Science 287:443–449

Farrer RA, Weinert LA, Bielby J, Garner TWJ and others (2011) Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. Proc Natl Acad Sci USA 108:18732–18736

Fisher MC, Garner TWJ (2007) The relationship between the emergence of *Batrachochytrium dendrobatidis*, the wildlife trade in amphibians and introduced amphibian species. Fungal Biol Rev 21:2–9

Forzán MJ, Gunn H, Scott P (2008) Chytridiomycosis in an aquarium collection of frogs: diagnosis, treatment, and control. J Zoo Wildl Med 39:406–411

Frías-Alvarez P, Vredenburg VT, Familiar-López M, Longcore JE and others (2008) Chytridiomycosis survey in wild and captive Mexican amphibians. EcoHealth 5: 18–26

Gaertner JP, Forstner MR, O'Donnell L, Hahn D (2009) Detection of *Batrachochytrium dendrobatidis* in endemic salamander species from central Texas. EcoHealth 6: 20–26

Garner TWJ, Perkins MW, Govindarajulu P, Seglie D, Walker S, Cunningham AA, Fisher MC (2006) The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. Biol Lett 2: 455–459

Garner TWJ, Stephen I, Wombwell E, Fisher MC (2009) The amphibian trade: bans or best practice? EcoHealth 6: 148–151

Goka K, Okabe K, Yoneda M, Niwa S (2001) Bumblebee commercialization will cause worldwide migration of parasitic mites. Mol Ecol 10:2095–2099

Goka K, Yokoyama J, Une Y, Kuroki T and others (2009) Amphibian chytridiomycosis in Japan: distribution, haplotypes, and possible route of entry into Japan. Mol Ecol 18:4757–4774

Hanselmann R, Rodriguez A, Lampo M, Fajardo-Ramos L and others (2004) Presence of an emerging pathogen of amphibians in introduced bullfrogs (*Rana catesbeiana*) in Venezuela. Biol Conserv 120:115–119

Harris RN, Brucker RM, Walke JB, Becker MH and others (2009) Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. ISME J 3: 818–824

Holsbeek G, Mergeay J, Hotz H, Plötner J, Volckaert F, De Meester L (2008) A cryptic invasion within an invasion and widespread introgression in the European water frog complex: consequences of uncontrolled commercial trade and weak international legislation. Mol Ecol 17: 5023–5035

IUCN (International Union for Conservation of Nature) (2004) Global amphibian assessment. Available at http://data.iucn.org/Themes/ssc/biodiversity_assessments/gaa/index_gaa.htm (accessed 18 April 2013)

James TY, Litvintseva AP, Vilgalys R, Morgan JA and others (2009) Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. PLoS Pathog 5:e1000458

Johnson ML, Speare R (2003) Survival of *Batrachochytrium dendrobatidis* in water: quarantine and disease control implications. Emerg Infect Dis 9:922–925

Karesh WB, Cook RA, Bennett EL, Newcomb J (2005) Wildlife trade and global disease emergence. Emerg Infect Dis 11:1000–1002

Keitzer SC, Goforth R, Pessier AP, Johnson AJ (2011) Survey for the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* in southwestern North Carolina salamander populations. J Wildl Dis 47:455–458

Kriger KM, Hero JM (2009) Chytridiomycosis, amphibian extinctions, and lessons for the prevention of future panzootics. EcoHealth 6:6–10

Kusrini MD, Skerratt LF, Garland S, Berger L, Endarwin W (2008) Chytridiomycosis in frogs of Mount Gede Pangrango, Indonesia. Dis Aquat Org 82:187–194

Laurance WF, McDonald KR, Speare R (1996) Epidemic disease and the catastrophic decline of Australian rain forest frogs. Conserv Biol 10:406–413

Lips KR (1999) Mass mortality and population declines of anurans at an upland site in western Panama. Conserv Biol 13:117–125

Lips KR, Diffendorfer J, Mendelson JR, Sears MW (2008) Riding the wave: reconciling the roles of disease and climate change in amphibian declines. PLoS Biol 6:e72

Longo AV, Rodriguez D, da Silva Leite D, Toledo LF, Mendoza Almeralla C, Burrowes PA, Zamudio KR (2013) ITS1 copy number varies among *Batrachochytrium dendrobatidis* strains: implications for qPCR estimates of infection intensity from field-collected amphibian skin swabs. PLoS ONE 8:e59499

Mazzoni R, Cunningham AA, Daszak P, Apolo A, Perdomo E, Speranza G (2003) Emerging pathogen of wild amphibians in frogs (*Rana catesbeiana*) farmed for international trade. Emerg Infect Dis 9:995–998

McLeod DS, Sheridan JA, Jiraungkoorskul W, Khonsue W (2008) A survey for chytrid fungus in Thai amphibians. Raffles Bull Zool 6:199–204

Ministry of the Environment Government of Japan (2004) The Invasive Alien Species Act. Available at www.env.go.jp/en/nature/as.html (accessed 18 April 2013)

Ministry of the Environment Government of Japan (2012) Red list of amphibians. Available at www.env.go.jp/press/file_view.php?serial=20553&hou_id=15619 (accessed 18 April 2013)

Morehouse EA, James TY, Ganley AR, Vilgalys R, Berger L, Murphy PJ, Longcore JE (2003) Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Mol Ecol 12:395–403

Morgan JA, Vredenburg VT, Rachowicz LJ, Knapp RA and others (2007) Population genetics of the frog-killing fungus *Batrachochytrium dendrobatidis.* Proc Natl Acad Sci USA 104:13845–13850

Murray KA, Skerratt LF, Speare R, McCallum H (2009) Impact and dynamics of disease in species threatened by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis.* Conserv Biol 23:1242–1252

Mutschmann F, Berger L, Zwart P, Gaedicke C (2000) Chytridiomycosis on amphibians: first report from Europe. Berl Münch Tierärztl Wochenschr 113:380–383 (in German with English abstract)

Nijman V, Shepherd CR (2011) The role of Thailand in the international trade in CITES-listed live reptiles and amphibians. PLoS ONE 6:e17825

OIE (World Organisation for Animal Health) (2012) Aquatic Animal Health Code. OIE, Paris. Available at www.oie.int/en/international-standard-setting/aquatic-code/ (accessed 18 April 2013)

Peel AJ, Hartley M, Cunningham AA (2012) Qualitative risk analysis of introducing *Batrachochytrium dendrobatidis* to the UK through the importation of live amphibians. Dis Aquat Org 98:95–112

Phillips BL, Puschendorf R (2013) Do pathogens become more virulent as they spread? Evidence from the amphibian declines in Central America. Proc R Soc Lond B Biol Sci 280:20131290

Picco AM, Collins JP (2008) Amphibian commerce as a likely source of pathogen pollution. Conserv Biol 22:1582–1589

Picco AM, Karam AP, Collins JP (2010) Pathogen host switching in commercial trade with management recommendations. EcoHealth 7:252–256

Rollins-Smith LA, Doersam JK, Longcore JE, Taylor SK, Shamblin JC, Carey C, Zasloff MA (2002) Antimicrobial peptide defenses against pathogens associated with global amphibian declines. Dev Comp Immunol 26: 63–72

Rowley JJL, Chan SKF, Tang WS, Speare R and others (2007) Survey for the amphibian chytrid *Batrachochytrium dendrobatidis* in Hong Kong in native amphibians and in the international amphibian trade. Dis Aquat Org 78:87–95

Savage AE, Grismer LL, Anuar S, Onn CK and others (2011) First record of *Batrachochytrium dendrobatidis* infecting four frog families from Peninsular Malaysia. EcoHealth 8: 121–128

Schlaepfer MA, Hoover C, Dodd CK (2005) Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. Bioscience 55:256–264

Schloegel LM, Picco A, Kilpatrick AM, Davies AJ, Hyatt AD, Daszak P (2009) Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and *Ranavirus* infection in imported North American bullfrogs (*Rana catesbeiana*). Biol Conserv 142: 1420–1426

Schloegel LM, Daszak P, Cunningham AA, Speare R, Hill B (2010) Two amphibian diseases, chytridiomycosis and ranaviral disease, are now globally notifiable to the World Organization for Animal Health (OIE): an assessment. Dis Aquat Org 92:101–108

Schloegel LM, Toledo LF, Longcore JE, Greenspan SE and others (2012) Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. Mol Ecol 21:5162–5177

Skerratt LF, Berger L, Speare R, Cashins S and others (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134

Smith KF, Behrens M, Schloegel LM, Marano N, Burgiel S, Daszak P (2009) Reducing the risks of the wildlife trade. Science 324:594–595

Spitzen-van der Sluijs A, Martel A, Wombwell E, Rooij PV and others (2011) Clinically healthy amphibians in captive collections and at pet fairs: a reservoir of *Batrachochytrium dendrobatidis.* Amphib-Reptilia 32:419–423

Swei A, Rowley JJL, Rödder D, Diesmos ML and others (2011) Is chytridiomycosis an emerging infectious disease in Asia? PLoS ONE 6:e23179

Tamukai K, Une Y, Tominaga A, Suzuki K, Goka K (2011) Treatment of spontaneous chytridiomycosis in captive amphibians using itraconazole. J Vet Med Sci 73: 155–159

Une Y, Kadekaru S, Tamukai K, Goka K, Kuroki T (2008) First report of spontaneous chytridiomycosis in frogs in Asia. Dis Aquat Org 82:157–160

Vörös J, Satasook C, Bates P, Wangkulangkul S (2012) First record of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* in Thailand. Herpetol Notes 5:519–521

Voyles J, Rosenblum EB, Berger L (2011) Interactions between *Batrachochytrium dendrobatidis* and its amphibian hosts: a review of pathogenesis and immunity. Microbes Infect 13:25–32

Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ (2010) Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proc Natl Acad Sci USA 107:9689–9694

Weldon C (2002) Chytridiomycosis survey in South Africa. Froglog 51:2

Weldon C, du Preez LH, Hyatt AD, Muller R, Spears R (2004) Origin of the amphibian chytrid fungus. Emerg Infect Dis 10:2100–2105

Weldon C, De Villiers AL, Du Preez LH (2007) Quantification of the trade in *Xenopus laevis* from South Africa, with implications for biodiversity conservation. Afr J Herpetol 56:77–83

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