

Survey for the amphibian chytrid *Batrachochytrium dendrobatidis* in Hong Kong in native amphibians and in the international amphibian trade

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ABSTRACT: Chytridiomycosis, caused by the pathogen *Batrachochytrium dendrobatidis*, is responsible for many amphibian declines and has been identified in wild amphibian populations on all continents where they exist, except for Asia. In order to assess whether *B. dendrobatidis* is present on the native amphibians of Hong Kong, we sampled wild populations of *Amolops hongkongensis*, *Paa exilispinosa*, *P. spinosa* and *Rana chloronota* during 2005–2006. Amphibians infected with *B. dendrobatidis* have been found in the international trade, so we also examined the extent and nature of the amphibian trade in Hong Kong during 2005–2006, and assessed whether *B. dendrobatidis* was present in imported amphibians. All 274 individuals of 4 native amphibian species sampled tested negative for *B. dendrobatidis*, giving an upper 95% confidence limit for prevalence of 1.3%. Approximately 4.3 million amphibians of 45 species from 11 countries were imported into Hong Kong via air over 12 mo; we did not detect *B. dendrobatidis* on any of 137 imported amphibians sampled. As *B. dendrobatidis* generally occurs at greater than 5% prevalence in infected populations during favorable environmental conditions, native amphibians in Hong Kong appear free of *B. dendrobatidis*, and may be at severe risk of impact if it is introduced. Until it is established that the pathogen is present in Hong Kong, management strategies should focus on preventing it from being imported and decreasing the risk of it escaping into the wild amphibian populations if imported. Further research is needed to determine the status of *B. dendrobatidis* in Hong Kong with greater certainty.

KEY WORDS: *Batrachochytrium dendrobatidis* · Amphibian chytrid fungus · Chytridiomycosis · Asia · China · Disease · Wildlife trade · Frogs

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INTRODUCTION

In recent decades, a number of pathogenic infectious diseases that represent a substantial global threat to human health have emerged, including

Acquired Immunodeficiency Syndrome, Severe Acute Respiratory Syndrome, and H5N1 Avian Influenza. In addition to those that are direct threats to human health, infectious diseases affecting wildlife and plant populations are emerging at unusually high rates and

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currently pose a great threat to the conservation of global biodiversity (Harvell et al. 1999, Ward & Lafferty 2004). Species that are thought to have declined or been eliminated due to disease are taxonomically diverse and include island land birds (Warner 1968, van Riper et al. 1986), grassland mammals (Thorne & Williams 1988, Ginsberg et al. 1995), snails (Daszak & Cunningham 1999) and frogs (Berger et al. 1998, Bosch et al. 2001, Bradley et al. 2002, Weldon & Du Preez 2004, Lips et al. 2006, Rachowicz et al. 2006, Schloegel et al. 2006).

Perhaps the most alarming wildlife disease known at present is chytridiomycosis, a potentially fatal disease of amphibians caused by the pathogen *Batrachochytrium dendrobatidis* (Berger et al. 1998, Lips et al. 2003a). Chytridiomycosis is an emerging infectious disease implicated in mass mortalities, population declines and extinctions of amphibian populations and species around the world (Berger et al. 1998, Lips 1999, Bosch et al. 2001, Bradley et al. 2002, Weldon & Du Preez 2004, Lips et al. 2006, Rachowicz et al. 2006). To date, *B. dendrobatidis* has been identified in all continents where wild amphibian populations exist, except for Asia. However, there has been little survey effort for *B. dendrobatidis* in Asia.

The origin of *Batrachochytrium dendrobatidis* is controversial, with some groups proposing that *B. dendrobatidis* is a virulent pathogen spreading through amphibian populations (e.g. Skerratt et al. 2007) and others arguing that the evidence is not yet strong enough to determine whether *B. dendrobatidis* has recently been dispersed or was widely endemic in amphibian populations prior to their decline (e.g. Rachowicz et al. 2005). If it has recently been dispersed from a single origin, it may have escaped from South Africa via the scientific and medical trade in the African clawed frog *Xenopus laevis* (Weldon et al. 2004) and become established in North America in the early 1960s (Ouellet et al. 2005) and Australia in the late 1970s (Berger et al. 1999). It may also have been dispersed by international trade in amphibians for other purposes (OIE 2006). Amphibians infected with *B. dendrobatidis* have been found in the scientific (Reed et al. 2000, Parker et al. 2002, Weldon et al. 2004), pet (Groff et al. 1991, Berger et al. 1999, Mutschmann et al. 2000, Cunningham et al. 2005), food (Mazzoni et al. 2003, Hanselmann et al. 2004, Garner et al. 2006) and zoological trades (Nichols et al. 1998, 2001, Pessier et al. 1999, Raverty & Reynolds 2001, Oevermann et al. 2005). Therefore, the trade and human-assisted movement of amphibians may cause disease outbreaks within continents by introducing *B. dendrobatidis* into new areas, or into areas with different strains of the pathogen.

The amphibian trade often presents an ideal opportunity for dispersal and transport of pathogens. Amphibians are transported in crowded conditions, in contact with multiple other species from many source regions, and may escape or be deliberately released into natural habitats carrying their associated pathogens and parasites. Therefore, it is extremely important to assess the risk posed by the amphibian trade, particularly in areas not presently known to contain *Batrachochytrium dendrobatidis*.

Hong Kong is one of the major world centres for the global amphibian trade, largely due to its strong transport and economic links with mainland China and many other countries, its open trading policy, economic success and high demand for wildlife consumption (Lau et al. 1995). In addition, the keeping of amphibians as pets is becoming increasingly popular in Hong Kong, with amphibians imported for both local sale and re-export (Lau et al. 1995). Many Asian countries, such as China, Thailand and Indonesia, are major breeders of food amphibians, producing more than 1000 tonnes per country per year (Daszak et al. 2006). Although the trade in amphibians as food in Hong Kong has largely been dominated by Chinese bullfrogs *Hoplobatrachus rugulosus* from Southeast Asia and mainland China, amphibians for the pet trade are imported from around the world (Lau et al. 1995). In addition, Hong Kong is also a transit port for Chinese goods intended for international markets (Lau et al. 1995). If *Batrachochytrium dendrobatidis* is present on amphibians imported into Hong Kong, this may pose a serious threat to the native amphibians of the region. Hong Kong contains 24 native amphibian species, including a number of species that are endangered, near threatened or vulnerable (Chan et al. 2005). The climate of the region is likely to be suitable for *B. dendrobatidis* (Ron 2005). All these factors make Hong Kong an ideal location to conduct the first systematic survey for *B. dendrobatidis* in mainland Asia.

We therefore aimed to conduct the first systematic survey for *Batrachochytrium dendrobatidis* in China, and the most comprehensive to date in Asia, to determine whether *B. dendrobatidis* occurs in the native amphibians of Hong Kong. In addition, we aimed to determine the extent and nature of the amphibian trade in Hong Kong, and to assess whether *B. dendrobatidis* was present in amphibians sold in Hong Kong's markets as part of the food and pet trades.

MATERIALS AND METHODS

Native amphibians of Hong Kong. In order to assess whether *Batrachochytrium dendrobatidis* was present in the native amphibians of Hong Kong, we selected 4

species of native frogs that we considered most likely to be infected with *B. dendrobatidis* based upon the characteristics of infected amphibians in other countries: the Hong Kong cascade frog *Amolops hongkongensis* (Ranidae; World Conservation Union [IUCN] near threatened), the lesser spiny frog *Paa exilispinosa* (Ranidae; IUCN vulnerable), the giant spiny frog *P. spinosa* (Ranidae; IUCN vulnerable) and the green cascade frog *Rana chloronota* (Ranidae; IUCN least concern). All species breed in hill and mountain streams rather than ponds, spend a large proportion of their time in association with the stream environment, and occur at relatively high elevations; these characteristics are correlated with *B. dendrobatidis*-related declines in other countries (McDonald & Alford 1999, Lips et al. 2003b). Such species comprise 9 out of the 24 native amphibians in Hong Kong. At least 80 individuals of each species were sampled for *B. dendrobatidis*, except for *P. spinosa*, for which we were unable to collect enough samples due to its rarity and restricted distribution in Hong Kong (a single site). We attempted to sample individuals from localities spanning the geographic range of each species within Hong Kong; no more than 20 frogs were collected from a single site (1 km²), to ensure that sampling was as representative of the frog population as was possible (Speare et al. 2005). We surveyed 13 sites on Lantau Island, Hong Kong Island and the New Territories (Fig. 1).

Frogs were sampled between December 2005 and October 2006. We attempted to sample all frogs during the winter (December–January in Hong Kong), when higher host infection prevalence and mortality are typically observed (Bradley et al. 2002, Berger et al. 2004, Retallick et al. 2004, McDonald et al. 2005, Woodhams & Alford 2005). However, during winter, frogs were inactive and not easily detected, and surveys continued 1 to 5 times per month until sufficient swabs (see below) were collected.

During nocturnal and occasional diurnal surveys, frogs were captured individually by hand, with a new pair of disposable plastic bags for each frog covering the hands in order to avoid cross-contamination between individuals. In most cases, the number of frogs observed at a site was low, and all frogs observed during each survey were captured and swabbed. If more than 20 individuals were observed at a site, the first 20 frogs encountered were caught for swabbing.

Immediately after capture, the ventral surface of each frog was swabbed using a sterile cotton swab (Medical Wire & Equipment). To avoid re-sampling the same individual, frogs were captured, swabbed and then released at 100 m intervals along the stream. If the number of samples collected in a single trip was inadequate, more samples were collected from different sections of the same stream or different streams at the same site.

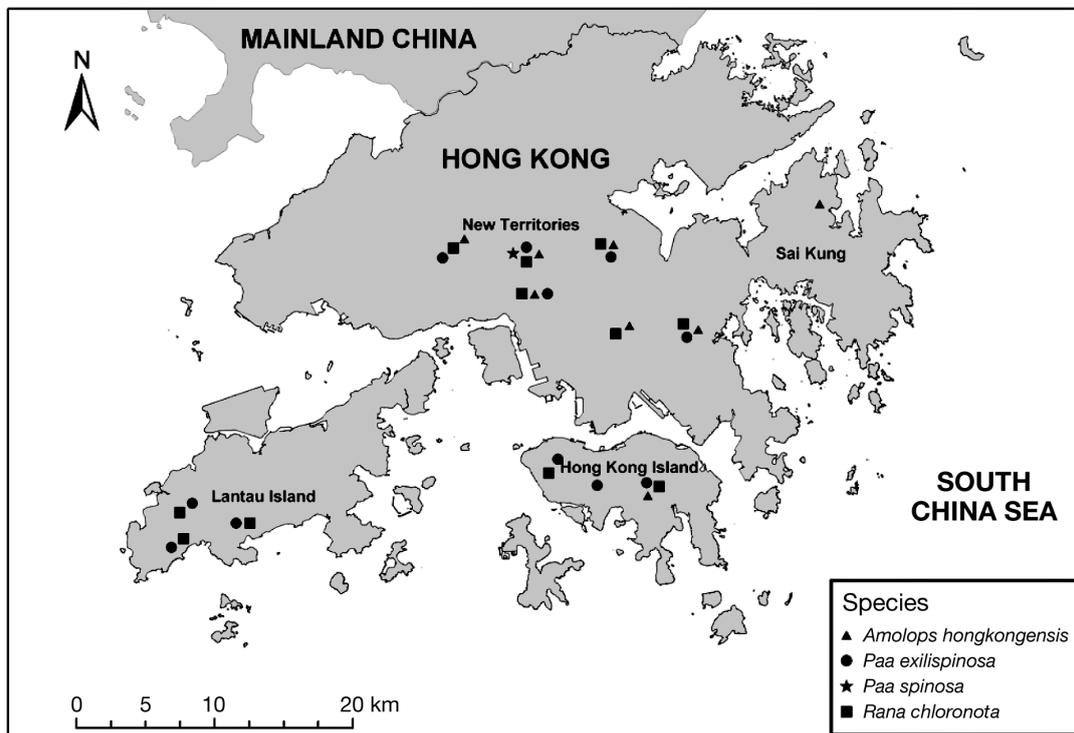


Fig. 1. Survey sites for *Batrachochytrium dendrobatidis* in 4 native amphibian species in Hong Kong

All samples were kept away from sunlight and stored frozen (–12 to –16°C) between 1 and 6 h after collection for 1 to 10 wk before shipping to Australia. Samples were evaluated for the presence of *Batrachochytrium dendrobatidis* using Taqman diagnostic quantitative PCR (Boyle et al. 2004). DNA was extracted with PrepMan Ultra, and amplified using primers ITS1-3 Chytr and 5.8S Chytr (Boyle et al. 2004). Each sample was tested in triplicate, and was only recorded as positive if all 3 replicates indicated the presence of *B. dendrobatidis*. Individual PCR tests were not examined for the presence of inhibitors to PCR although positive and negative controls were included in each test run. Similar swabbing and testing methods have been used to regularly detect *B. dendrobatidis* on Australian frogs (Speare et al. 2005). All diagnostic PCR was performed within 14 wk of swab collection.

Amphibians imported into Hong Kong. In order to assess the scale and nature of the amphibian trade in Hong Kong, and the risk that this trade might pose to the native amphibians of Hong Kong, we obtained records of the number of live amphibians imported into Hong Kong via Hong Kong International Airport in the food and non-food (pet/scientific) trades. We obtained this information from the Agriculture, Fisheries and Conservation Department in Hong Kong for the 1 yr period of 1 December 2005 to 30 November 2006. For food frogs and a number of non-food amphibian shipments, weight (kg), rather than number of individuals was recorded. For these species, we obtained the maximum body weight for each species from the literature and divided the shipment weight by this value. We did not include import data for live amphibians entering Hong Kong via land from mainland China, or those amphibians in transit in Hong Kong before re-export to other countries.

In order to determine whether *Batrachochytrium dendrobatidis* was present in frogs imported to Hong Kong and sold at local markets, samples from frogs imported for both food and pets were collected at Hong Kong International Airport and at 7 different food and pet markets. During market frog surveys, no more than 10 frogs were sampled from the same shipment or container of frogs. Samples were collected, stored and analysed in the same manner as for native frogs.

RESULTS

Native amphibians of Hong Kong

We sampled 274 individuals of 4 species of frogs native to Hong Kong (99 *Amolops hongkongensis*, 81 *Paa exilispinosa*, 11 *P. spinosa* and 83 *Rana chloronota*). All samples tested negative for *Batrachochytrium dendrobatidis* in all 3 replicates (Table 1).

Amphibians imported into Hong Kong

The majority of live amphibians imported into Hong Kong via Hong Kong International Airport between 1 December 2005 and 30 November 2006 were part of the food trade. During this period, over 2 100 000 kg of live amphibians were imported to Hong Kong for human consumption. The vast majority of these were imported from Thailand, with only 18 kg from the USA. Assuming that all amphibians from Thailand are *Hoplobatrachus rugulosus*, and all amphibians from the USA are *Rana catesbeiana*, this mass represents approximately 4 260 000 amphibians.

Numbers of live amphibians imported into Hong Kong as part of the pet or scientific trade were considerably smaller, but encompassed a much wider range of species and source countries. In total, about 33 000 individuals of at least 45 species of amphibians, sourced from 11 countries, were imported into Hong Kong (Table 2). Source countries were located in Asia, Africa, Europe and North America (Table 2). Although the IUCN Red Book status of most of the species was 'least concern', 4 were listed above this with one species each listed as 'vulnerable' (*Leptopelis barbouri*), 'near threatened' (*Ceratophrys ornata*), 'endangered' (*Conraua goliath*) and 'critically endangered' (*Neurergus kaiseri*) (Table 2).

We sampled 137 individuals of 3 species of frogs sold at food and pet markets in Hong Kong (129 *Hoplobatrachus rugulosus*, 1 *Occidozyga martensii* and 7 *Xenopus laevis*). All samples tested negative for *Batrachochytrium dendrobatidis* in all 3 replicates (Table 3).

DISCUSSION

We did not detect *Batrachochytrium dendrobatidis* on any samples from the 274 individuals of 4 native amphibian species sampled. Although we cannot rule out the presence of *B. dendrobatidis* in native amphibians, if we assume that all species are equally likely to carry the infection, the upper 95% confidence limit for prevalence is 1.3% at the time of sampling. This may be an underestimate, since susceptibility is very likely to differ among species, however, when species are considered separately, upper 95% confidence limits for prevalence are still very low: 3.7% for *Amolops hongkongensis*, 4.5% for *Paa exilispinosa*, 28.5% for *P. spinosa* and 4.4% for *Rana chloronota*. Because we targeted species at high risk for chytridiomycosis, the infection prevalence for the other frog species in Hong Kong is likely to be much lower if *B. dendrobatidis* is present. Given that in other regions *B. dendrobatidis* generally occurs at greater than 5% prevalence when endemic and weather conditions are favourable (Hop-

kins & Channing 2003, Retallick et al. 2004, McDonald et al. 2005, Speare et al. 2005, Woodhams & Alford 2005, Kriger & Hero 2006, Puschendorf et al. 2006), it appears that native amphibians in Hong Kong are free of *B. dendrobatidis*. Amphibian population trends in Hong Kong also support the absence of *B. dendrobatidis* in the region, with no observations of the unexplained mass mortality events or population declines of amphibians usually associated with emergence of *B. dendrobatidis* in naïve populations (S. K. F. Chan unpubl. data). The reasons for the absence of *B. dendrobatidis* from our samples are not certain. It may be entirely absent from Hong Kong and the source populations of all the imported animals we examined. However, it is known that susceptibility to *B. dendrobatidis* infection varies among species (Berger et al. 1999); some amphibian species are known to produce antimicrobial peptides or carry cutaneous bacteria that can inhibit the growth of *B. dendrobatidis in vitro* (Rollins-Smith et al. 2002, Harris et al. 2006). Thus, the species

we examined may not be susceptible to *B. dendrobatidis* infection. Further research is needed to determine the status of *B. dendrobatidis* in Hong Kong with greater certainty.

The scale of the amphibian trade in Hong Kong is large in terms of numbers of individual frogs, and the diversity of species and source countries. Between 1 December 2005 and 30 November 2006, at least 4.3 million individuals of 45 amphibian species from around the world were imported into Hong Kong via air alone. A number of amphibians were imported to Hong Kong from countries within their native range, and were likely wild-caught (e.g. *Pyxicephalus adspersus* and *Hyperolius tuberilinguis*), but most were imported from countries outside their native range, and were either captive bred (e.g. *Litoria caerulea* and *Ambystoma mexicanum*) or their recorded country of origin was a trans-shipment point. Although 4 of the species imported into Hong Kong had IUCN Red Book Listing above least concern, none were on the

Table 1. Native frogs sampled for *Batrachochytrium dendrobatidis* in Hong Kong

Species	Zone	Site	Elevation (m)	Number of samples	
<i>Amolops hongkongensis</i>	Hong Kong Island	Violet Hill Catchwater (Tai Tam Area)	160–250	20	
	New Territories	Tai Lam Wu Catchwater	100–160	19	
	New Territories	Lion Rock Catchwater	140–160	1	
	New Territories	Shek Kong Catchwater	100–200	17	
	New Territories	Tai Mo Shan-Shing Mun Catchwater	200–220	8	
	New Territories	Tai Po Kau Nature Reserve	120–160	13	
	New Territories	Tai Mo Shan (Sze Lok Yuen)	600–700	10	
	New Territories	Sai Kung-Hau Tong Kai	100–160	11	
			100–700	99	
<i>Paa exilispinosa</i>	Hong Kong Island	Violet Hill Catchwater (Tai Tam Area)	200–240	10	
	Hong Kong Island	Aberdeen (Yue Kwong Tsuen)	120–160	4	
	Hong Kong Island	Pok Fu Lam Country Park	200–340	14	
	Lantau Island	Shek Pik & Lantau Trail Catchwater	80–160	11	
	Lantau Island	Keung Shan Catchwater	60–100	11	
	New Territories	Shek Kong Catchwater	120–160	5	
	New Territories	Tai Po Kau Nature Reserve	140–160	3	
	New Territories	Tai Lam Wu	380–420	11	
	New Territories	Tai Mo Shan (Sze Lok Yuen)	620–780	10	
	New Territories	Tai Mo Shan Shing Mun Catchwater	200–220	2	
			60–780	81	
<i>Paa spinosa</i>	New Territories	Tai Mo Shan (Sze Lok Yuen)	580–660	11	
			580–660	11	
<i>Rana chloronota</i>	Hong Kong Island	Violet Hill Catchwater (Tai Tam Area)	60–250	14	
	Hong Kong Island	Pok Fu Lam Country Park	200–340	10	
	Lantau Island	Shek Pik & Lantau Trail Catchwater	100–200	10	
	Lantau Island	Keung Shan Catchwater	80–160	10	
	New Territories	Tai Lam Wu Catchwater	100–400	4	
	New Territories	Shek Kong Catchwater	120–140	3	
	New Territories	Tai Mo Shan-Shing Mun Catchwater	180–220	9	
	New Territories	Tai Mo Shan (Sze Lok Yuen)	620–640	1	
	New Territories	Tai Po Kau Nature Reserve	120–160	10	
	New Territories	Lion Rock Catchwater	140–160	12	
				80–640	83
	Total				274

Table 2. Live amphibians imported to Hong Kong for the pet/scientific trade via Hong Kong International Airport from 1 December 2005 to 30 November 2006

Order	Family	Species	Source	Number of frogs	
Anura	Arthroleptidae	<i>Leptopelis barbouri</i>	Cameroon	20	
	Ceratophryidae	<i>Ceratophrys cornuta</i>	USA	39	
		<i>Ceratophrys cranwelli</i>	Canada	140	
			USA	1062	
		<i>Ceratophrys ornata</i>	Japan	20	
			USA	186	
		<i>Ceratophrys</i> sp.	USA	50	
		<i>Lepidobatrachus laevis</i>	Japan	20	
			USA	6	
		Hemisotidae	<i>Hemisus marmoratus</i>	Thailand	20
		Hylidae	<i>Agalychnis callidryas</i>	Canada	55
				USA	12
			<i>Dendropsophus sarayacuensis/triangulum</i>	Germany	6
			<i>Hyla gratiosa</i>	USA	12012
	<i>Hyla japonica</i>		Japan	40	
	<i>Hypsiboas boans</i>		Germany	3	
	<i>Litoria caerulea</i>		Canada	12	
			USA	4732	
	<i>Osteopilus septentrionalis</i>		USA	5	
	<i>Phyllomedusa bicolor</i>		Germany	5	
	<i>Phyllomedusa hypochondrialis</i>		USA	6	
	<i>Phyllomedusa sauvagii</i>		USA	5	
	<i>Phyllomedusa tomopterna</i>		Germany	2	
	<i>Phyllomedusa vaillantii</i>		Germany	1	
	<i>Hypsiboas punctatus</i>		Germany	10	
	Hyperoliidae		<i>Hyperolius marmoratus</i>	Tanzania	150
			<i>Hyperolius parkeri</i>	Cameroon	10
			<i>Hyperolius tuberilinguis</i>	Tanzania	100
			<i>Kassina maculata</i>	Thailand	20
	Limnodynastidae		<i>Limnodynastes salmini</i>	USA	5
	Megophryidae	<i>Brachytarsophrys carinensis</i>	Japan	2	
		<i>Megophrys nasuta</i>	USA	6	
	Microhylidae	<i>Dyscophus guineti</i>	Canada	5	
			USA	38	
		<i>Dyscophus insularis</i>	USA	3040	
		<i>Kaloula pulchra</i>	Vietnam	169	
		<i>Phrynomantis bifasciatus</i>	Ghana	20	
	Petropedetidae	<i>Conraua goliath</i>	Japan	5	
	Pipidae	<i>Xenopus laevis</i>	France	20	
			USA	220	
	Pyxicephalidae	<i>Pyxicephalus adspersus</i>	Canada	25	
			Tanzania	100	
			USA	1	
	Ranidae	<i>Rana catesbeiana</i>	USA	20	
	Rhacophoridae	<i>Polypedates otilophus</i>	USA	7	
		<i>Rhacophorus arboreus</i>	Japan	100	
		<i>Theloderma corticale</i>	USA	12	
Caudata	Ambystomatidae	<i>Ambystoma maculatum</i>	USA	10034	
		<i>Ambystoma mexicanum</i>	Czech Republic	100	
			Germany	100	
		<i>Ambystoma tigrinum</i>	USA	112	
	Salamandridae	<i>Cynops pyrrhogaster</i>	Japan	20	
		<i>Neurergus kaiseri</i>	Germany	3	
		<i>Salamandra salamandra</i>	USA	30	
	<i>Triturus karelinii</i>	Japan	50		
Total				32993	

Table 3. Market frogs sampled for *Batrachochytrium dendrobatidis* in Hong Kong

Species	Survey location	Origin of frogs	Number of samples
<i>Hoplobatrachus rugulosus</i>	Fanling Wet Market	Guangzhou, China	2
		Thailand	6
	Hong Kong International Airport (Importer: Kong Kang Fruits Lann)	Thailand	36
	Hong Kong International Airport (Importer: Man Kee Hong)	Central and Southern Thailand	43
	Kwun Tong Wet Market	China	4
		Guangzhou, China	2
		Thailand	4
	Mong Kok Wet Market	China	10
		Guangzhou, China	2
	Sai Kung Wet Market	China	4
	Shatin Wet Market	China	4
		Thailand	2
	Tai Po Wet Market	China	2
Thailand		8	
<i>Occidozyga martensii</i>	Mong Kok (Tung Choi Street)	Southeast Asia	1
<i>Xenopus laevis</i>	Mong Kok (Tung Choi Street)	China	7
Total			137

Convention on International Trade in Endangered Species (CITES) Appendices I–III and no CITES permits were required for their movement.

Based on the scale and nature of amphibian imports into Hong Kong, the potential for the introduction of amphibian pathogens and parasites, including *Batrachochytrium dendrobatidis*, appears high. Releases of captive animals or interactions between captive and wild animals have caused outbreaks of disease in wild populations in other animal groups (Fischer et al. 1997, Hartup et al. 2001, Krkosek et al. 2006). Imported amphibians may escape into the wild or be released as part of religious customs such as Buddhist merit release (Smith 1999), or as unwanted pets (Karesh et al. 2005). In Hong Kong, *Hoplobatrachus rugulosus* and other non-native frogs were found at our survey sites on several occasions between 2001 and 2006. These frogs were typically found in a poor state of health, and are thought to have recently been released into the wild. In addition, at least 9 of the species imported into Hong Kong are known to be susceptible to *B. dendrobatidis* infection in the wild and/or in captivity in other countries, and amphibians from almost all major source countries are known to carry the pathogen (with the notable exceptions of China and Thailand; Speare & Berger 2000). However, all individuals sampled during our survey of market frogs tested negative for *B. dendrobatidis*. Therefore, if *B. dendrobatidis* was present on amphibians imported into Hong Kong during our survey period, and we assume that all species sampled are equally likely to carry the infection, the

upper 95% binomial confidence limit for its prevalence is 2.7%. As with native frogs, the true upper limit is probably higher, since susceptibility probably varies among species, but it suggests that *B. dendrobatidis* was absent from the groups of imported frogs we sampled, or at least from imported *H. rugulosus*; for this species considered alone the upper 95% binomial confidence limit is 2.8%. Our survey of imported market frogs was limited in that all animals examined originated from Asia (Table 3) where chytridiomycosis has not been reported. It was also limited because 129 of the 137 animals sampled belonged to the single species *H. rugulosus*; if this species is resistant to infection by *B. dendrobatidis* our results will be biased. We would expect that amphibians imported from countries and regions where chytridiomycosis is endemic (such as North America, Africa and Europe) would be more likely to carry *B. dendrobatidis*. For example, Weldon (2005) found that 10% of *Xenopus laevis* intended for export from South Africa were infected with *B. dendrobatidis*. Further *B. dendrobatidis* surveys should focus on amphibians of species other than *H. rugulosus* imported into Hong Kong from countries where *B. dendrobatidis* is endemic, to assess the risk of imports from these regions.

The failure to find *Batrachochytrium dendrobatidis* in wild amphibians in Hong Kong has significant implications for wildlife management and for border control strategies for amphibian disease. It is safest to assume that *B. dendrobatidis* is absent from Hong Kong. In this case, the native amphibians may be at severe risk of

impact if it is introduced. As long as *B. dendrobatidis* is not known to occur in Hong Kong, management strategies should focus on preventing its importation, decreasing the risk of it escaping into the wild amphibian populations if imported, and decreasing the risk of transmission between amphibians if it does escape. Since the likely vehicles for escape of *B. dendrobatidis* are infected frogs and water that has been in contact with infected frogs (Johnson & Speare 2003), protocols should be implemented to prevent escape or release of imported frogs into the wild and to encourage disinfection of water in which frogs have been held. An emergency response plan which includes disease surveillance for chytridiomycosis in the most vulnerable wild populations should also be formulated to deal with any introduction. Other strategies include implementation of hygiene protocols by scientists and wildlife managers having contact with wild amphibians. The OIE is currently evaluating the need for health requirements for amphibians being traded globally (OIE 2006). Countries or regions such as Hong Kong that do not have chytridiomycosis will benefit if strategies are implemented in the global amphibian trade to decrease the likelihood of frogs infected with *B. dendrobatidis* being moved between countries.

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