

## Short Communication

**Mercy or messy: distribution and differentiation of native and released Chinese bullfrogs (*Hoplobatrachus rugulosus*) in Hong Kong using genetic and morphological analyses**Wing-Him Lee<sup>1</sup>, Jonathan J. Fong<sup>1</sup>, Wing-Ho Lee<sup>2</sup> and Yik-Hei Sung<sup>1,\*</sup><sup>1</sup>Science Unit, Lingnan University, Hong Kong SAR, China<sup>2</sup>Croucher Institute for Environmental Sciences, Department of Biology, Hong Kong Baptist University, Hong Kong SAR, ChinaAuthor e-mails: [henrylee@ln.hk](mailto:henrylee@ln.hk) (WHIL), [jonfong@ln.edu.hk](mailto:jonfong@ln.edu.hk) (JJF), [leehoken1989@gmail.com](mailto:leehoken1989@gmail.com) (WHL), [yhsung@ln.edu.hk](mailto:yhsung@ln.edu.hk) (YHS)

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**Abstract**

The Chinese bullfrog, *Hoplobatrachus rugulosus* (Wiegmann, 1834), naturally distributed in Thailand, Laos, Vietnam and China, is commonly used in the religious practice of mercy release. The release of *H. rugulosus* is complicated by the fact that it likely represents a cryptic species complex (multiple species under a single name). In Hong Kong, although there are native and released individuals, the impact of release has not been investigated. In this study, we conduct genetic and morphological analyses of *H. rugulosus* across Hong Kong to determine the source (native or released) of frogs, the distribution of released populations and identify morphological characteristics that can be used to differentiate native and released individuals. We found that *H. rugulosus* individuals in Hong Kong belong to the two major genetic clades identified in previous studies (Clade A and Clade B). We consider individuals in Clade A as released frogs based on the genetic result that cluster these individuals with wild *H. rugulosus* from western, central and eastern Thailand, and individuals from Chinese frog farms. Individuals from the two genetic clades differ based on morphology (dorsal color, coloration of loreal region, presence of wounds and coloration on lateral sides), that can be useful for identification. However, there are four individuals in Clade A that are morphologically similar to native frogs, raising the possibility of hybridization between individuals in the two clades. We strongly recommend that the mercy release of *H. rugulosus* and other exotic animals should be strictly prohibited by law in Hong Kong and other parts of the world. Eradication guidelines should be developed by experts, as a references for governments, conservation groups and the public.

**Key words:** amphibian, introduced species, mercy release, phylogeny, religious release**Introduction**

Mercy release refers to the Buddhist and Taoist practice of releasing captive animals for good karma (Shiu and Stokes 2008). Such practice is common in many countries, and the release of large numbers of animals has led to establishment and propagation of invasive species (Liu et al. 2012; Su et al. 2016). The scale and impacts of mercy release have been poorly studied, hindering effective management of native species and released populations. Some potential negative impacts of mercy release are

genetic pollution (Pagano et al. 2003), pathogen spillover (Kolby et al. 2014) and predation of native species (Wiles et al. 2003).

Mercy release often involves species farmed for commercial use because of their abundance and availability in food markets. One such species in Asia is the Chinese bullfrog, *Hoplobatrachus rugulosus* (Wiegmann, 1834). The release of *H. rugulosus* is common and is complicated by the fact that this species likely represents a cryptic species complex (multiple species under a single name). Genetic studies of *H. rugulosus* (Pansook et al. 2012; Yu et al. 2015) found that there are considerable differences between populations, with populations from western, central and eastern Thailand in one clade (Clade A) and populations from northern and northeastern Thailand, Laos, Vietnam and China in another clade (Clade B), following the names used in Pansook et al. (2012). Given that breeding stocks of farmed *H. rugulosus* are sourced from western, central and eastern Thailand (Yu et al. 2015), the release of farmed frogs into the wild may result in unnatural hybridization if done within the geographic range of Clade B. We study *H. rugulosus* populations in Hong Kong to evaluate the potential negative impacts of release.

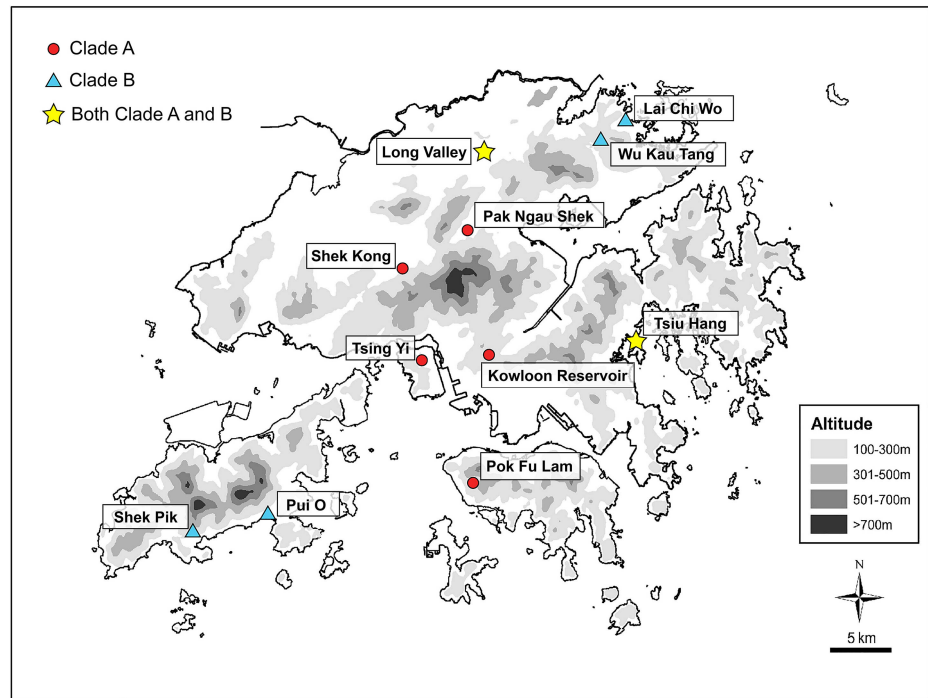
In Hong Kong, naturalists infer the identity of *H. rugulosus* individuals in the field based primarily on coloration, with lighter individuals considered as released (Clade A) and darker individuals considered as native (Clade B). However, this assumption has never been formally evaluated. By using genetic and morphological data, we evaluate whether morphological characteristics can differentiate native from released individuals.

In this study, we conducted genetic and morphological analyses of *H. rugulosus* across Hong Kong. Based on genetic data, we determine the source (native or released) of individuals and distribution of released populations. Further, we identify morphological characteristics that can be used to differentiate native and released individuals. Last, we identify locations where native and released *H. rugulosus* co-occur and pose a risk of genetic pollution. These results will provide recommendations on the management of released *H. rugulosus* to minimize the negative impacts of mercy release.

## Materials and methods

### *Sampling*

This study was done in the Hong Kong Special Administrative Region, China (22°09'–22°37'N; 113°50'–114°30'E). We selected 11 study sites (Figure 1, Table 1), of which six (Lai Chi Wo, Shek Pik, Wu Kau Tang, Long Valley, Pui O, and Tsiu Hang) consist of suitable habitat for *H. rugulosus* (marshes and agricultural fields), and five (Shek Kong, Kowloon Reservoir, Pokfulam, Tsing Yi, and Pak Ngau Shek) consist of reservoirs, catchwater and urban parks that are hotspots of mercy release with frequent sightings of released animals (e.g., *H. rugulosus*, *Trachemys scripta elegans* and exotic fish).



**Figure 1.** Survey sites for this study. Individuals were categorized as Clade A (released) and Clade B (native) based on the genetic analysis. Two sites (Long Valley, Tsiu Hang) had individuals from both Clade A and Clade B living sympatrically.

**Table 1.** Information on the surveys for *Hoplobatrachus rugulosus* in Hong Kong.

Sampling locality	Number of surveys	Number of specimens	Habitat type
Shek Kong	5	2	Reservoir
Kowloon Reservoir	5	2	Reservoir
Pok Fu Lam	1	1	Reservoir
Tsing Yi	1	1	Urban park
Tsiu Hang	2	3	Pond
Wu Kau Tang	2	3	Freshwater wetland
Long Valley	1	4	Freshwater wetland
Pak Ngau Shek	1	2	Stream
Pui O	2	1	Freshwater wetland
Lai Chi Wo	1	2	Freshwater wetland
Shek Pik	1	2	Catchwater
Mong Kok	1	1	Wet Market
Lok Fu	1	1	Wet Market

We surveyed for *H. rugulosus* using active searching to capture frogs at night (Crump and Scott, 1994) during the wet season (May to September) in 2016 and 2017 (Fei et al. 2010). In addition, we acquired two individuals from two food markets (Mong Kok and Lok Fu wet markets) as genetic references for farmed frogs. We classified individuals into two preliminary groups, potentially native and released. As *H. rugulosus* sold in local wet markets are usually pale yellow in color and have wounds on the snout and skin, individuals with these characteristics were classified as released and euthanized. Euthanasia of frogs was done by immersing them in a solution of tricaine methanesulfonate (MS-222; Argent Laboratories, Redmond, WA USA). To minimize the negative impacts on native populations, individuals classified as native were released immediately after taking a

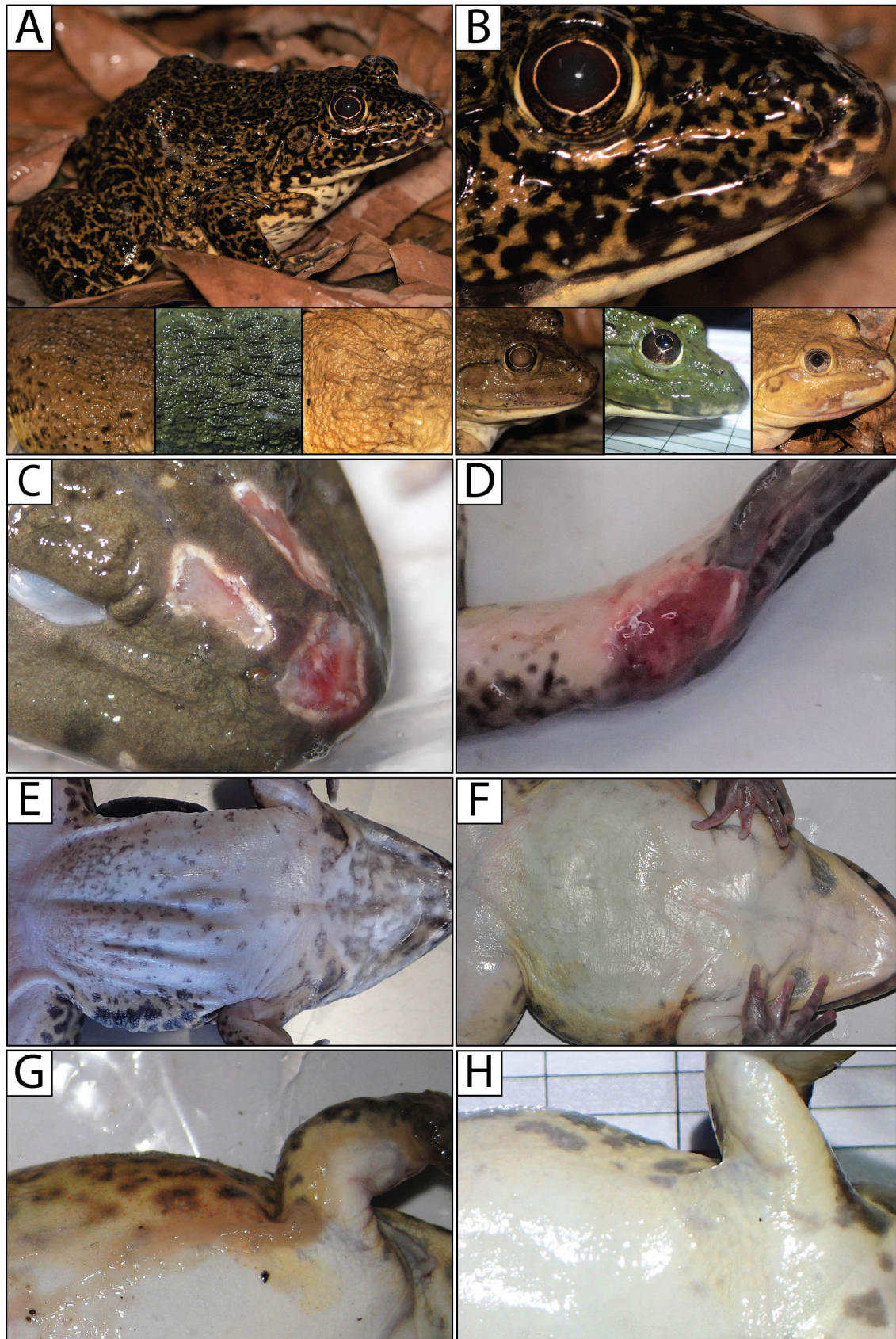
tissue sample and morphological measurements. For all individuals, we preserved a toe tip in 95% ethanol for genetic analysis.

### *Genetic analysis*

We extracted DNA from tissues using a kit (Qiagen DNeasy Blood & Tissue Kit), and amplified a 750-bp fragment of the mitochondrial cytochrome b gene (*cytb*) by using the L-14841 (5'-CTC CCA GCC CCA TCC AAC ATC TCA GCA TGA TGA AAC TTC G-3') and CB3-H (5'-GGC AAA TAG GAA GTA TCA TTC TG-3') primer pair (Kosuch et al. 2001). PCR amplifications were performed in 20- $\mu$ L volumes with the following cycling conditions: an initial denaturing step at 94 °C for 5 min; 35 cycles of denaturing at 94 °C for 45 s, annealing at 53 °C for 45 s and extending at 72 °C for 1 min; and a final extending step of 72 °C for 10 min. PCR products were purified using a commercial kit (Bioneer AccuPrep PCR Purification Kit) and sequenced in the forward and reverse directions using the PCR primers and a BigDye Terminator v3.1 Cycle Sequencing Kit at Beijing Genomics Institute (Hong Kong). New sequences from 25 individuals and those downloaded from GenBank (representing individuals from mainland China, Vietnam, Laos and Thailand) were aligned using MUSCLE (Edgar, 2004) in Geneious R11 (Kearse et al. 2012). A phylogenetic tree was constructed using the maximum likelihood algorithm (with 1000 bootstrap replicates) in RAxML v.8 (Stamatakis 2014). We included *Hoplobatrachus litoralis* (Hasan et al. 2012) and *Hoplobatrachus tigerinus* (Daudin, 1802) as outgroups. We used PopART (Leigh and Bryant 2015) to infer a haplotype network using the median-joining network algorithm (Bandelt et al. 1999), setting epsilon to zero. Individuals were grouped based on geographic origin.

### *Morphological measurement*

We chose seven morphological characteristics that can be examined easily and are potentially useful for distinguishing native and released *H. rugulosus* in Hong Kong (Figure 2): dorsal color, color of loreal region, wounds on snout, wounds on body, wounds on limbs, speckled pattern on belly and red/orange color on the lateral sides of body. Additionally, we took 20 morphological measurements from live specimens using digital calipers to the nearest 0.1 mm (Fei et al. 2010; Sung et al. 2014): snout-vent length (SVL), head length from tip of snout to rear of jaws (HDL), head width at the commissure of the jaws (HDW), snout length from tip of snout to the anterior corner of eye (SNT), horizontal diameter of the exposed portion of the eyeball (EYE), interorbital distance (IOD), horizontal diameter of tympanum (TMP), distance from anterior edge of tympanum to posterior corner of the eye (TEY), tibia length with the hindlimb flexed (TIB), foot length from proximal edge of inner metatarsal tubercle to tip of fourth toe (FOT), manus length from tip of third digit to proximal edge of inner palmar tubercle (ML), length of adpressed first finger (F1L), length of



**Figure 2.** Morphological characteristics of *Hoplobatrachus rugulosus* captured in Hong Kong. (A) dorsal color (dark brown [top], brown [bottom left], green [bottom middle] and yellow [bottom right]); (B) color of loreal region (dark brown [top], brown [bottom left], green [bottom middle] and yellow [bottom right]); (C) wounds on snout; (D) wounds on limbs; (E) speckled pattern on belly; (F) speckled pattern absent on belly; (G) red/orange pigment on lateral sides of body; (H) red/orange pigment absent on lateral sides of body. Photo by Ivan Lam, Henry Lee and Ken Lee.

adpressed second finger (F2L), length of adpressed third finger (F3L), length of adpressed fourth finger (F4L), length of adpressed first toe (T1L), length of adpressed second toe (T2L), length of adpressed third toe (T3L), length of adpressed fourth toe (T4L), length of adpressed fifth toe (T5L) and forearm and hand length (FHL). For consistency, all measurements were taken by the same person from the right side of the specimen's body. We could not obtain morphological measurement of five individuals (Pui O 119, Shek Pik 120, Shek Pik 121, Lok Fu Market 123 and Mong Kok Market 125), so these individuals were excluded from the morphological analysis (Supplementary material Table S1).

#### *Analysis of morphological measurement*

We removed seven morphological measurements (F2L, F3L, F4L, T1L, T2L, T3L and T4L) from the analysis because of the high ( $\geq 60\%$ ) correlation with other measurements. To control for differences due to body size, all measurements were normalized using snout-vent length (SVL) (Pawłowski et al. 2019). We used the genetic results to classify each individual (native and released) and determine which characteristics can be used to distinguish native and released frogs using three methods. First, we checked if the range of morphological measurements overlap between native and released frogs. Second, we used Mann-Whitney  $U$  tests to compare morphological measures between native and release individuals. As SVL-normalized measurements may differ between sex and age groups and confound the results, we compared the morphological measurements between age and sex groups (females, juveniles and males) and between sexes (females and males) using Wilcoxon tests for 120 wild *H. rugulosus* captured for a diet study in Hong Kong (including the 25 individuals sequenced this study). We excluded nine (out of 13) and two morphological characteristics that differed between age and sex groups and between sexes, respectively, from the Mann-Whitney  $U$  tests (Table S2). Further, as we detected putative hybrids from genetic analysis, we excluded the hybrids and ran separate tests. The results were similar with and without the putative hybrids (Table 2). Third, we applied a multivariate approach to compare the measurements. We used nonmetric multidimensional scaling (NMDS) to visualize, and an analysis of similarities (ANOSIM) to test the significance of the difference between native and released individuals (Clarke and Warwick, 2001). Similarity percentage analysis (SIMPER) was applied to determine the contribution of individual measurement to the difference.

## **Results**

### *Genetic analysis*

A total of 25 new individuals were included in the genetic analysis. These sequences were deposited in GenBank (Accession number = MZ322508–

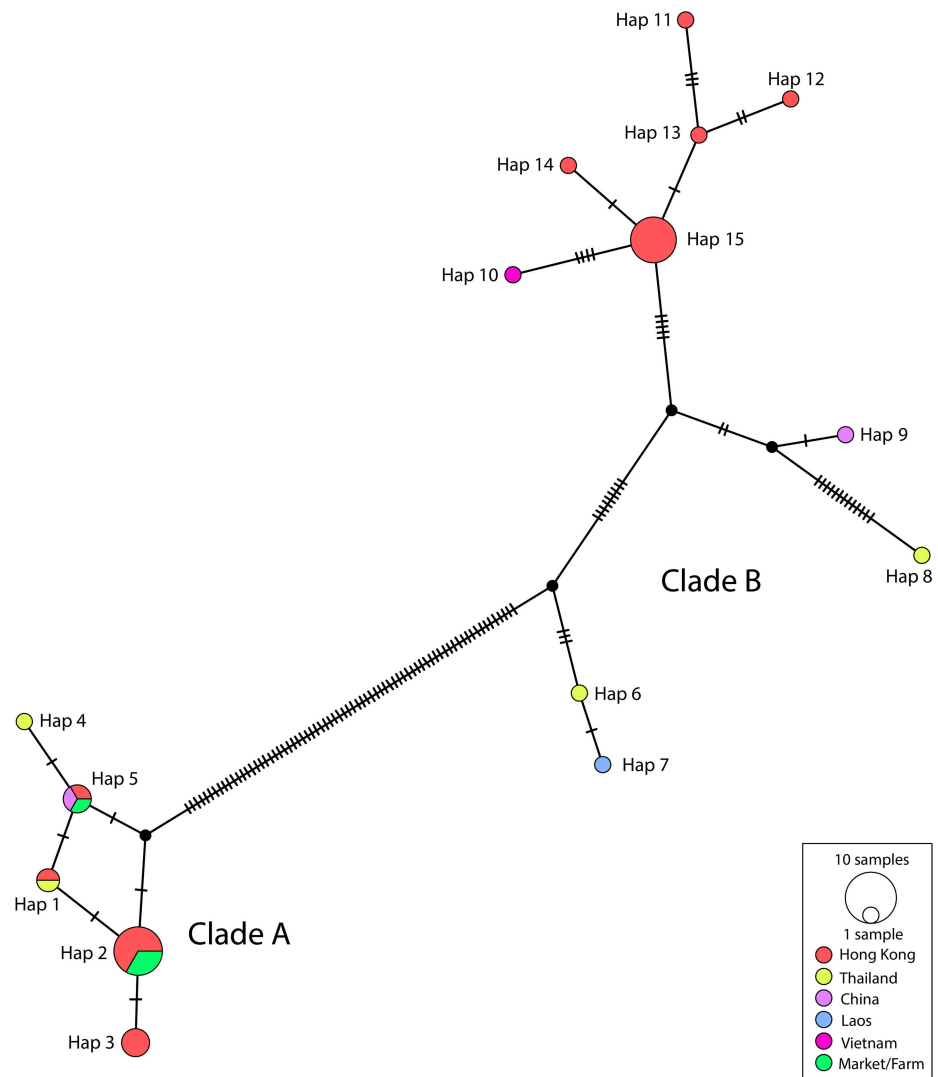


**Figure 3.** Maximum likelihood phylogenetic tree of *Hoplobatrachus rugulosus* inferred from sequence data of the mitochondrial cytochrome b gene (*cytb*). *Hoplobatrachus littoralis* and *Hoplobatrachus tigerinus* were used as outgroups. Bootstrap values for the major clades are shown above the major branches. Clade names follow Pansook et al. (2012). Samples from this study are denoted with bold text. Colored symbols indicate the key morphological characteristics to distinguish native and released frogs. Putative hybrids are highlighted in red.

MZ322532; Figure 3). All *H. rugulosus* individuals (including GenBank) formed a strongly-supported, monophyletic group (bootstrap=100; Figure 3). There were two major clades within *H. rugulosus* (bootstrap = 83–100), which corresponded to Clade A and Clade B of Pansook et al. (2012). The pairwise percent identity within clades (Clade A: 0–0.2%, Clade B: 0–1.4%) was lower than between clades (13.7–14.7%). The individuals collected from Hong Kong were found in both clades. Clade A includes 14 from seven locations in Hong Kong (Kowloon Reservoir, Long Valley, Pak Ngau Shek, Pok Fu Lam, Shek Kong, Tsing Yi and Tsiu Hang; Figure 1) and the two frogs acquired from food markets, clustering with wild *H. rugulosus* from western, central and eastern Thailand and individuals from Chinese frog farms (Figure 3). Clade B includes 11 individuals from six locations in Hong Kong (Lai Chi Wo, Long Valley, Pui O, Shek Pik, Tsiu Hang and Wu Kau Tang), clustering with wild *H. rugulosus* from China, Vietnam, Laos, northern and northeastern Thailand. Notably, frogs collected from two locations, Long Valley and Tsiu Hang, belong to both Clade A and B. Figure 4 shows a haplotype network between Clade A and Clade B, color-coded based on their geographic origin.

#### *Morphological examination and measurement*

We compared morphological characteristics and measurements between *H. rugulosus* from Hong Kong that belong to Clades A and B based on the



**Figure 4.** Median-joining network of *Hoplobatrachus rugulosus* haplotypes based on a sequence data of the mitochondrial cytochrome b gene (*cytb*) constructed in PopART. Haplotypes are represented by circles and the sizes are proportional to the number of individuals. Different colors represent the geographic origin of samples. Hong Kong samples in Clade A are released individuals. A \* indicates the haplotypes (Hap 2 and Hap 3) that contain putative hybrid individuals.

genetic results (Table 2). For dorsal color, 21% of Clade A individuals (released) were in yellow, whereas all native individuals were in brown, dark brown or green (Table 3; Figure 2). For color of loreal region, no native frogs were brown or yellow, while half of released frogs were in brown or yellow. For native individuals, no wounds were observed, whereas 21%, 29% and 43% of released individuals did on snout, body and limbs, respectively. Speckled pattern on belly was present in both released (50%) and native frogs (64%). 64% of released frogs had red/orange color on the lateral sides of body but absent in native frogs.

For the morphological measurements (Table 2), the ranges overlapped, but four were significantly different between Clade A and Clade B: HDL ( $W = 65$ ;  $P = 0.027$ ), TMP ( $W = 64.5$ ;  $P = 0.018$ ), TEY ( $W = 21$ ;  $P = 0.026$ ) and FOT ( $W = 65$ ;  $P = 0.028$ ). For multivariate analysis (NMDS), the morphological measurements differed between native and released individuals



**Table 2.** Morphological measurements (mean  $\pm$  SD [range]) of native and released *Hoplobatrachus rugulosus* in Hong Kong. Five individuals included in the genetic analysis were excluded from the morphological measurements (Lok Fu Market 123, Mong Kok Market 125, Pui O 119, Shek Pik 120, Shek Pik 121). All measurements were SVL-normalized to remove the influence of body size. Abbreviations of the measurements follow the text. Bold text indicates measurements significantly different between groups. We excluded morphological measurements that differ between age and sex groups (refer to Table S2).

SVL-normalized measurement	Native (Clade B)	Released (Clade A)	Mann-Whitney <i>U</i> Test (All)		Mann-Whitney <i>U</i> Test (Adults only)		Mann-Whitney <i>U</i> Test (Adults only and exclude putative hybrids)	
			<i>U</i>	<i>P</i>	<i>U</i>	<i>P</i>	<i>U</i>	<i>P</i>
HDL	0.244 $\pm$ 0.030 (0.216–0.296)	0.251 $\pm$ 0.075 (0.193–0.396)			<b>65</b>	<b>0.027</b>	<b>56</b>	<b>0.009</b>
HDW	0.334 $\pm$ 0.025 (0.298–0.380)	0.345 $\pm$ 0.035 (0.306–0.421)	41	0.613	58	0.114	49	0.066
SNT	0.159 $\pm$ 0.020 (0.133–0.193)	0.145 $\pm$ 0.009 (0.134–0.162)			57	0.122	43.5	0.199
EYE	0.091 $\pm$ 0.020 (0.059–0.119)	0.076 $\pm$ 0.016 (0.055–0.098)						
IOD	0.066 $\pm$ 0.012 (0.039–0.081)	0.053 $\pm$ 0.005 (0.048–0.062)			46	0.611	34	0.837
TMP	0.073 $\pm$ 0.104 (0.058–0.085)	0.06 $\pm$ 0.006 (0.046–0.071)			<b>64.5</b>	<b>0.018</b>	<b>49.5</b>	<b>0.039</b>
TEY	0.043 $\pm$ 0.0046 (0.037–0.053)	0.05 $\pm$ 0.007 (0.038–0.057)	<b>21</b>	<b>0.026</b>	33.5	0.555	22.5	0.313
TIB	0.416 $\pm$ 0.020 (0.387–0.450)	0.397 $\pm$ 0.026 (0.347–0.454)						
ML	0.209 $\pm$ 0.013 (0.188–0.231)	0.212 $\pm$ 0.019 (0.168–0.236)			38.5	0.924	30	0.907
FOT	0.464 $\pm$ 0.460 (0.407–0.530)	0.411 $\pm$ 0.043 (0.319–0.472)	72	0.068	<b>65</b>	<b>0.028</b>	<b>51</b>	<b>0.041</b>
F1L	0.133 $\pm$ 0.021 (0.104–0.150)	0.124 $\pm$ 0.027 (0.872–0.176)			55	0.191	46	0.130
T5L	0.258 $\pm$ 0.023 (0.222–0.283)	0.223 $\pm$ 0.056 (0.145–0.298)			57	0.141	49	0.071
FHL	0.359 $\pm$ 0.021 (0.327–0.385)	0.368 $\pm$ 0.028 (0.323–0.401)	38	0.460	44.5	0.720	36.5	0.631

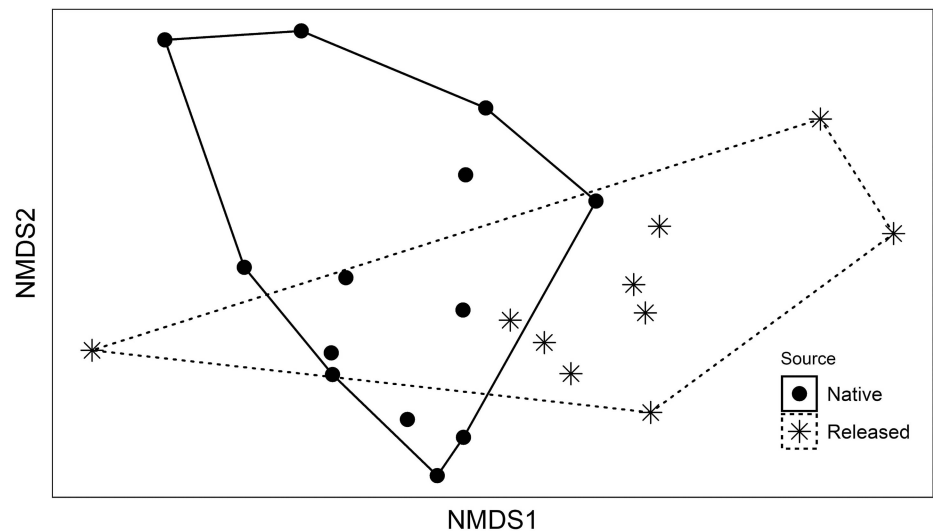
**Table 3.** Comparison of morphological characteristics between Clade A and Clade B *Hoplobatrachus rugulosus* in Hong Kong. Classification as Clade A (released) and B (native) is based on the genetic analysis.

Characteristics		Native (Clade B)	Released (Clade A)
Dorsal color	Brown	9.1%	28.6%
	Dark brown	63.6%	21.4%
	Green	27.3%	28.6%
	Yellow	0%	21.4%
Color of loreal region	Brown	0%	28.6%
	Dark brown	54.5%	21.4%
	Green	45.5%	28.6%
	Yellow	0%	21.4%
Wounds on snout	Yes	0%	21.4%
Wounds on body	Yes	0%	28.6%
Wound on limbs	Yes	0%	42.9%
Speckled pattern on belly	Yes	63.6%	50.0%
Red/orange pigment on lateral sides of body	Yes	0%	64.3%

( $R = 0.335$ ;  $P < 0.001$ ; Figure 5). The top five measurements that contributed to the dissimilarity were FOT (14.7%), T5L (14.3%), HDL (12.3%), T1B (7.8%) and F1L (7.2%).

## Discussion

In this study, we investigated mercy release of *H. rugulosus* in Hong Kong using genetic and morphological data. Genetically we identified the presence



**Figure 5.** Two-dimensional non-metric multidimensional scaling (NMDS) representing Bray-Curtis distances between native and released *Hoplobatrachus rugulosus* in Hong Kong.

of the two major clades in Hong Kong, one that is native (Clade B) and one non-native (Clade A). The two clades differ by 13.7–14.7%, which indicates that they likely represent different species. This will need to be addressed with a study focusing on this issue. For our study, aiming to identify the source of individuals in Hong Kong, the non-native individuals were likely released individuals sourced from wet markets. We compared the morphology of individuals from the two genetic clades and identify morphological characteristics (dorsal color, coloration of loreal region, presence of wounds, coloration on lateral sides) and measurements (IOD, TMP, TEY, FOT) that can be useful for identification. We discuss these results in detail, as well as provide recommendations on the management of released *H. rugulosus*.

In China, consumption of *H. rugulosus* is supplied primarily by breeding farms sourced with wild individuals from Thailand (Yu et al. 2015). We believe that frogs sold in Hong Kong wet markets are from the same sources, since the two market exemplars in our study (Lok Fu Market 123, Mong Kok Market 125) were genetically members of Clade A (Figure 3). These frogs from wet markets are the likely source of released animals in Hong Kong because *H. rugulosus* captured from the mercy release hotspots (Pak Ngau Shek, Pok Fu Lam, Kowloon Reservoir, Shek Kong and Tsing Yi) were members of Clade A. *Hoplobatrachus rugulosus* is not the only species released at these sites, and other introduced species are also commonly found, such as the Red-eared slider, *Trachemys scripta elegans* (Wied, 1838) and Chinese water dragon, *Physignathus cocincinus* (Cuvier, 1829). To prevent or eliminate the negative impacts of introduced organisms on local environments, extra effort needs to be made at these sites to both stop mercy release and eradicate released animals.

There were two sites, Long Valley and Tsiu Hang, where both native and released individuals were found. Both sites are co-managed by non-governmental organizations and the government for conservation and

environmental education, and are not mercy release hotspots. Long Valley is composed of freshwater wetland and farmland whereas Tsiu Hang is a protected area with artificial ponds and freshwater wetlands. The presence of released individuals at these sites indicates that mercy release is widespread in Hong Kong and may include natural and/or conservation-focused environments. Morphologically, the released individuals (Tsiu Hang 117, Long Valley 1, Long Valley 2 and Long Valley 3) at these sites look similar to native frogs, since they do not have any wounds and have a dark brown dorsal color. Although not in the scope of our study, this raises the possibility of hybridization. Such hybridization can occur in farms or in the wild, with the latter resulting from mercy release. Hybridization between native and released frog will cause genetic pollution in local populations (Pagano et al. 2003). Additional genetic analysis using nuclear genes needs to be done to verify whether hybridization can and does occur in the wild.

We found that three morphological characteristics are useful in distinguishing native and released *H. rugulosus* – body color, the presence of wounds and lateral color. The body color of *H. rugulosus* is highly variable (Figure 2). However, there are some general guidelines that can be followed; the dorsal color of native individuals is darker in color, and paler for the released individuals. Although only a small proportion of released individuals were yellow (21%), no native individuals were yellow. For wounds, at least 66% of released frogs had them on their snout, body or limbs, while native individuals had none. Such wounds are usually a result of being kept in cages, and are frequently seen on individuals in wet markets. For lateral color, red or orange pigment is present in 58% of released frogs, but absent in all native frogs. In summary, lighter color, the presence of wounds and red/orange color on the lateral sides of the body are likely characters of released individual, but the absence of these characters does not guarantee the individuals are native. Although we identified three morphological measurements were significantly different between native and released frogs (IOD, TMP, TEY and FOT), they may not be as useful in the field, as it would take time to measure and there is overlap between the two groups. We believe that the combination of the three morphological characters (body color, wounds and lateral color) can be used for a quick field assessment to identify some (but not all) released individuals for eradication. However, in sensitive situations where more precision is needed, such as a declining population, we suggest to also incorporate genetic data for identification.

We strongly suggest that the mercy release of *H. rugulosus* should be strictly prohibited. In addition to the potential of genetic pollution of native populations, exotic amphibians may introduce disease, such as chytrid fungus, *Batrachochytrium dendrobatidis* (Longcore et al. 1999), into the native ecosystems. Chytrid fungus has caused dramatic amphibian population declines globally (Skerratt et al. 2007). A previous study in

China has investigated the presence of chytrid fungus in both introduced American bullfrogs, *Lithobates catesbeianus* (Shaw, 1802) and native amphibian species (Bai et al. 2010). Moreover, released *H. rugulosus* may prey on native species (Wiles et al. 2003) and compete with the native conspecifics (Ings et al. 2006). More studies are needed to understand the ecological impacts of the released Chinese bullfrogs in Hong Kong, but we have already identified the potential for hybridization between native and released *H. rugulosus*. Therefore, we suggest the immediate ban on mercy release and prohibiting by law the release of any kinds of exotic species. This should be supplemented with educational activities that raise public awareness on the negative aspects of mercy release and introduced species, such as talks and eco-tours. Eradication guidelines should also be developed by experts, as a reference for governments, conservation groups and the public.

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### Authors' contribution

Wing-Ho Lee and Yik-Hei Sung conceptualized the research; Jonathan J. Fong, Wing-Ho Lee and Yik-Hei Sung prepared the sampling design and methodology; Wing-Him Lee, Wing-Ho Lee and Yik-Hei Sung participated in the investigation and data collection; Wing-Him Lee, Jonathan J. Fong and Yik-Hei Sung performed data analysis and interpretation; Yik-Hei Sung prepared the ethics approval; Yik-Hei Sung received funding; and Wing-Him Lee, Jonathan J. Fong, Wing-Ho Lee and Yik-Hei Sung participated in writing and editing the manuscript.

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### Ethics and permits

Methods used in this study were approved by the committee on the use of human and animal subjects of Hong Kong Baptist University (permit number: HASC/15-16/0010).

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### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Information and morphological measurements of the specimens included in the phylogenetic analysis.

**Table S2.** Results of the comparison of morphological measurements between sex and age groups using Kruskal-Wallis *H* and Mann-Whitney *U* tests.

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