TOPIC AREA

QUALITY SEEDSTOCK DEVELOPMENT

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GENETIC DIVERSITY OF STRIPED SNAKEHEAD (*CHANNA STRIATA*) IN CAMBODIA AND VIETNAM

Quality Seedstock Development/Study/16QSD01UC

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ABSTRACT

Striped snakehead (*Channa striata*) culture has recently been allowed again in Cambodia after a ban of 12 years. In Vietnam, this species has been domesticated since 1990s. Domestication of this species in Cambodia requires genetic information to maintain good genetic resources for sustainable aquaculture. For this purpose, we collected wild samples of striped snakehead from eight locations in Cambodia including five localities around the Tonle Sap Lake (Battambang, Siem Reap, Pursat, Kampong Thom, and Kampong Chnang,) and three in the Mekong River floodplains (Kampong Cham, Kandal, and Prey Veng). We also analyzed three domesticated and wild populations in Vietnam. Sequence data of two mitochondrial markers (Cytochrome b and D-loop region) revealed that the highest level of genetic diversity was found in wild snakehead populations in Cambodia, and the lowest was in Vietnamese domesticated populations. There were no significant genetic differences among domesticated populations, but collectively domesticated populations were significantly different from wild populations. These results indicated that genetic diversity of domesticated snakehead in Vietnam decreased along with the number of domestication generations, providing useful information for snakehead farming in Cambodia. In Cambodia, suitable management of broodstock should be provided to farmers to minimize the genetic loss due to inbreeding and random genetic drift associated with the domestication process. In addition, genetic diversity of broodstock in hatcheries should be monitored periodically in order to have genetic improvement strategies in time. On the other hand, the low genetic diversity of hatchery populations in Vietnam indicated an urgent need for genetic improvement programs to prevent inbreeding depression.

INTRODUCTION

Striped snakehead (*Channa striata*) is an economically important species in both culture and capture fisheries throughout Southeast Asian countries, particularly in the Lower Mekong River basin of Cambodia and Viet Nam. Snakehead farming history in Cambodia and Viet Nam provides a typical example of species domestication for aquaculture. In Vietnam, commercial farming of striped snakehead started in the 1990s (Sinh *et al.* 2014) and rapidly developed recently due to achievements in formulated feed development (Hien *et al.* 2015, 2017a) and feeding technology, especially weaning methods for fish larvae (Hien *et al.* 2016, 2017b). In Cambodia, however, the government put a ban on snakehead farming in September 2004. Reasons for this ban were: 1) potential negative impacts

on wild snakehead populations from wasteful snakehead seed collection and on other fish species diversity, particularly the freshwater small-sized fish used as feed for snakehead aquaculture, and 2) potential negative effects on poor consumer groups from decreased availability of small-sized fish due to dependence of snakehead aquaculture on small-sized fish (So 2009). The ban was lifted in June 2016 thanks to research on artificial propagation and larval and grow-out culture of striped snakehead with formulated feed (So *et al.* 2011; Nen *et al.* 2015). For sustainable aquaculture, it is critical to choose good sources with high levels of genetic diversity for domestication and breeding and appropriate broodstock management in captive conditions (Tave 1993; Dunham 2011).

Striped snakehead is a nesting-spawning and short-lived species with one-year generation time. These biological characteristics (Ellegren *et al.* 2016) and breeding techniques in captive conditions (Tave 1993; Dunham 2011) can affect species genetic diversity. The spawning season takes place from April to August, most prominently from April to June (So *et al.* 2011; Duong *et al.* 2014). In hatcheries, breeding of this species can be either semi-artificial or hormone-induced. At the main spawning season, semi-artificial methods are usually applied by farmers. One or two pairs of mature males and females are stocked in a small pond (around 10 m^2) with artificial nests using aquatic plants or artificial materials. Fish spawn after several days of daily water exchange. At a larger scale, mature fish can be induced by hormones (Marimuthu *et al.* 2007)). Striped snakehead seed production in the Viet Nam Mekong Delta is mainly supplied by local farmers at small scales (Sinh *et al.* 2014). Thus, effective breeding numbers can be predictably low, contributing to rapid decrease of genetic diversity of hatchery broodstock.

Different situations of domestication and wild sources of striped snakehead between Cambodia and Vietnam can affect differently genetic diversity of this species. In the wild, genetic diversity of populations can be threatened by over-exploitation, habitat fragmentation, introduced species, and also by interbreeding with escaped hatchery-bred individuals (Hutchings & Fraser 2008; Laikre *et al.* 2010). In aquaculture, low genetic diversity of broodstock can result in bad seed quality such as high mortality and susceptibility to diseases, or low growth rates. Long-term domestication can result in low levels of genetic diversity of hatchery-bred fish populations due to small population sizes and inappropriate broodstock management (Tave 1999; Hallerman 2008). Further genetic improvement of such populations requires genetic information of these populations and other possible sources for genetic exchange. On the other hand, in the process of domestication of potential cultured species, evaluating genetic diversity of different sources is an important step to establish good base populations (Eknath *et al.* 2007; Dunham 2011).

OBJECTIVE

Objectives include:

1. To characterize and compare genetic diversity of (1) wild (non-domesticated) snakehead populations collected from different natural water bodies in Cambodia, and (2) Cambodia wild (non-domesticated) striped snakehead and Vietnamese domesticated striped snakehead (*Channa striata*) collected from different hatcheries in the Mekong Delta, inferred from mitochondrial DNA markers.
2. To provide basic information and science-based recommendations for (1) striped snakehead domestication and selective breeding and farming in Cambodia, and (2) possible exchange of snakehead genetic resources between Cambodia and Vietnam

MATEIALS AND METHODS

Sample collection

Fish samples were collected from the wild in Cambodia and from wild and hatchery-bred populations in Vietnam (Figure 1). In Cambodia, wild (non-domesticated) snakehead samples were collected from 5 locations (including Siem Reap, SR; Battambang, BB; Kampong Thom, KT; Pursat, PS; and

Kampong Chnang, CHN) in the Tonle Sap Lake, the largest and most productive lake in South-East Asia, and three locations in the Mekong River floodplains (Kampong Cham, KCH; Kandal, KD; Prey Veng, PV). In Vietnam, fish were collected in 3 hatcheries located in the main areas of snakehead farming and reproduction (including Dong Thap DT, An Giang AG, and Hau Giang HG) and wild populations in two conservation parks (Long An LA and Ca Mau CM) and in an aquaculture area (Hau Giang HG).

Genetic analysis

DNA from fin clips from 20-30 individuals of each population was extracted using Wizard® SV Genomic DNA Purification kit (Promega, USA). DNA extracts were amplified (polymerase chain reaction, PCR) for two mitochondrial genes (mtDNA) including Cytochrome b and D-loop (or the control region) using universal primer pairs L15803/H16461 (Briolay *et al.* 1998) and D-loop-Thr-F/ D-loop-Phe-R (Cheng *et al.* 2012), respectively. The PCR thermal cycles for Cytochrome b included 1 cycle of 94°C at 2 minutes, 1 cycle of 50°C at 1 minute, 1 cycle of 71°C at 1 minute, and 38 cycles of 94°C at 30 seconds, 50°C at 30 seconds and 71°C at 30 seconds, and the last extension of 71°C at 10 minutes. D-loop was amplified at the following conditions: 1 cycle of 94°C at 4 minutes, 35 cycles of 94°C at 30 seconds, 55°C at 30 seconds, 72°C at 90 seconds, and the last step of 1 cycle of 72°C at 5 minutes (Cheng *et al.* 2012). The PCR ingredients were based on Tsigenopoulos and Berrebi (2000) for Cytochrome b and Cheng et al. (2012) for D-loop region. PCR products were visualized on 1.7% agarose gels and then sent for DNA sequencing at First BASE Laboratories Sdn Bhd (Selangor, Malaysia).

Data analysis

Sequences were first aligned by ClustalW and checked for ambiguous bases for each population using MEGA7 (Kumar *et al.* 2016) and Finch TV version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com). The program MEGA was employed to test the best fitting of the nucleotide substitution models based on the lowest Akaike Information Criterion (AIC) (Posada & Buckley 2004). The Tamura and Nei model with the lowest AIC was chosen for further analyses. DnaSP 5.0 (Librado & Rozas 2009) was used to estimate molecular genetic diversity indices (including the number of haplotypes, haplotype diversity, nucleotide diversity) for each population and for three groups: Cambodian, Vietnamese wild and Vietnamese domesticated populations.

For phylogenetic analyses, haplotype distribution was first obtained from Arlequin ver. 3.5 (Excoffier & Lischer 2010). Haplotype data were then constructed into a phylogenetic tree based on the Tamura and Nei model with 1000 bootstrappings (using MEGA7) and the Neighbor-Joining (NJ) tree using NETWORK software (fluxus-engineering.com) to examine the phylogenetic relationship among haplotypes.

Genetic structure of snakehead in the Lower Mekong Basin was evaluated using genetic distances and F_{st}. Genetic distances were based on the Tamura and Nei model, which was determined based on the lowest AIC when testing the best fitting of the nucleotide substitution models (Posada & Buckley 2004). Within- and between-group genetic distances were calculated using MEGA 7.0. Estimation of F_{st}-based genetic distances among populations was performed with 5000 permutations using Arlequin ver. 3.5 (Excoffier & Lischer 2010).

RESULTS

Genetic diversity of striped snakehead across populations

A total of 262 sequences of Cytochrome b and 279 sequences of D-loop with the final trimmed sequences of 585 bp and 914 bp in length, respectively, were obtained from striped snakehead wild and cultured populations in Cambodia and Vietnam. The nucleotide frequencies of Cytochrome b

were 22.91% (A), 30.93% (T), 32.83% (C), and 13.33% (G), and those of D-loop were 34.32% (A); 28.94% (T); 22.08% (C), and 14.66% (G). The estimated transition/transversion bias (R) was 20.74 for Cytochrome b and 12.08 for D-loop region.

All sequences of Cytochrome b revealed 26 polymorphic sites (11 singleton variable sites and 15 parsimony informative sites) generating 28 haplotypes (Annex 1), of which 23 haplotypes were found in Cambodian wild populations, 8 haplotypes were in Vietnamese wild populations and 2 haplotypes were in Vietnamese domesticated fish (Table 2). Other molecular genetic diversity indices including haplotype diversity and nucleotide diversity of Cambodian striped snakehead populations (0.760±0.033 and 0.00239, respectively) were also highest, whereas those of Vietnamese domesticated populations were lowest (0.034±0.033 and 0.00006, respectively).

D-loop sequence data revealed similar results (Table 2). There were 128 haplotypes found in all samples with 102 haplotypes for Cambodian populations, 29 for Vietnamese wild and 5 for Vietnamese domesticated populations. Overall haplotype diversity of striped snakehead was 0.946±0.101.

At a smaller scale, genetic diversity of each population was compared with others within and between countries. In Cambodia, five populations in Tonle Sap floodplains overall had significantly higher diversity indices compared to those in the Mekong River floodplains (KCH, KD and PV) and this result was more obvious in Cytochrome b compared to D-loop sequences. In Vietnam, genetic diversity within hatchery populations was very low, especially the one in Dong Thap (DT) where there were only one haplotype of Cytochrome b and two haplotypes of D-loop region. Although Cambodian populations had higher diversity indices than wild ones in Vietnam, at least one Vietnam population (in LA) was more genetically diverse than those in KD and PV (e.g. nucleotide diversity based on Cytochrome b was 0.00152 for LA, 0.00101 for KD and 0.00045 for PV, Table 2).

Phylogeographic relationships among haplotypes

Haplotypes of Cytochrome b and D-loop were region-specific. Cambodian populations had 19 (of 28) unique haplotypes for Cytochrome b and 95/128 for D-loop, while those of Vietnamese wild populations were 4/28 and 22/128; and of Vietnamese domesticated populations were 1/28 and 2/128, respectively.

In the diagram of median-joining network based on Cytochrome b, H-3 with the highest frequency (60.31%) could be the most ancestral haplotype and was shared by three groups of populations (Figure 2). Other haplotypes presented at low frequencies (0.38-9.54%) and were not clearly structured. The most frequent D-loop haplotype was H-10 (22.22%), which is the only haplotype shared by three fish groups. Because of the large number of haplotypes (n=128) with low frequencies (0.36-2.87%), D-loop haplotypes were impractical to visualize by a network.

Genetic structure of striped snakehead in the Lower Mekong Basin

Genetic distances based on the Tamura-Nei parameter using Cytochrome b data were significant in most pairwise comparisons among wild populations, except for pairwise comparisons of domesticated populations among themselves and between these and PV in Cambodia (Annex 2). Two populations in the Mekong River floodplains in Cambodia had the smallest genetic differences with five wild and domesticated populations along the Mekong River in Vietnam (means of genetic distances range 0.0002 – 0.0010), except the CM population (0.0016) far from the River.

Values of F_{st} based on pairwise differences in Cytochrome b showed slightly different results where populations were geographically structured. Five populations in the Tonle Sap floodplains (Cambodia) were not significantly different from zero ($P>0.05$) and some of them differed ($P<0.05$)

from populations in the Lower Mekong KD and PV (Table 3). Non-significant values of F_{st} were found between KD and PV, pairwise domesticated populations, and between Vietnamese wild populations LS and HG. Cambodian KCH and Vietnam CM populations showed the highest F_{st} (ranging from 0.155-0.456 for KCH and 0.395-0.780 for CM), significantly different from all other populations ($P<0.01$).

Genetic distance (Annex 3) and F_{st} based on D-loop haplotypes showed some similar results. The Vietnamese population CM (but not Cambodian KCH as Cytochrome b) also had the highest values of F_{st}, ranging from 0.090-0.559 (Table 4). Low and no pairwise genetic differences were found among populations in Tonle Sap floodplains, two populations in Cambodian Mekong floodplains (KD and PV), and Vietnam domesticated fish.

However, when populations in Cambodia and Vietnam were compared, two genes revealed different inferences. Tamura-Nei genetic distances and F_{st} based on Cytochrome b indicate that Cambodian KD and PV populations were genetically closed to Vietnam populations along the Mekong River (except for CM) whereas D-loop data (Table 4 and Annex 2) showed that all Cambodian populations differed from Vietnam wild and domesticated populations (except wild HG). Domesticated populations were highly diverged in the D-loop region from most wild populations in both countries.

DISCUSSION

Genetic diversity between wild and domesticated striped snakehead populations

Results of the study revealed (i) significantly low levels of genetic diversity in domesticated striped snakehead populations in Vietnam compared to wild populations, and (ii) the overall highest genetic diversity in Cambodian wild populations. However, in smaller geographic areas, two populations in the Cambodian Mekong floodplains had lower levels of genetic diversity compared to five populations in the Tonle Sap floodplains and one Vietnam wild population.

Low genetic diversity in hatchery-bred populations has also been reported in various species such as barramundi, *Lates calcarifer* (Frost *et al.* 2006), gilthead sea bream, *Sparus aurata* (Brown *et al.* 2005), Japanese flounder, *Paralichthys olivaceus* (Sekino *et al.* 2002); and ayu, *Plecoglossus altivelis* (Iguchi *et al.* 1999). In barramundi, for example, loss of genetic diversity in cultured populations was attributed to hatchery practices including small effective population sizes of broodstock, high variation in offspring survivals and offspring contributions among breeders (Frost *et al.* 2006). These factors could also be the case for striped snakehead cultured in Vietnam. Most of the seed suppliers are small-scale farmers who own small numbers of broodstock (100-300 individuals), and a portion of fish farmers (4.3%, N= 635) can propagate seed for themselves using broodstock selected from their grow-out ponds (Sinh *et al.* 2014). Due to small breeder population sizes, genetic diversity can decrease rapidly as a result of genetic drift and inbreeding (Tave 1993; Allendorf & Luikart 2007). Negative effects of small population sizes become more severe as striped snakehead in Vietnam has been domesticated for many generations, >25 generations (domestication since 1990s with one-year generation time), evidenced by smaller haplotype diversity and nucleotide diversity than wild populations. Several other species were also reported to have low effective population sizes (N_e) in hatchery broodstock populations. For instance, N_e ranged from 14 to 18 for gilthead sea bream (Brown *et al.* 2005), or from 3 to 30 for Indian carps and thus inbreeding increased from 2% to 17% per year (Eknath & Doyle 1990). Besides, variation in offspring contribution (due to differences in offspring produced and their survival) among breeders, a common phenomenon in fish (Hedrick 2005), could lessen effective population sizes and thus lower genetic diversity of striped snakehead. In addition, differences in offspring among striped snakehead families can be caused by cannibalism behavior (Qin & Fast 1996; Abol-Munafi *et al.* 2004). Another factor that reduces N_e is unbalanced sex ratios of breeders, as reported in other aquaculture species such as gilthead sea bream, *Sparus aurata* (Brown *et al.* 2005). However, in snakehead, breeding behavior requires pairing where both

parents build their nest and take care of offspring. Therefore, equal sex is also applied in artificial reproduction, and thus does not affect genetic diversity of this species.

Wild populations in the Lower Mekong River basin generally have high levels of genetic diversity compared to other fish species using similar markers. In another snakehead species *Channa marulius*, Habib et al. (2011) found that wild fish collected from three rivers in India had haplotype diversity (***Hd***) of 0.763 and nucleotide diversity (***pi***) value of 0.0128 based on sequences of 307 bp Cytochrome b mtDNA. Three populations of bighead catfish *Clarias macrocephalus* sampled in Peninsular Malaysia had lower ***pi*** (0.003) and ***Hd*** (varying from 0.657 to 0.765) (Nazia *et al.* 2010) compared to striped snakehead based on the same two genes cytochrome b and D-loop in this study (mean **pi** of two genes from 0.004 – 0.008, and **Hd** from 0.748 – 0.875). Different levels of genetic diversity among striped snakehead wild populations can be related to population sizes and different pressures of exploitation because overfishing is a main factor causing the decrease of genetic diversity in wild fish populations (Pinsky & Palumbi 2014). Among wild populations, five populations in the Tonle Sap Lake (Cambodia) showed the highest level of genetic diversity, consistent with the abundance of the species in the most productive and largest lake (Lim *et al.* 1999; Campbell *et al.* 2006).

Phylogeographic relationships and genetic structure of striped snakehead in the Lower Mekong River basin

Striped snakehead has the capacity for long-distance migration through physical connectivity (Adamson *et al.* 2010; Tan *et al.* 2012) and can also perform strong localized migration (Amilhat & Lorenzen 2005). Long migration can explain a close genetic relationship between two populations, KD and PV, in Cambodia with wild populations located along the Mekong River in Vietnam (i.e. wild HG and LA). In addition, gene flow among populations between the two countries can be caused by anthropogenic factors such as transportation along Mekong River or striped snakehead trading from Vietnam to Cambodia (Sinh *et al.* 2014). Migration of striped snakehead is also supported by the dense network of rivers and canals in the Lower Mekong basin. Although the CM population in Vietnam genetically differed from the Cambodian wild populations, data from mtDNA markers showed that the number of migrants per generation (Nm) between Cambodian striped snakehead populations with CM were less than 1 based on Cytochrome b but varied from 2-5 based on D-loop, indicating that the CM population was not isolated from the other wild populations. Further studies sampling striped snakehead from other rivers outside the Mekong River should be carried out to understand phylogeographic relationships among striped snakehead populations in the native distribution range.

Three groups (Cambodian-, Vietnam wild- and domesticated- groups) of striped snakehead had unique haplotypes for both Cytochrome b and D-loop. However, there is no relationship between geographic distances and haplotype tree or network. Most of the unique haplotypes are diverged from the common haplotype (e.g., Hap-3 in Cytochrome b), indicating they are recent mutants (Posada & Crandall 2001). Adamson et al. (2010) found that conspecific divergence of striped snakehead could have occurred in the late Miocene, about 7 million years ago.

Implications for snakehead domestication and genetic improvement

Findings on the genetic diversity of striped snakehead in the Mekong basin revealed important implications for breeding and broodstock selection of striped snakehead in Cambodia, where domestication of this species has just been started. Wild snakehead populations in the Tonle Sap can be good sources for breeding and domestication programs. A lesson from Vietnam striped snakehead farming, that genetic diversity of striped snakehead cultured populations decreases rapidly, mainly due to small effective population sizes, indicates that genetic monitoring should be regularly carried

out in Cambodia during the domestication programs with the participation of small-scale fish farmers. This lesson is also useful for other fish species newly used in aquaculture. On the other hand, low genetic diversity of hatchery populations in Vietnam requires an urgent need of genetic improvement programs to prevent inbreeding depression. Replacing or supplementing local wild individuals to current hatchery broodstock can be one of the solutions (Vuorinen 1984; Garcia-Marin *et al.* 1991) in addition to increasing population sizes (Tave 1999).

Given relatively large genetic differences among Vietnamese and Cambodian populations and high levels of genetic diversity in most wild populations, genetic resource exchange of striped snakehead between the two countries is not necessary. Such action can lead to outbreeding depression (McClelland & Naish 2007; Whiteley *et al.* 2015). Moreover, results from this study have changed the intention (before genetic information was available) of using the Vietnamese domesticated strain for breeding and farming striped snakehead in Cambodia because of their superiority in growth, survival and artificial feed intake compared to Cambodian wild strains (Nen *et al.* 2015). Based on high genetic diversity, Tonle Sap populations are recommended to be used as broodstock resources for domestication in Cambodia.

CONCLUSIONS

Genetic diversity was highest in Cambodia wild populations and lowest in Vietnam domesticated populations. Domesticated populations genetically differed from wild fish in different locations. The lesson that genetic diversity of cultured snakehead in Vietnam has decreased rapidly mainly due to small-scale seed production with small effective population sizes along with a long history of domestication can be useful for snakehead farming in Cambodia. Genetic structure of the species in a large range of their distribution along the Mekong River basin was shaped by natural density of rivers/canals and anthropogenic factors.

QUANTIFIABLE ANTICIPATED BENEFITS

Cambodian and Vietnamese agencies can use this result for training fish farmers on the genetic issues in fish farming and breeding.

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TABLES AND FIGURES

Table 1. Sampling locations and population character (wild W or domesticated D) of striped snakehead populations in Cambodia and Vietnam

Locality	Abbr.	Latitude	Longtitude	Population character	Number of samples sequenced	
					Cytochrome b	D-loop
In Cambodia						
1. Siem Reap	SR	13.230908	103.835629	W	19	20
2. Battambang	BB	13.232911	103.658981	W	14	20
3. Kampong Thom	KT	12.741941	104.260975	W	19	20
4. Pursat	PS	12.568085	104.240538	W	23	20
5. Kampong Chnang	CHN	12.510229	104.449958	W	19	19
6. Kampong Cham	KCH	11.928478	105.425273	W	21	21
7. Kandal	KD	11.243789	105.023308	W	16	20
8. Prey Veng	PV	11.32384	105.287469	W	14	20
In Vietnam						
9. Long An	LA	10.774585	105.710519	W	21	20
10. Hau Giang	HG	9.726790	105.72621	W	20	20
11. Ca Mau	CM	9.273056	104.988583	W	18	20
12. An Giang	AG	10.6823	105.101584	D	20	20
13. Dong Thap	DT	10.75603	105.34919	D	17	19
14. Hau Giang	HG	9.651031	105.559292	D	21	20

Note: Locations were identified by GCS WGS84

Table 2. Summary of genetic diversity (number of haplotype **H**, haplotype diversity **Hd**, and nucleotide diversity **pi**) of snakehead populations based on Cytochrome b and D-loop sequences

Abbr.	Cytochrome b			D-loop		
	H	**Hd**	**pi**	**H**	**Hd**	**pi**
In Cambodia						
SR	6	0.784±0.059	0.00192	16	0.979±0.021	0.01234
BB	6	0.802±0.090	0.00270	15	0.968±0.00064	0.01362
KT	10	0.784±0.098	0.00270	17	0.979±0.024	0.01025
PS	10	0.866±0.049	0.00307	18	0.989±0.019	0.01305
CHN	9	0.813±0.081	0.00246	18	0.994±0.00037	0.01005
KCH	4	0.710±0.060	0.00251	8	0.829±0.00418	0.01114
KD	4	0.525±0.137	0.00101	16	0.979±0.021	0.01255
PV	2	0.264±0.136	0.00045	17	0.979±0.024	0.01136
Average	***23***	***0.760±0.033***	***0.00239***	***104***	***0.990 ± 0.002***	***0.01205***
In Vietnam						
Wild LA	2	0.095±0.084	0.00016	9	0.705±0.111	0.00689
Wild HG	8	0.647±0.120	0.00152	19	0.995±0.0003	0.01183
Wild CM	2	0.366±0.112	0.00063	8	0.816±0.071	0.00838
Average	***8***	***0.561±0.003***	***0.00152***	***29***	***0.936 ± 0.019***	***0.01058***
Domes. AG	2	0.100±0.088	0.00017	4	0.553±0.111	0.00305
Domes. DT	1	0.000±0.000	0.00000	2	0.199±0.112	0.00261
Domes. HG	1	0.000±0.000	0.00000	4	0.505±0.126	0.00375
Sub-total	***2***	***0.034±0.033***	***0.00006***	***5***	***0.431 ± 0.076***	***0.00314***
Total	**28**	**0.620±0.034**	**0.00169**	**130**	**0.946±0.0106**	**0.01104**

Note: Domes. = domestication

Table 3. Values of pairwise F_{st} based on pairwise difference in cytochrome b among striped snakehead populations in Cambodia and Vietnam (Population abbreviations are presented in Table 1)

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. SR	0													
2. BB	0.0096	0												
3. KT	-0.014	-0.016	0											
4. PS	-0.013	0.046	0.033	0										
5. CHN	-0.032	-0.030	-0.030	0.007	0									
6. KCH	0.232**	0.204**	0.155**	0.222**	0.204**	0								
7. KD	0.087*	0.015	0.010	0.128**	0.010	0.254**	0							
8. PV	0.044	0.038	0.006	0.072*	0.010	0.242**	0.056	0						
9. W-LA	0.177**	0.110**	0.066**	0.187**	0.086**	0.317**	0.027	0.075	0					
10. W-HG	0.088**	0.041	0.034*	0.119**	0.037	0.219**	0.010	-0.008	0.003	0				
11. W-CM	0.487**	0.429**	0.395**	0.410**	0.420**	0.456**	0.565**	0.653**	0.731**	0.408**	0			
12. D-AG	0.172**	0.105**	0.063*	0.182**	0.087*	0.310**	0.059*	0.071	0.000	0.011	0.725**	0		
13. D-DT	0.167**	0.097**	0.054**	0.173**	0.079**	0.303**	0.059*	0.097	-0.010	0.003	0.759**	-0.008	0	
14. D-HG	0.191**	0.120**	0.070**	0.194**	0.098**	0.330**	0.077*	0.120	0.000	0.014**	0.780**	0.003	0	0

Table 4. Pairwise F_{st} based on pairwise difference in Dloop sequences among striped snakehead populations in Cambodia and Vietnam (Population abbreviations are presented in Table 1)

Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1. SR	0													
2. BB	0.020	0												
3. KT	0.018	0.070*	0											
4. PS	-0.004	0.028	0.067*	0										
5. CHN	-0.014	0.042	-0.015	0.023	0									
6. KCH	0.060*	0.068**	0.017	0.084**	0.022	0								
7. KD	0.053*	0.056*	-0.003	0.086**	0.017	-0.003	0							
8. PV	0.016	0.047*	-0.005	0.025	-0.009	0.027	0.016	0						
9. W-LA	0.209**	0.226**	0.130*	0.220**	0.174**	0.112**	0.127**	0.134**	0					
10. W-HG	0.046	0.063*	0.033	0.055*	0.024	0.023	0.023	0.003	0.071*	0				
11. W-CM	0.177**	0.090**	0.212**	0.163**	0.162**	0.140**	0.132**	0.151**	0.359**	0.154**	0			
12. D-AG	0.405**	0.395**	0.321**	0.382**	0.379**	0.259**	0.311**	0.307**	0.073*	0.210**	0.559**	0		
13. D-DT	0.398**	0.389**	0.313**	0.375**	0.374**	0.252**	0.303**	0.301**	0.036	0.232**	0.554**	0.049	0	
14. D-HG	0.364**	0.363**	0.276**	0.348**	0.334**	0.228**	0.272**	0.267**	0.024	0.182**	0.524**	-0.038	-0.007	0

Annex 1. Haplotypes with positions of polimorphism of 585- bp cytochrome b

Position	**3**	**4**	**5**	**5**	**5**	**7**	**1**	**1**	**1**	**1**	**2**	**2**	**2**	**3**	**3**	**3**	**3**	**3**	**3**	**4**	**4**	**4**	**4**	**4**	**5**	**5**
	0	**6**	**2**	**3**	**8**	**0**	**0**	**4**	**8**	**9**	**2**	**4**	**7**	**0**	**2**	**3**	**5**	**6**	**7**	**0**	**1**	**3**	**3**	**7**	**2**	**2**
Haplotypes	**5**	**6**	**2**	**3**	**8**	**0**	**3**	**0**	**7**	**6**	**7**	**4**	**4**	**7**	**5**	**7**	**8**	**1**	**0**	**3**	**5**	**0**	**3**	**8**	**3**	**4**
Hap_1	G	T	T	C	C	C	C	C	G	A	C	T	C	T	G	A	C	G	C	G	G	T	G	G	T	C
Hap_2	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_3	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_4	.	.	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
Hap_5	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
Hap_6	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	A	.	.	.	.	.
Hap_7	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_8	A	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_9	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_10	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
Hap_11	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
Hap_12	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
Hap_13	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_14	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T
Hap_15	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
Hap_16	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
Hap_17	.	.	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_18	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_19	.	.	.	T	.	.	.	.	.	G	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
Hap_21	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_22	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
Hap_23	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_24	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_25	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
Hap_26	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
Hap_27	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.
Hap_28	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Annex 2. Genetic distances (estimated using the Tamura and Nei model) based on Cytochrome b gene among Cambodian and VN (wild W, and domesticated D) populations

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. SR		0.0009	0.0009	0.0010	0.0009	0.0013	0.0007	0.0007	0.0006	0.0007	0.0015	0.0006	0.0006	0.0006
2. BB	0.0023		0.0009	0.0011	0.0009	0.0012	0.0007	0.0007	0.0006	0.0007	0.0014	0.0006	0.0006	0.0006
3. KT	0.0023	0.0027		0.0010	0.0008	0.0012	0.0006	0.0006	0.0005	0.0006	0.0014	0.0005	0.0005	0.0005
4. PS	0.0024	0.0030	0.0030		0.0010	0.0014	0.0009	0.0009	0.0009	0.0009	0.0016	0.0009	0.0009	0.0009
5. CHN	0.0021	0.0025	0.0025	0.0028		0.0012	0.0007	0.0006	0.0005	0.0006	0.0014	0.0005	0.0005	0.0005
6. KCH	0.0029	0.0033	0.0031	0.0036	0.0031		0.0011	0.0011	0.0010	0.0011	0.0016	0.0010	0.0010	0.0010
7. KD	0.0016	0.0019	0.0019	0.0024	0.0018	0.0024		0.0004	0.0003	0.0004	0.0013	0.0003	0.0003	0.0003
8. PV	0.0013	0.0016	0.0016	0.0019	0.0015	0.0021	0.0008		0.0002	0.0004	0.0013	0.0003	0.0002	0.0002
9. W-LA	0.0013	0.0016	0.0015	0.0020	0.0014	0.0020	0.0006	0.0003		0.0003	0.0013	0.0001	0.0001	0.0001
10. W-HG	0.0019	0.0022	0.0022	0.0026	0.0021	0.0026	0.0013	0.0010	0.0008		0.0013	0.0003	0.0003	0.0003
11. W-CM	0.0025	0.0028	0.0028	0.0032	0.0027	0.0030	0.0019	0.0016	0.0014	0.0018		0.0013	0.0013	0.0013
12. D-AG	0.0013	0.0016	0.0015	0.0020	0.0014	0.0020	0.0006	0.0003	0.0002	0.0009	0.0014		0.0001	0.0001
13. D-DT	0.0012	0.0015	0.0014	0.0019	0.0014	0.0019	0.0005	0.0002	0.0001	0.0008	0.0013	0.0001		0.0000
14. D-HG	0.0012	0.0015	0.0014	0.0019	0.0014	0.0019	0.0005	0.0002	0.0001	0.0008	0.0013	0.0001	0.0000	

Annex 3. Genetic distances (estimated using the Tamura and Nei model) based on D-loop region among Cambodian and VN (wild W, and domesticated D) populations

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. SR		0.0021	0.0019	0.0020	0.0019	0.0022	0.0021	0.0020	0.0022	0.0021	0.0022	0.0024	0.0024	0.0024
2. BB	0.0122		0.0021	0.0022	0.0021	0.0023	0.0023	0.0022	0.0024	0.0022	0.0021	0.0026	0.0026	0.0025
3. KT	0.0104	0.0130		0.0022	0.0020	0.0022	0.0021	0.0020	0.0021	0.0020	0.0022	0.0021	0.0022	0.0021
4. PS	0.0115	0.0138	0.0126		0.0021	0.0024	0.0023	0.0022	0.0024	0.0022	0.0023	0.0026	0.0026	0.0025
5. CHN	0.0099	0.0125	0.0101	0.0118		0.0022	0.0021	0.0021	0.0022	0.0020	0.0021	0.0023	0.0024	0.0023
6. KCH	0.0121	0.0141	0.0118	0.0138	0.0116		0.0023	0.0023	0.0022	0.0022	0.0023	0.0022	0.0023	0.0022
7. KD	0.0120	0.0141	0.0115	0.0141	0.0116	0.0131		0.0022	0.0022	0.0022	0.0022	0.0023	0.0023	0.0023
8. PV	0.0109	0.0133	0.0109	0.0126	0.0107	0.0124	0.0123		0.0021	0.0021	0.0022	0.0022	0.0022	0.0022
9. W-LA	0.0108	0.0134	0.0100	0.0128	0.0103	0.0111	0.0113	0.0107		0.0019	0.0025	0.0012	0.0012	0.0012
10. W-HG	0.0115	0.0138	0.0116	0.0133	0.0113	0.0125	0.0127	0.0118	0.0102		0.0022	0.0018	0.0019	0.0019
11. W-CM	0.0113	0.0123	0.0120	0.0129	0.0111	0.0126	0.0122	0.0118	0.0121	0.0121		0.0029	0.0028	0.0028
12. D-AG	0.0110	0.0140	0.0099	0.0131	0.0105	0.0110	0.0115	0.0105	0.0054	0.0095	0.0131		0.0008	0.0010
13. D-DT	0.0106	0.0136	0.0095	0.0126	0.0101	0.0107	0.0111	0.0102	0.0050	0.0096	0.0126	0.0030		0.0009
14. D-HG	0.0109	0.0138	0.0098	0.0129	0.0104	0.0109	0.0114	0.0104	0.0055	0.0096	0.0129	0.0033	0.0032	

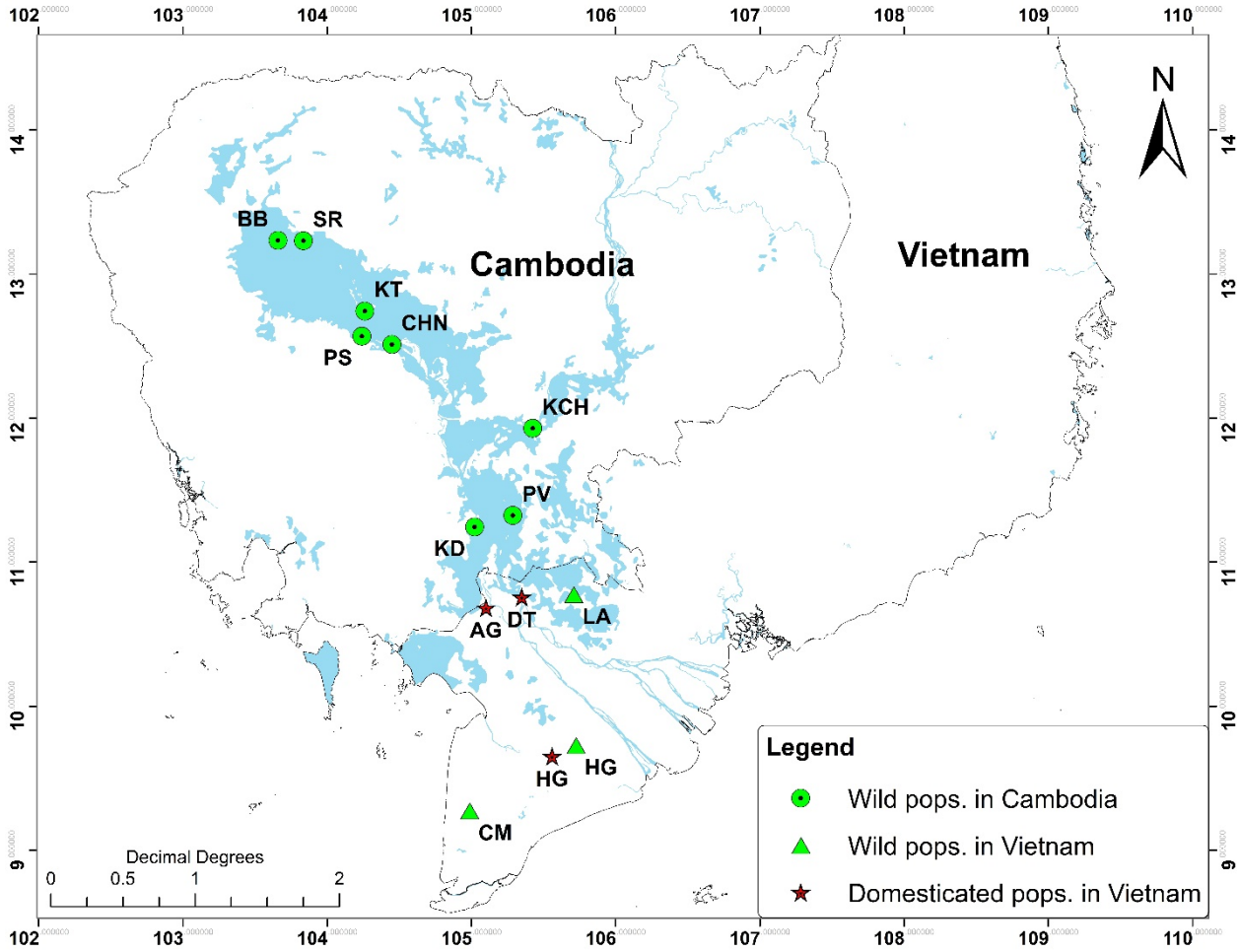

Figure 1. Sampling locations for striped snakehead in Cambodia and Vietnam.

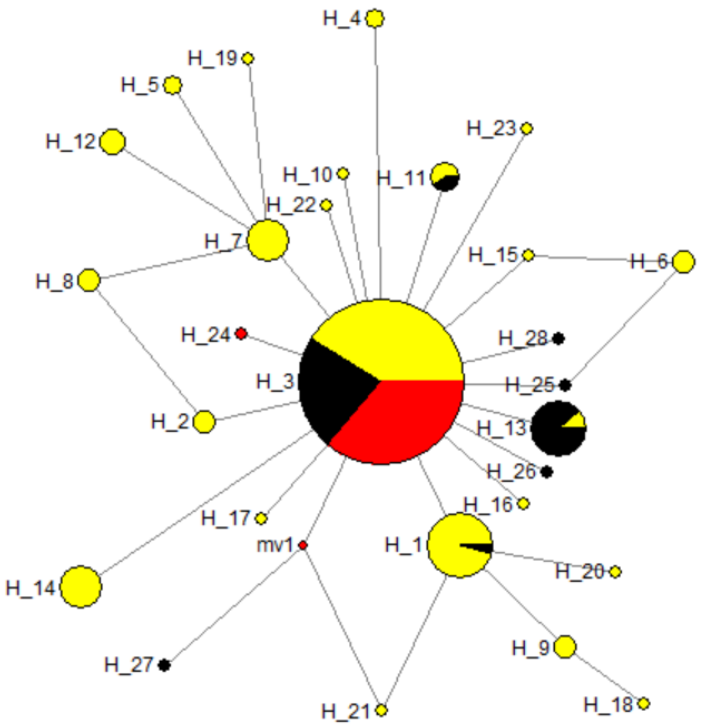

Figure 2. Median-joining network of Cytochrome b haplotypes of striped snakehead in Cambodia and Vietnam. Sizes of circles represent the haplotype frequencies. Yellow, black and red stand for Cambodian wild-, Vietnam wild- and domesticated-populations, respectively.