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Genetic diversity and structure of striped snakehead (*Channa striata*) in the Lower Mekong Basin: Implications for aquaculture and fisheries management

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ABSTRACT

Striped snakehead (*Channa striata*) has been cultured for several decades in Viet Nam while only recently cultured in Cambodia. A cross-country comparison of genetic diversity in wild and cultured striped snakehead populations provided novel insights for improved genetic resource management and cultivation in Lower Mekong region. We collected striped snakehead samples from three wild and three cultured populations in the Vietnamese Mekong Delta for comparison to samples from eight wild populations in Cambodia (n = 5 and 3 from Tonle Sap Lake and Mekong River floodplain, respectively). Sequencing of cytochrome b (585 bp) and D-loop (874 bp) from 270 individuals yielded 28 and 128 haplotypes, respectively, resulting in 150 concatenated haplotypes. Mean genetic diversity indices of concatenated sequences were highest in wild Tonle Sap Lake populations (haplotype diversity **Hd** = 0.994 ± 0.004, nucleotide diversity **pi** = 0.0077 ± 0.0009), intermediate in wild Cambodian Mekong River populations (**Hd** = 0.925 ± 0.097, **pi** = 0.0076 ± 0.0005) and wild Vietnamese Mekong River populations (**Hd** = 0.832 ± 0.152, **pi** = 0.0061 ± 0.0018), and lowest in cultured Vietnamese populations (**Hd** = 0.451 ± 0.198, **pi** = 0.0021 ± 0.0002). The wild Tonle Sap Lake and cultured Vietnamese populations differed significantly in all genetic diversity indices ($P < 0.05$). The unique haplotypes and significant genetic divergence ($P < 0.01$) among striped snakehead populations from each habitat (e.g., lake or floodplain, wild or cultured) suggests habitat-specific genetic structure. Genetic differences among all wild populations were also positively correlated with hydrological distance in the range of 600 km ($P < 0.01$), suggesting isolation by distance. These findings have important implications for appropriate management of wild and cultured *C. striata* in Viet Nam and Cambodia.

1. Introduction

Striped snakehead fish (*Channa striata*, Bloch 1793) is an economically important species in both capture fisheries and farming in the Lower Mekong River Basin of Cambodia and Viet Nam, due to its popularity as a food and high market values. It is one of the top ten species of inland fisheries in Cambodia, mostly from Tonle Sap Lake (Lamberts, 2001). In Viet Nam, wild snakehead is also over-exploited in paddy areas (Cong et al., 2009) although catch production is low, mainly serving for local consumption (Sinh et al., 2014). Differences in fishing pressure, areas of water bodies, and hydrology practices may affect population sizes of snakehead differently between the two countries. In aquaculture, snakehead farming in these two countries has a different history of domestication. In Viet Nam, commercial farming of striped snakehead has been carried out with hatchery-reared fingerlings since the 1990s (Sinh et al., 2014) and has developed rapidly in recent years due to achievements in formulated feed development (Hien et al., 2017a, 2015) and feeding technology, especially weaning methods for fish larvae (Hien et al., 2017b, 2016). In Cambodia, before 2004, striped snakehead culture was based on wild stock, and small-size fish (or 'trash fish') were used as feed. In September 2004, the Cambodian government put a ban on snakehead farming. Main reasons for this ban were the potential negative impacts on wild snakehead populations from wasteful seed collection and on the diversity of small-sized

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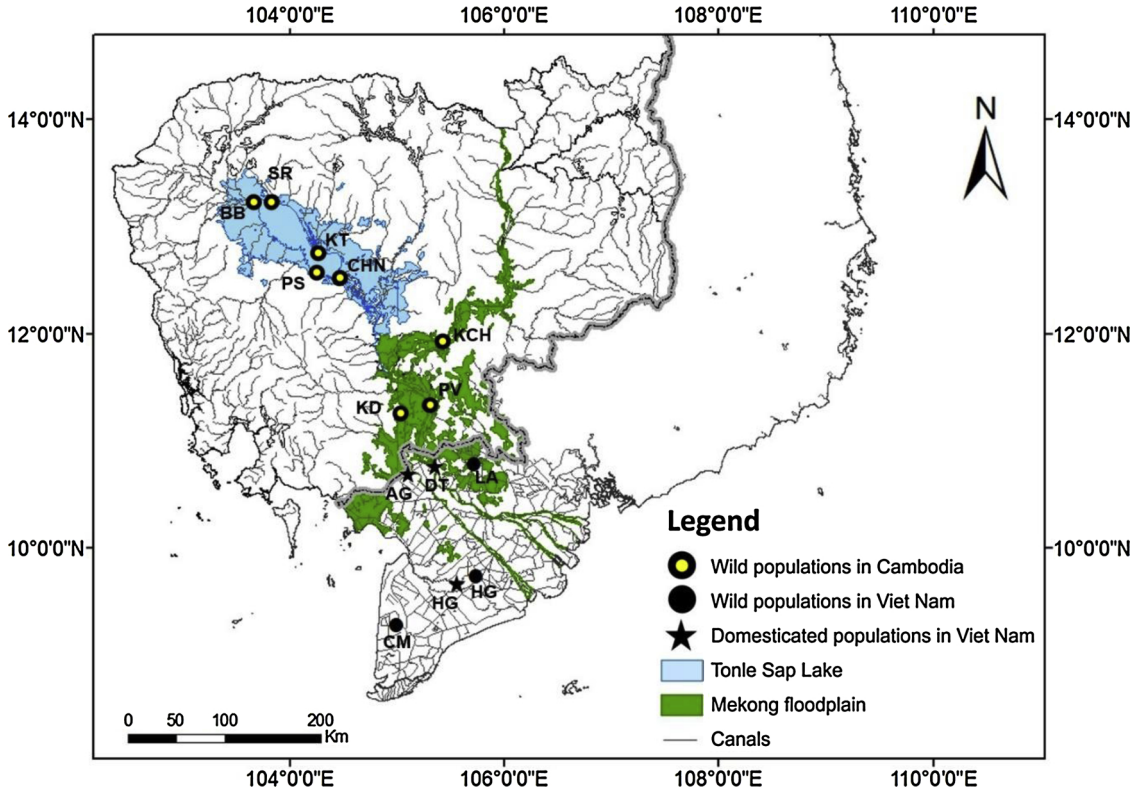

Fig. 1. Sampling locations for striped snakehead in Cambodia and Viet Nam.

freshwater fish used as feed for snakehead (Hien et al., 2015; Nen et al., 2015). The ban was recently lifted in June 2016 (Decision 325 by Cambodian Ministry of Agriculture Forestry and Fishery) after successful collaborative studies on artificial propagation, larval production, and grow-out of striped snakehead with formulated feed (Nen et al., 2015; So et al., 2011). For sustainable striped snakehead farming in Cambodia, it is critical to choose good broodstock sources with high levels of genetic diversity for domestication and breeding, and appropriate broodstock management in captive conditions (Dunham, 2011; Tave, 1993).

Genetic diversity of striped snakehead can be affected by the species' biology, hatchery practice and seed production scales. Snakehead is characterized as a nesting-spawning species where parents provide care for young offspring. Romiguier et al. (2014) reported that such parental care species can have lower genetic diversity (based on nucleotide diversity) compared to broadcasting spawning organisms. In addition, snakehead can mature within one year. Short generation time can positively correlate with genetic diversity of wild populations (Ellegren et al., 2016). However, in hatcheries, early maturation increases the number of generations over a period of domestication, and together with breeding practices such as unbalanced sex ratios or/and pooling gametes can decrease levels of genetic diversity (Dunham, 2011; Tave, 1993). Hatchery-bred snakehead seed can be produced by semi-artificial or hormone-induced propagation. During their peaking spawning season from June to August (Courtenay and Williams, 2004), semi-artificial methods are commonly applied by small-scale farmers. One or two pairs of mature males and females are stocked in a small pond (around 10 m^2) containing artificial nests made of aquatic plants or artificial materials. Snakehead breeders spawn after several days of daily water exchange. On a larger scale or at early and late spawning season, snakehead propagation can be induced by hormones (Marimuthu et al., 2007). However, striped snakehead seed in the Vietnam Mekong Delta is mainly produced by small-scale farmers (Sinh et al., 2014).

Genetic management and improvement of snakehead culture in Vietnam require genetic information of captive populations and other possible wild sources for genetic exchange. On the other hand, in the process of domestication of potential cultured species in Cambodia, evaluating genetic diversity of different sources is an important step to establish genetically sound base populations (Dunham, 2011; Eknath et al., 2007).

Previous genetic studies of striped snakehead mainly focused on phylogeographic features of wild populations. Tan et al (2012) found that snakehead collected from different isolated regions in Malaysia were structured mainly by natural physical barriers and anthropogenic activities. Another study based on Cytochrome C oxidase subunit I (COI) found similar genetic differentiation among Malaysia and Indonesia (Sumatra island) populations (Siti-Balkhis et al., 2011; Tan et al., 2015). In India, eight riverine populations are genetically structured as a result of isolation by geographic distance (Baisvar et al., 2018). However, the geographic distance used in the above studies may not reflect the water distance which is directly involved in the natural migration of fish species. In addition, there is little information on genetic diversity of the species in captive and natural conditions, especially in the Lower Mekong River Basin, where aquaculture and capture fisheries of snakehead occur intensively and concurrently.

This study aimed to quantify and compare genetic diversity between wild striped snakehead populations in Cambodia with wild and domesticated fish populations in Viet Nam and to understand the genetic structure of this species in the water network of the Lower Mekong River Basin. Investigations of genetic diversity of striped snakehead in cultured and wild environments in both countries can provide lessons not only for domestication and conservation strategies of snakehead but also for other fish species in the region.

2. Materials and methods

2.1. Sample collection

Wild fish populations were collected from Cambodia and both wild

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

Locality	Abbr.	Latitude	Longtitude	Population character	Number of samples sequenced
In Cambodia					
1. Siem Reap	SR	13°13'51.3"N	103°50'08.3"E	W	20
2. Battambang	BB	13°13'58.5"N	103°39'32.3"E	W	20
3. Kampong Thom	KT	12°44'31.0"N	104°15'39.5"E	W	19
4. Pursat	PS	12°34'05.1"N	104°14'25.9"E	W	20
5. Kampong Chhnang	CHN	12°30'36.8"N	104°26'59.9"E	W	19
6. Kampong Cham	KCH	11°55'42.5"N	105°25'31.0"E	W	19
7. Kandal	KD	11°14'37.6"N	105°01'23.9"E	W	20
8. Prey Veng	PV	11°19'25.8"N	105°17'14.9"E	W	18
In Viet Nam					
9. Long An	LA	10°46'28.5"N	105°42'37.9"E	W	20
10. Hau Giang	HG	9°43'36.4"N	105°43'34.4"E	W	20
11. Ca Mau	CM	9°16'23.0"N	104°59'18.9"E	W	18
12. An Giang	AG	10°40'56.3"N	105°06'05.7"E	D	20
13. Dong Thap	DT	10°45'21.7"N	105°20'57.1"E	D	17
14. Hau Giang	HG	9°39'03.7"N	105°33'33.5"E	D	20

and hatchery-bred populations from Viet Nam (Fig. 1, Table 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 Chhnang, CHN) in the basin of the Tonle Sap Lake, which connects to the Mekong River, and three locations in the Mekong River floodplain (Kampong Cham, KCH; Kandal, KD; Prey Veng, PV). In Viet Nam, fish were collected from 3 hatcheries (located in main areas of snakehead fish farming and reproduction including Dong Thap DT, An Giang AG, and Hau Giang HG) and from three wild populations in the Mekong Delta (two populations in wetland conservation parks (Long An, LA and Ca Mau, CM) and one from an aquaculture area (Hau Giang, HG)).

2.2. Genetic analysis

Fin clips taken from 20 to 30 individuals of each population had DNA extracted using Wizard® SV Genomic DNA Purification kit (Promega, USA). DNA of each sample was amplified (polymerase chain reaction, PCR) for two mitochondrial markers (mtDNA) including Cytochrome b gene 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 ingredients and thermal cycles were based on Tsigenopoulos and Berrebi (2000) for Cytochrome *b* and Cheng et al. (2012) for D-loop region. PCR products were visualized on 1.5% agarose gels and then sent for DNA sequencing (two-way direction for two samples/population and one-way direction or the rest) at First BASE Laboratories Sdn Bhd (Selangor, Malaysia).

2.3. Data analysis

Sequences from each marker 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). Two markers (Cytochrome b and D-loop) were then concatenated for further analyses. The program MEGA was employed to test the best fitting of the nucleotide substitution models based on the lowest Akaike Information Criterion (AIC) (Posada and Buckley, 2004). The Tamura and Nei model with the lowest AIC was chosen for further analyses. DnaSP 5.0 (Librado and Rozas, 2009) were used to estimate molecular genetic diversity indices (including the number of haplotypes, haplotype diversity, and nucleotide diversity) for each population. Genetic diversity indices were compared among four groups of (i) Cambodian Tonle Sap Lake, (ii) Cambodian Mekong River floodplain; (iii) Vietnamese wild, and (iv) Vietnamese domesticated populations (Fig. 1) using non-parametric Kruskal-Wallis tests. Significant Kruskal-Wallis tests would be followed by Dunn tests using the Benjamini-Hochberg correction method for multiple comparisons. These statistical analyses were conducted in R (R Core Team, 2017).

For phylogenetic analyses, haplotype data for each marker and concatenated sequences were obtained from DnaSP 5.0. Haplotype data were then used to construct the Neighbor-Joining (NJ) phylogenetic tree based on the Tamura and Nei model with bootstrapping 1000 times (using MEGA7) and the Median-Joining (MJ) tree using NETWORK software (fluxus-engineering.com), to examine the phylogenetic relationship among haplotypes. NJ and MJ trees showed similar topologies, therefore, only MJ reconstruction was presented.

Genetic structure of snakehead in the Lower Mekong Basin was evaluated based on genetic distances, genetic differences (F_{ST}), and partitioning of genetic variation (AMOVA). Within and between groups genetic distances based on the Tamura and Nei model (estimating the number of base substitutions per site) were calculated using MEGA7. Estimation of F_{ST}-based genetic differences among populations was performed with 5000 permutations using Arlequin ver. 3.5 (Excoffier and Lischer, 2010). AMOVA analyses implemented in Arlequin software were performed to compare distribution patterns of genetic diversity within and among sampling locations in three alternative hypotheses of hierarchical population structure, including (i) two groups by countries; (ii) three groups of Cambodian wild, Vietnamese wild, and Vietnamese hatcheries; and (iii) four groups of lake and floodplain habitats in Cambodia, and Vietnamese wild and hatchery samples. In addition, we tested the "isolation by distance" hypothesis by using Program ISOLDE implemented in GENEPOP 4.0 (Raymond and Rousset, 1995; Rousset, 2008). Spearman Rank correlation between matrices of pairwise genetic distances (computed as $F_{ST}/(1 - F_{ST})$ values) and the logarithm of pairwise water (river/canal) distances (km) was tested by Mantel tests with 5000 permutations. The strength of a significant correlation between two matrices was evaluated based on parameters of the linear regression of $F_{ST}/(1 - F_{ST})$ on the natural logarithm of hydrological distances. Distances along rivers or canals between all pairs of sampling locations were estimated using Image J 1.x software (Schneider et al., 2012) based on the GIS (Geographical Information System) inland water data downloaded at http://www.diva-gis.org. ArcMap 10.2 program (ESRI, 2013) was used to combine GIS data to build a map with the water system of Cambodia and Viet Nam (Fig. 1).

3. Results

3.1. Genetic diversity of striped snakehead fish across populations

A total of 270 sequences concatenated from both Cytochrome b (585 bp) and D-loop (874 bp) were obtained from the striped snakehead wild and cultured populations in Cambodia and Viet Nam. Fragments of

Table 2
Summary of genetic diversity indices of snakehead populations based on Cytochrome b, D-loop sequences, and the concatenated sequences.

	Cyt B- H	Dloop-H	Concatenated sequences		
			H	Hd	pi
Cambodia – Tonle Sap Lake					
SR	6	16	19	0.995 ± 0.018	0.0069 ± 0.0006
BB	6	15	18	0.994 ± 0.019	0.0076 ± 0.0020
KT	10	17	18	0.994 ± 0.019	0.0075 ± 0.0009
PS	10	18	18	0.989 ± 0.019	0.0092 ± 0.0010
CHN	9	18	19	1.000 ± 0.017	0.0072 ± 0.0004
Average	***8.2 ± 2.0 b***	***16.8 ± 1.3 b***	***18.4 ± 0.5 b***	***0.994 ± 0.004 b***	***0.0077 ± 0.0009 b***
Cambodia – Mekong River floodplain					
KCH	4	8	8	0.813 ± 0.080	0.0073 ± 0.0007
KD	4	16	16	0.979 ± 0.021	0.0082 ± 0.0006
PV	2	17	17	0.982 ± 0.026	0.0073 ± 0.0004
Average	***3.3 ± 1.2 ab***	***13.7 ± 4.9 ab***	***13.7 ± 4.9 ab***	***0.925 ± 0.097 ab***	***0.0076 ± 0.0005 ab***
Viet Nam – Wild (W) populations					
W-LA	2	9	9	0.705 ± 0.111	0.0044 ± 0.0010
W-HG	8	19	20	1.000 ± 0.016	0.0080 ± 0.0007
W-CM	2	8	8	0.791 ± 0.087	0.0059 ± 0.0009
Average	***4.0 ± 3.5 ab***	***12.0 ± 6.1 ab***	***12.3 ± 6.7 ab***	***0.832 ± 0.152 ab***	***0.0061 ± 0.0018 ab***
Viet Nam – Domesticated (D) populations					
D-AG	2	4	5	0.616 ± 0.106	0.0020 ± 0.0008
D-DT	1	2	2	0.233 ± 0.126	0.0019 ± 0.0010
D-HG	1	4	5	0.505 ± 0.126	0.0024 ± 0.0009
Average	***1.3 ± 0.6 a***	***3.3 ± 1.2 a***	***4.0 ± 1.7 a***	***0.451 ± 0.198 a***	***0.0021 ± 0.0002 ab***
Total	**28**	**128**	**150**	**0.955 ± 0.009**	**0.0077 ± 0.0002**

Note: H = number of haplotypes; Hd = haplotype diversity; pi = nucleotide diversity.
Avearge values in the same column with the same letters are not significantly different (P > 0.05).

Cytochrome b revealed 26 polymorphic sites (11 singleton variable sites and 15 parsimonious informative sites) generating 28 haplotypes (accession numbers MK258875–MK258902). Meanwhile, 128 D-loop haplotypes (accession numbers MK258903–MK259030) resulted from 100 variable sites (32 singleton and 68 parsimonious informative sites). The concatenated mtDNA sequences generated 150 haplotypes, of which 81 were found in Tonle Sap samples (18 and 19 haplotypes per population), 39 in Cambodian Mekong floodplain (8–17 haplotypes per population), 31 in Vietnamese wild populations (8–20 haplotypes per population), and 18 in Vietnamese domesticated fish (Table 2). Other mean genetic diversity indices were highest in Tonle Sap Lake populations (haplotype diversity ***Hd*** = 0.994 ± 0.004 and nucleotide diversity ***pi*** = 0.0077 ± 0.0009), intermediate in wild Cambodian Mekong River (***Hd*** = 0.925 ± 0.097, ***pi*** = 0.0076 ± 0.0005) and wild Vietnamese populations (***Hd*** = 0.832 ± 0.152, ***pi*** = 0.0061 ± 0.0018), and lowest in cultured Vietnamese populations (***Hd*** = 0.451 ± 0.198, ***pi*** = 0.0021 ± 0.0002). The wild Tonle Sap Lake and cultured Vietnamese populations differed significantly in all genetic diversity indices (all P values = 0.017). Meanwhile, other pairwise comparisons among wild populations in Cambodia and Viet Nam, and between wild Mekong and Vietnamese domesticated populations were not significant (P > 0.05). However, the effect sizes between wild Mekong and domesticated groups were large, ranging from 1.84 to 3.64 folds (Table 2). Overall genetic diversity of striped snakehead in the Lower Mekong Basin was high with 0.955 ± 0.009 haplotype diversity and 0.0077 ± 0.0002 nucleotide diversity.

3.2. Phylogeographic relationships among haplotypes

Haplotypes of Cytochrome b, D-loop, and their concatenated sequences were region-specific. The number of unique haplotypes was highest in Tonle Sap group (15 for Cytochrome b (57.1% of total haplotypes), 59 for D-loop (46.1%), and 77 (51.3%) for concatenated haplotypes) and lowest in Vietnamese cultured populations (1, 3 and 4 haplotypes, respectively). Only one haplotype of Cytochrome b (H-3, accession number MK258884, with a frequency of 63.0%) was shared among all populations. Meanwhile, one D-loop haplotype (H-44, accession number MK258946, accounting for 22.2%) were present in three groups (indicated in Table 2) of Tonle Sap Lake, Vietnamese wild, and cultured populations (data not shown). The phylogenetic median-joining network of concatenated haplotypes (Fig. 2) showed that six concatenated haplotypes (4%) were shared at least two groups but no haplotype was common among four groups. The most common concatenated haplotype (the sequence combined of MK258884 and MK258946) with a frequency of 20.4% consisted of individuals from Tonle Sap Lake and Vietnamese wild and cultured populations, except those from Cambodian Mekong floodplain. Haplotypes were not phylogenetically clustered by geographic regions but more likely by wild and cultured conditions (Fig. 2).

3.3. Genetic structure of striped snakehead in the Lower Mekong Basin

Values of F_{ST} based on pairwise differences in concatenated mtDNA sequences showed that striped snakehead populations were geographically structured. Five populations in the Tonle Sap Lake were genetically similar with low values F_{ST} (P > 0.05); however, some of them differed (P < 0.05) from populations in the Cambodian Mekong floodplain (Table 3). Values of F_{ST} were not significant between KD and PV, and between domesticated populations. Cambodian KCH and Vietnamese CM populations were significantly different from all other populations (P < 0.01) with high values of F_{ST} (ranging from 0.097 to 0.351 for KCH and from 0.123 to 0.569 for CM). Slightly different from F_{ST}, pairwise genetic distances based on Tamura and Nei model among populations varied in a small range, from 0.0020 to 0.0102 (mean 0.0077, table data not shown).

Genetic variation partitioning supported the spatial genetic structure of striped snakehead. AMOVA results indicated that all fixation indices were significant (P < 0.001) and that the majority of genetic variation was from within populations (sampling locations), ranging from 78.4 to 80.9% across alternative groupings (Table 4). Between-country variation under two-group hypothesis contributed to 10% of total genetic variation, while within groups accounted for 11.1%

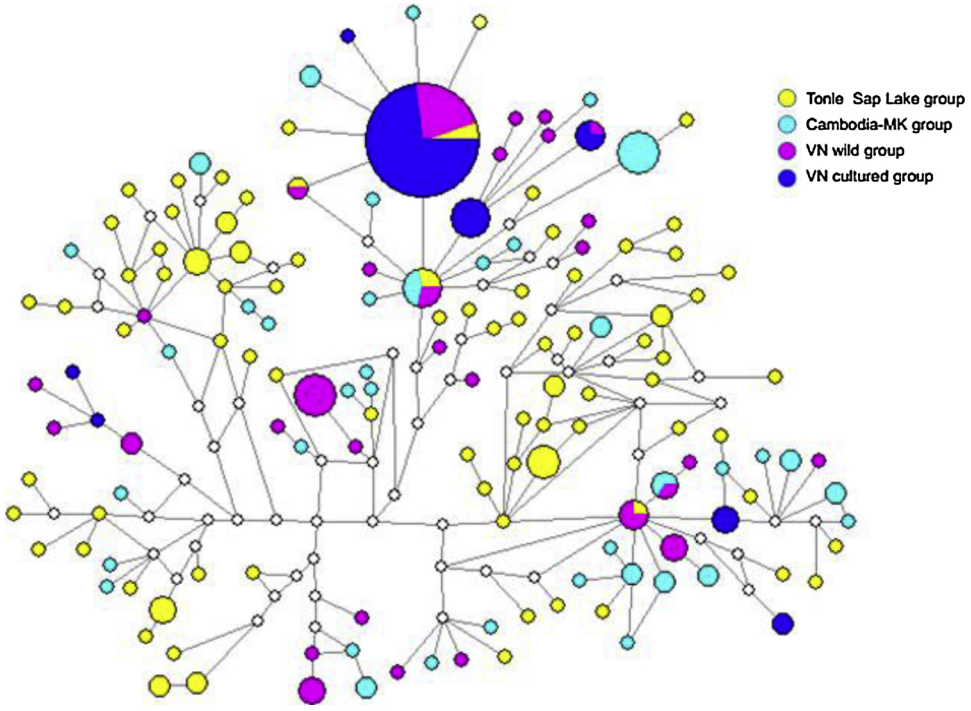

Fig. 2. Median-joining network of 150 concatenated haplotypes of four striped snakehead groups the Lower Mekong Basin. The size of nodes (circles) is proportional with the number of individuals. White dots represent median vectors (inferred and unsampled haplotypes).

variation. Hypothetical groupings in three and four groups showed larger portions of variation among compared to within groups, indicating better reflecting the hierarchical structure of striped snakehead in the Lower Mekong Basin. Four groups (including Tonle Sap Lake, Cambodian Mekong floodplain, Vietnamese wild and domesticated groups) had the lowest heterogeneity (6.9%) within groups and a relatively large variation among groups (12.5%).

Spatial genetic structure of wild striped snakehead was also evidenced by the positive correlation between pairwise genetic differences (F_{ST}) inferred from concatenated sequence data and water distances among 11 wild populations (Mantel test, $P < 0.01$, Table 5). The positive relationship between F_{ST} and water distances became weaker (with a smaller slope, $b = 0.026$, and determination coefficient, $R^2 = 0.071$; $P = 0.093$) among Cambodian populations compared to among all wild populations ($b = 0.060$, $R^2 = 0.174$, $P < 0.01$; Table 5, Fig. 3). When only Cambodian-Vietnamese pairwise populations were considered, the genetic differences increased faster with the increase of hydrological distances, implying limited gene flow among populations between the two countries (Fig. 3).

4. Discussion

4.1. Genetic diversity of striped snakehead populations

Concatenated sequence data of Cytochrome b and D-loop region reveal that wild striped snakehead populations in the Lower Mekong River Basin have high levels of genetic diversity compared to the same species in other regions and to other fish species using similar markers. Baisvar et al. (2018) found that eight striped snakehead populations in India have similar ranges of haplotype diversity (***Hd*:** 0.200–0.867) and nucleotide diversity (***pi*:** 0.0002–0.0073) in Cytochrome b gene. In another snakehead species *Channa marulius*, 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 Cytochrome b

Table 3
Values of F_{ST} based on pairwise difference in the concatenated mtDNA sequences (below diagonal line) and water distance (km, above diagonal) among striped snakehead populations in Cambodia and Viet Nam (population abbreviations are presented in Table 1).

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. SR	–	21	88	95	119	279	278	291	395	488	601	338	361	508
2. BB	0.02	–	73	87	107	250	261	277	389	481	596	326	327	508
3. KT	0.011	0.058*	–	22	32	177	189	195	303	412	537	253	255	429
4. PS	−0.005	0.032	0.061*	–	29	181	186	194	324	399	514	301	266	407
5. CHN	−0.016	0.036	−0.019	0.021	–	145	157	162	309	377	491	275	241	381
6. KCH	0.160**	0.143**	0.119**	0.156**	0.121**	–	101	91	212	293	417	176	173	344
7. KD	0.056*	0.053*	−0.0002	0.093*	0.0175	0.115**	–	36	135	224	330	65	79	225
8. PV	0.019	0.042	−0.007	0.038	−0.009	0.117**	0.009	–	124	225	348	84	80	254
9. W-LA	0.206**	0.217**	0.147**	0.218**	0.167**	0.195**	0.123**	0.124**	–	174	290	82	48	201
10. W-HG	0.049*	0.059*	0.0405	0.062*	0.026	0.097**	0.022	−0.001	0.068*	–	111	156	153	19
11. W-CM	0.208**	0.123**	0.229**	0.193**	0.194**	0.259**	0.164**	0.186**	0.381**	0.172**	–	269	269	100
12. D-AG	0.389**	0.378**	0.327**	0.365**	0.355**	0.351**	0.303**	0.297**	0.071*	0.1997**	0.569**	–	35	184
13. D-DT	0.348**	0.341**	0.284**	0.327**	0.314**	0.319**	0.261**	0.259**	0.013	0.191**	0.538**	0.039	–	176
14. D-HG	0.351**	0.349**	0.286**	0.335**	0.316**	0.320**	0.266**	0.258**	0.024	0.174**	0.539**	−0.037	−0.018	–

Note: * $P < 0.05$.
** $P < 0.01$.

Table 4
AMOVA results for the concatenated mtDNA sequences (between Cytochrome b and D-loop, 1450 bp) based on three hypothetical groupings of snakehead populations in Cambodia and Viet Nam.

Source of variations	df	Sum of squares	% of variation	Fixation indices
Two groups by countries				
Among groups	1	94.5	10.0	F_{CT} = 0.100***
Among populations within groups	12	205.7	11.1	F_{SC} = 0.123***
Within populations	256	1181.9	78.9	F_{ST} = 0.211***
Three groups (Cambodian wild -, Vietnamese wild -, and domesticated populations)				
Among groups	2	156.0	14.1	F_{CT} = 0.141***
Among populations within groups	11	144.2	7.5	F_{SC} = 0.087***
Within populations	256	1181.9	78.4	F_{ST} = 0.216***
Four groups (Tonle Sap lake, Cambodian Mekong floodplain, Vietnamese wild and domesticated populations)				
Among groups	3	178.0	12.5	F_{CT} = 0.125***
Among populations within groups	10	122.1	6.9	F_{SC} = 0.079***
Within populations	256	1181.9	80.6	F_{ST} = 0.194***

Note: *** P < 0.001.

Table 5
Parameters of linear regression Fst/(1-Fst) to a + b ln(water distance) based on concatenated sequences of wild striped snakehead populations in Cambodia and Viet Nam.

	a	b	Adjusted R^2	P*
Cambodia populations (n = 8)	−0.066	0.026	0.071	0.093
All wild populations (n = 11)	−0.198	0.060	0.174	< 0.01

* P values under the null hypothesis of no isolation by distance (Mantel tests, 5000 permutations).

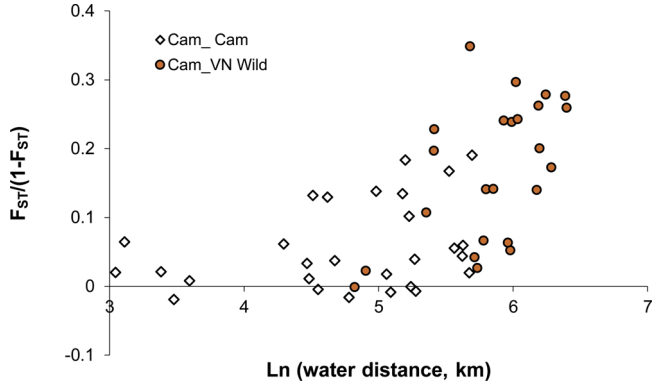

Fig. 3. A plot of genetic differentiation based on concatenated sequences of Cytochrome b and D-loop with hydrological distance among pairs of striped snakehead wild populations in Cambodia (Cam) and Viet Nam (VN).

(Habib et al., 2011). Three populations of bighead catfish *Clarias macrocephalus* sampled in Peninsular Malaysia had lower ***pi*** (0.003) and ***Hd*** (varying 0.657–0.765) (Nazia et al., 2010) compared to wild striped snakehead based on the same two genes Cytochrome b and D-loop in this study (***pi*** of concatenated sequences from 0.0044–0.0092, and ***Hd*** from 0.705 to 1, Table 2). D-loop sequences are more diverse than Cytochrome b (128 and 28 haplotypes, respectively, from the same 270 individuals investigated in the present study) or another mtDNA, ND5 gene (27 haplotypes from 280 individuals of striped snakehead in Malaysia (Tan et al., 2012).

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 and Palumbi, 2014). Among wild populations, five populations in the Tonle Sap Lake (Cambodia) have the highest level of genetic diversity indicated by genetic indices (Table 2) and the number of unique haplotypes (Fig. 2). High genetic diversity of Tonle Sap populations is consistent with the species abundance in the most productive and largest lake in the Mekong River basin (Campbell et al., 2006; Lim et al., 1999).

The present study also found that genetic diversity in domesticated striped snakehead populations was significantly lower with a large magnitude of difference compared to wild populations. Genetic diversity indices of domesticated populations were 2–4 times smaller than wild populations, given similar sample size means (N˜19). Such a remarkable decrease in genetic diversity of domesticated fish stock has not been previously reported although lower genetic diversity in hatchery-bred populations than wild populations was found in various species such as bighead catfish, *Clarias macrocephalus* (Duong and Scribner, 2018), cachama, *Colossoma macropomum* (Santos et al., 2016), barramundi, *Lates calcarifer* (Frost et al., 2006), gilthead sea bream, *Sparus aurata* (Brown et al., 2005). The most likely cause of genetic diversity loss in Vietnamese domesticated snakehead can be due to founder effects. Several other species were also reported to have low effective population sizes (Ne) in hatchery broodstock populations. For instance, Ne ranged from 14 to 18 for gilthead sea bream (Brown et al., 2005) and from 3 to 30 for Indian carps thus inbreeding coefficient increased from 2% to 17% per year (Eknath and Doyle, 1990). In snakehead farming in Viet Nam, most seed suppliers are small-scale farmers with small numbers of broodstock (100–300 individuals), and many fish farmers propagate seed for themselves using broodstock selected from their grow-out ponds (Sinh and Chung, 2009). Self-seed supply and small numbers of breeders can lead to a rapid decrease in genetic diversity as a result of genetic drift and inbreeding (Allendorf and Luikart, 2007; Tave, 1993). The negative effects of small population sizes become more severe because striped snakehead has been domesticated for more than 25 generations (since the 1990s with one-year generation time) with limited broodstock renewal from the wild, mostly from hatchery-bred sources. Domesticated fish are preferred for broodstock supplementation because they have become adapted to formulated feed and captive conditions. Different from striped snakehead, cultured bighead catfish, another freshwater species domesticated for a long time in the Mekong Delta, shows relatively small genetic diversity reduction because of yearly renewal of males (Duong and Scribner, 2018). In addition, cannibalism at early life stages can contribute to differences in offspring survival among striped snakehead families (Abol-Munafi et al., 2004; Qin and Fast, 1996) leading to lessen effective population sizes, thus lowering genetic diversity of striped snakehead.

4.2. Genetic structure of striped snakehead in the Lower Mekong River Basin

A pattern of F_{ST} values based on concatenated sequences shows that genetic differences between populations are low within each habitat group but moderate to high among groups (from 0.022 to 0.569) of snakehead populations in the Lower Mekong River Basin. Similar to higher values of F_{ST} based on Cytochrome b were reported among snakehead populations in India, ranging from 0.243 to 0.998 (Baisvar et al., 2018). Estimates of genetic differences can vary among mtDNA sequences due to differences in mutation rates (Broughton et al., 2001; Mcmillan and Palumbi, 1997). In the present study, F_{ST} values based on Cytochrome b (0.037–0.780) are generally higher than those based on D-loop region (0.024–0.559, data tables not shown), because Cytochrome b sequences are more conservative (Broughton et al., 2001).

Results of relatively high genetic differences (F_{ST}), genetic variation components (AMOVA), and significant tests of “isolation by distance” consistently support that striped snakehead populations in the region

are structured by living environments (lake and river floodplains, wild and hatcheries). Migration capacity of the species, hydrological connectivity, and possible anthropogenic factors can be attributed to this observed genetic structure of the species. Previous studies reported that striped snakehead has the capacity for long-distance migration (> 500 km) through historical physical connectivity (Adamson et al., 2010; Tan et al., 2012). They also exhibit strong localized migration (Amilhat and Lorenzen, 2005). Data presented in this study are more likely to support the latter migration pattern of striped snakehead. The significant isolation by hydrological distances and the lower genetic variation within compared to among three or four groups (Table 4) indicate limited gene flow among locations of maximal range of 600 km (Fig. 1 and Table 3), due to short migration capacity of the species. Meanwhile, Baisvar et al. (2018) found evidence of isolation by geographic distances from 500 to 2500 km among striped snakehead populations in India. In addition, higher genetic divergences of KCH and CM from the other wild populations suggest that physical barriers could decrease gene flow among these populations. Limited gene flow among habitats also explains the pattern of haplotype distribution, where each group is characterized many unique haplotypes and shared a few haplotypes (Fig. 2) with the other groups. On the other hand, a small number of common haplotypes among groups of populations could result from historical migration (Adamson et al., 2010; Tan et al., 2012). Moreover, gene flow among populations between the two countries could be affected by anthropogenic factors such as transportation along the Mekong River or striped snakehead trading from Viet Nam to Cambodia (Sinh et al., 2014).

4.3. Implications for snakehead domestication and genetic improvement

Findings on genetic diversity of striped snakehead in the Mekong basin reveal important implications for breeding and broodstock selection of striped snakehead in Cambodia, where domestication of this species has just been started. Before conducting the present study, snakehead seed production in Cambodia was proposed to use Vietnamese domesticated broodstock because of their higher performance in growth, survival, and feed utilization compared to Cambodian wild strains (Nen et al., 2015). However, evidence of low genetic diversity of Vietnamese cultured broodstock indicates that this source should not be used for breeding programs in Cambodia. Instead, wild Tonle Sap populations with high levels of genetic diversity can be good baselines for breeding and domestication programs. In addition, a lesson from striped snakehead farming in Viet Nam is that genetic diversity in cultured populations can decrease rapidly, mainly due to small numbers of breeders in small-scale hatcheries. This indicates that genetic monitoring should be carried out regularly in Cambodia during domestication programs with participating small-scale fish farmers or government hatcheries. On the other hand, the low genetic diversity of hatchery populations in Viet Nam indicates an urgent need for genetic improvement programs to prevent inbreeding depression. The most obvious solutions would be replacing or supplementing current hatchery broodstock with local wild individuals (Garcia-Marin et al., 1991; Vuorinen, 1984) together with increasing the size of the breeding population (Tave, 1999).

In terms of fisheries management, given relatively large genetic differences among Vietnamese and Cambodian wild populations and high levels of genetic diversity in most investigated wild populations, regional strategies should be developed to prevent striped snakehead translocation between the two countries. Such genetic exchanges can lead to outbreeding depression (McClelland and Naish, 2007; Whiteley et al., 2015). In addition, fishing pressures, which are intensive in Tonle Sap (Campbell et al., 2006) and other places in the Mekong Basin should be controlled in all populations, especially in those with low genetic diversity (i.e. CM and LA in Viet Nam).

Declarations of interest

None.

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