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Duong, T.Y., Uy, S., Chheng, P., So, N., Tran, T.H.T., Nguyen, N.T.T., Pomeroy, R. and Egna, H., 2019. Genetic diversity and structure of striped snakehead (*Channa striata*) in the Lower Mekong Basin: Implications for aquaculture and fisheries management. *Fisheries Research*, 218, pp.166-173.



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Fisheries Research

journal homepage: [www.elsevier.com/locate/fishres](http://www.elsevier.com/locate/fishres)



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



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### ARTICLE INFO

Handled by J. Viñas

**Keywords:**

*Channa striata*

Genetic diversity

Domestication

Population structure

mtDNA

### 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  $Hd = 0.994 \pm 0.004$ , nucleotide diversity  $\pi = 0.0077 \pm 0.0009$ ), intermediate in wild Cambodian Mekong River populations ( $Hd = 0.925 \pm 0.097$ ,  $\pi = 0.0076 \pm 0.0005$ ) and wild Vietnamese Mekong River populations ( $Hd = 0.832 \pm 0.152$ ,  $\pi = 0.0061 \pm 0.0018$ ), and lowest in cultured Vietnamese populations ( $Hd = 0.451 \pm 0.198$ ,  $\pi = 0.0021 \pm 0.0002$ ). 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.

# Introduction

- Lower Mekong River region of focus  
– Cambodia and Vietnam
- Striped snakehead (*Channa striata*)
  - Over-exploited for local consumption
  - Cultured in Vietnam since 1990s
  - Farming was banned in Cambodia in 2004 – illegal fishing of small fish for feed – ban lifted in 2016
- **Problem:** need good broodstock sources with high genetic diversity for Cambodia!



Fig.1: Striped snakehead (*Channa striata*) photo extracted from <https://inaturalist.ala.org.au/taxa/97280-Channa-striata>



# Materials & Methods

## Sample collection:

- Cambodia: **8** wild populations
  - **5** Tonle Sap Lake population
  - **3** Mekong River floodplain
- Vietnam: **6** populations
  - **3** wild population
  - **3** domesticated population

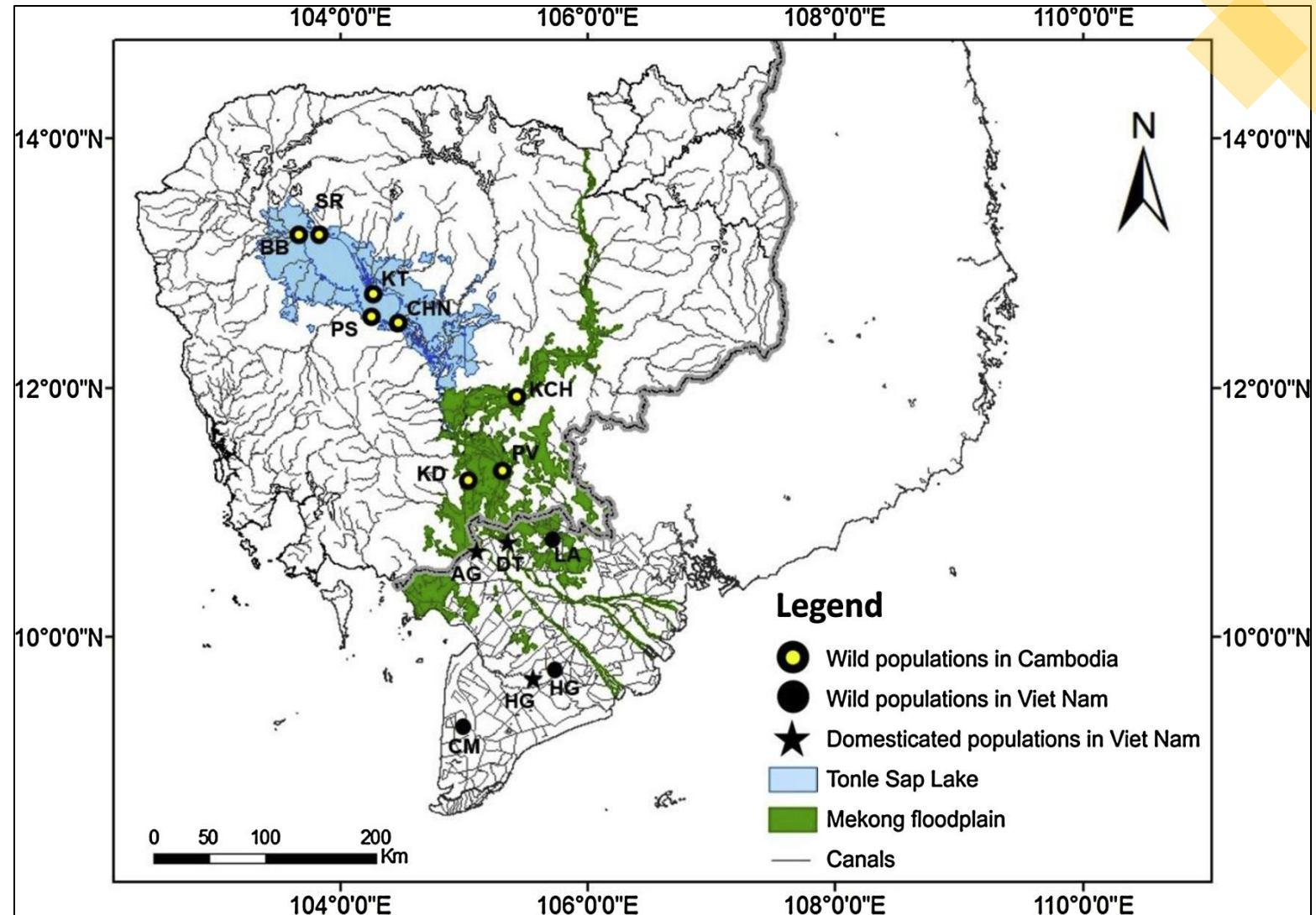
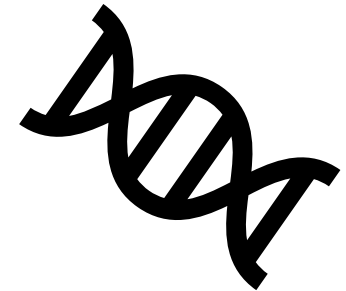


Fig. 2: Sampling locations for striped snakehead

# Materials & Methods

## Genetic analysis:

- Extracted DNA from 20 to 30 individuals of each population
- Used two mitochondrial markers for DNA amplification:
  - Cytochrome b gene
  - D-loop
- Polymerase chain reaction (PCR)
- Sent off for DNA sequencing



# Materials & Methods



## Data analysis:

- Used bioinformatics software to:
  - align the sequences
  - estimate genetic diversity indices (GDI) – number of haplotypes, haplotype diversity, and nucleotide diversity
- Used non-parametric Kruskal-Wallis tests to compare GDI
- Constructed median-joining phylogenetic tree based on Tamura and Nei model with bootstrapping 1000 times
- Evaluate genetic structure based on genetic distances, genetic differences ( $F_{ST}$ ), and partitioning of genetic variation (AMOVA)

# Results

## Genetic diversity

- Obtained 270 sequences
- Cytochrome b generated 28 haplotypes and 26 polymorphic sites
- D-loop generated 128 haplotypes and 100 polymorphic sites

# Results

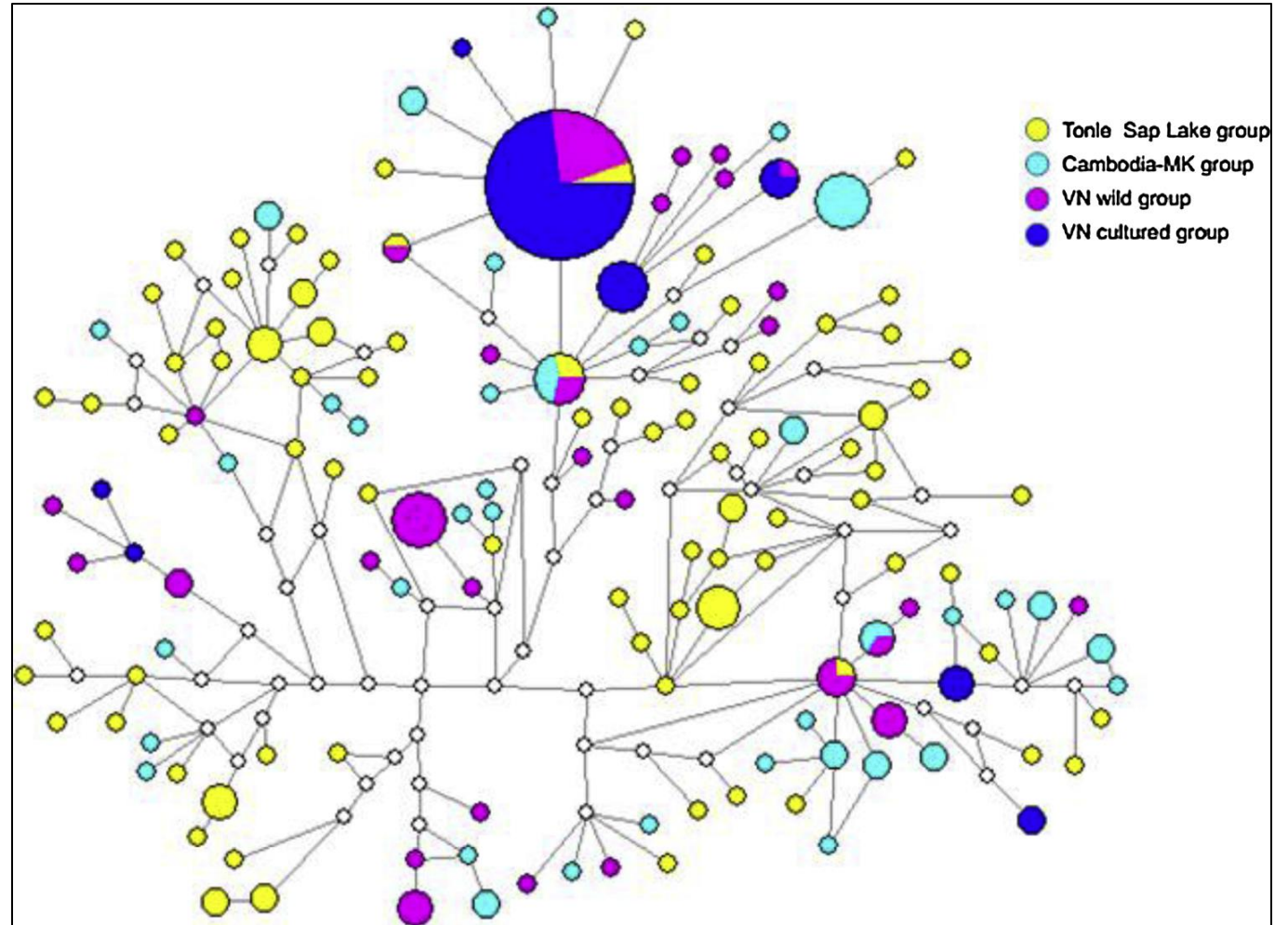
## Genetic diversity

- Population GDI from highest to lowest:
  - Tonle Sap lake (haplotype diversity  $Hd = 0.994 \pm 0.004$  and nucleotide diversity,  $pi = 0.0077 \pm 0.0009$ )
  - Cambodian Mekong River ( $Hd = 0.925 \pm 0.097$ ,  $pi = 0.0076 \pm 0.0005$ )
  - Vietnamese wild ( $Hd = 0.832 \pm 0.152$ ,  $pi = 0.0061 \pm 0.0018$ )
  - Vietnamese domesticated ( $Hd = 0.451 \pm 0.198$ ,  $pi = 0.0021 \pm 0.0002$ )
- Tonle Sap lake vs Vietnamese domesticated population differ significantly in all GDI ( $P = 0.017$ )
- Overall, genetic diversity was high
  - $0.955 \pm 0.009$  haplotype diversity
  - $0.0077 \pm 0.0002$  nucleotide diversity

# Results

## Phylogeographic

- Number of unique haplotypes:
  - highest in Tonle Sap group
  - Lowest in Vietnamese domesticated group
- Only one haplotype shared among all populations
- Phylogenetics showed no common haplotype among the 4 groups
- Phylogenetically clustered by wild vs domesticated, not geography



*Fig. 3:* Median-joining network of haplotypes of the striped snakehead groups. The size circles is proportional with the number of individuals. White dots represent median vectors (inferred and unsampled haplotypes).



# Results

## Genetic structure

- Low  $F_{ST}$  within habitat group
- Moderate to high  $F_{ST}$  among group
- AMOVA showed that all fixation indices were significant ( $P < 0.001$ )
  - Majority of genetic variation was from sampling locations
- The four groups had the lowest heterogeneity (6.9%) within groups and a relatively large variation among groups (12.5%)
- Pairwise populations among the two countries show genetic differences increase faster with the increase of hydrological distances → limited gene flow

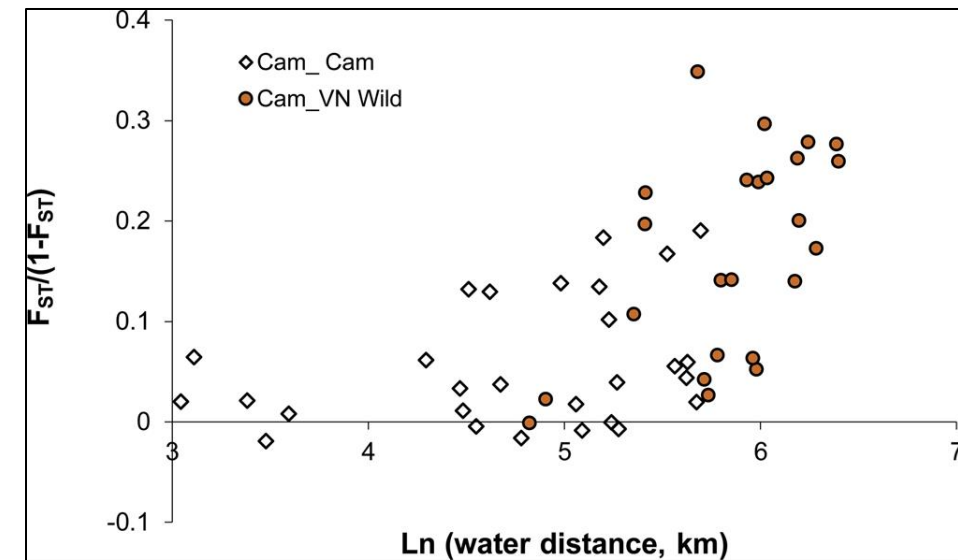


Fig. 4: Plot of genetic distance vs hydrological distance

# Discussion

## Genetic diversity

- Different levels of genetic diversity in the wild can be related to:
  - Population sizes
  - Different pressures of exploitation (overfishing)
- Founder effects in the Vietnamese domesticated population (small-scale farming)

# Discussion

## Genetic structure

- Both  $F_{ST}$  and AMOVA support that there is genetic structure by living environments
  - Migration capacity
  - Hydrological connectivity
  - Possible anthropogenic factors
- Gene flow could be affected by transportation of striped snakehead from Vietnam to Cambodia

# Discussion

## Implications

- Should NOT use Vietnamese broodstock for Cambodian domestication because of the low genetic diversity
  - Use Tonle Sap population
- Genetic monitoring
- Genetic improvement in Vietnam to prevent inbreeding depression
  - Supplement local wild individuals
  - Increase size of the breeding population
- Should not translocate between the two countries (outbreeding depression)
- Control fishing pressures



# Similar studies

## **[1] Boonkusol and Tongbai, 2016**

- Striped snakehead populations in Central Thailand
- 396 bp in mitochondria Cytochrome c oxidase subunit I
- Median-joining tree did not show that haplotype clades correspond to the geographic distribution
- Suggest a high genetic variation in this area – high gene flow between regional populations due to fish dispersal by flood and aquaculture

## **[2] Hornick and Plough, 2021**

- Chesapeake Bay eastern oysters (*Crassostrea virginica*)
- Investigated the broader genetic impacts of large-scale hatchery-based bivalve restoration
- Diversity increased when using large numbers of local, natural broodstock in hatchery

[1] Boonkusol, D. and Tongbai, W., 2016. Genetic variation of striped snakehead fish (*Channa striata*) in river basin of Central Thailand inferred from mtDNA COI gene sequences analysis.

[2] Hornick, K.M. and Plough, L.V., 2021. Genome-wide analysis of natural and restored eastern oyster populations reveals local adaptation and positive impacts of planting frequency and broodstock number. *Evolutionary applications*, 15(1), pp.40-59.

# Conclusion

It is a sound solution to use the Tonle Sap population for striped snakehead broodstock in Cambodia.