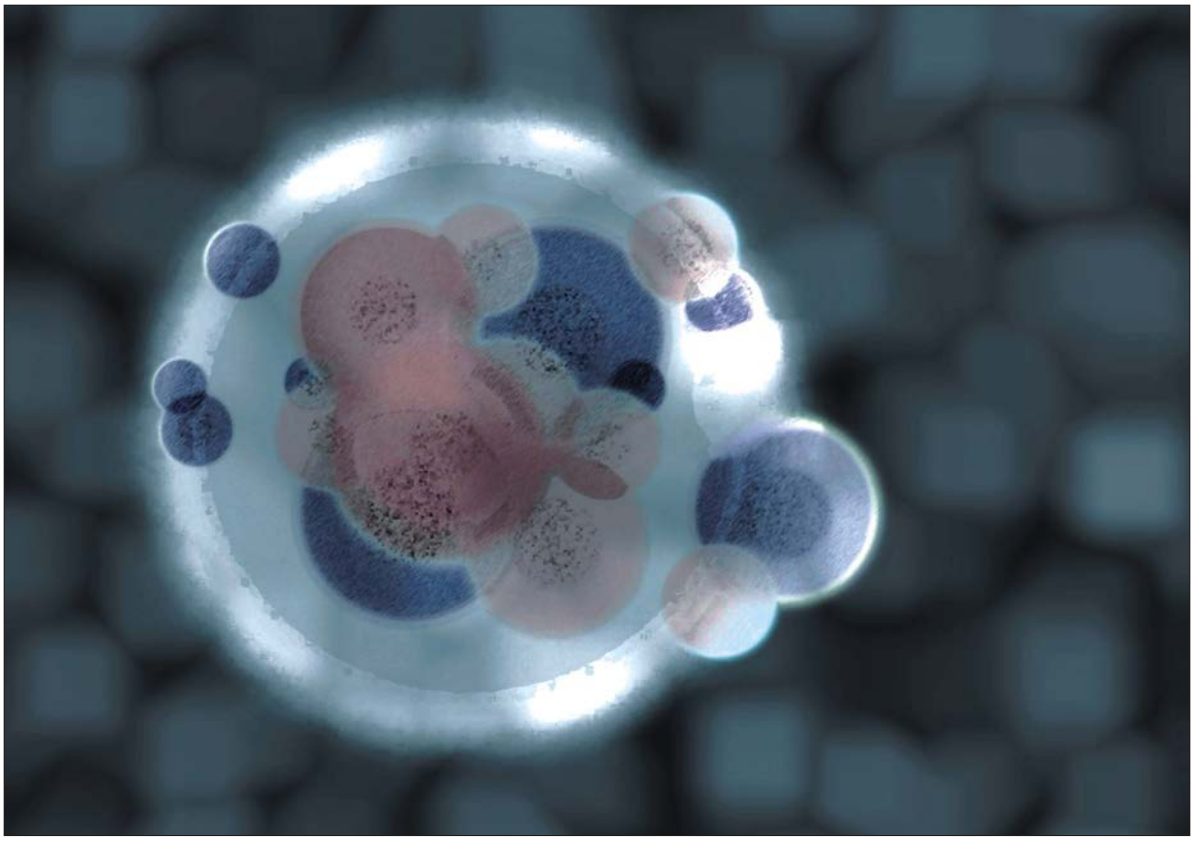

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Research Article
Genetic Variation of Striped Snakehead Fish (*Channa striata*) in River Basin of Central Thailand Inferred from mtDNA COI Gene Sequences Analysis

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Abstract

Genetic variation of striped snakehead fish (*Channa striata*) was examined using nucleotide sequence analysis of 396 bp in mitochondria Cytochrome c oxidase subunit I (COI) of 66 samples collected from 7 natural populations in river basin of central Thailand. Sequencing of 396 bp COI fragments revealed the presence of 33 haplotypes with haplotype diversity value of 0.976 and nucleotide diversity value of 0.01810. Population specific haplotypes were observed for 19 haplotypes in samples from Lopburi (7 haplotypes), Singburi (5 haplotypes), Kamphaeng Phet (3 haplotypes) and Phichit (4 haplotypes) and shared population haplotypes were observed for 14 haplotypes in samples from Ayutthaya, Angthong, Lopburi, Singburi and Suphanburi. High genetic variability in each population were revealed from their haplotype and nucleotide diversity values. Median-joining network demonstrated that haplotype clades were not corresponding to the geographic distribution of this species. The present results suggest a high genetic variability of striped snakehead fish in this area, probably due to high gene flow between regional populations due to fish dispersal by flood and aquaculture. The newly obtained sequences are a valuable addition to the database relevant for identification of *Channa* species in the future.

Key words: Striped snakehead fish, genetic variation, mtDNA, COI gene, *Channa striata*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Channa striata locally known as "Pla Chon", is economically important in fisheries and aquaculture industries in Thailand and Indo-China and also cultivated in India and Pakistan (Hossain *et al*., 2008). *Channa striata* has a wide range of habitats ranging from rivers, swamps, ponds, canals, lakes and rice paddy fields. This carnivorous snakehead fish is able to tolerate adverse environments due to its hardiness and air-breathing capabilities assisted by a suprabranchial chamber, an air-breathing organ (Chandra and Banerjee, 2004) which is presented in all species of family Channidae. The local market demand on *C. striata* is greatly expanding due to its commercial value, agreeable flavor (Hossain *et al*., 2008) and its postoperative medicinal applications enhancing wound healing and reducing postoperative pain and discomfort (Mat Jais *et al*., 1997; Zakaria *et al*., 2004). Studies by Michelle *et al*. (2004) showed that a *C. striata* extract might be useful as an alternative treatment in osteoarthritis.

Conservation is imperative for the Asian snake heads, as the species has been over fished due to high market demands. Information on the population structure of *C. striata* is urgently needed for better handling this species in aquaculture and to provide a permanent conservation area for this species. To date, the exact taxonomic status at the level of species of the genus Channa remains unclear as *C. striata* was placed as one of the "species complexes" under the above mentioned genus (Courtenay and Williams, 2004). This has raised interest in identifying *C. striata* species genetic variation.

Different mtDNA gene sequences have been used to determine variation at interspecific and intraspecific levels in fish. The higher mutation rate of base substitution in mtDNA compared to nuclear DNA (Tang *et al*., 2006) coupled with maternal inheritance have made it an efficient genetic marker for studying genetic differentiations. The mitochondrial cytochrome c oxidase subunit I (COI) gene is the prime DNA barcoding region for taxonomically identifying animals, such as fish, insects, amphibians and sponges (Erpenbeck *et al*., 2006; Seifert *et al*., 2007; Alessandrini *et al*., 2008; Rock *et al*., 2008; Smith *et al*., 2008) and is sometimes useful for investigating phylogeographic groups within a single species (Li *et al*., 2009).

The objective of this study was to determine genetic variation and divergence of *C. striata* populations from seven different wild locations, through DNA sequencing of COI of mtDNA.

MATERIALS AND METHODS

Sample collection: A total of 66 specimens of *C. striata* with eight to ten individuals as representatives from each of seven locations (Table 1) were collected and used in this study. Samples were caught by simple fishing methods such as nets. Collection was made from November, 2013 to June, 2014. Species level was identified at the time of collection. The GPS location of collection sites was recorded (Table 1) and locations of sampling sites were showed in Fig. 1. Fin tissues (2 x 2 mm) of each were collected and stored individually in 95% ethanol. The voucher specimens were collected in Thepsatri Rajabhat University.

Mitochondrial DNA analysis: Total genomic DNA was extracted from fin samples using an Aquagenomic Kit (Bio-Syntech, Salt Lake City, Utah, USA), according to the manufacturer's instructions. DNA was amplifiedusing Universal Primers for mtDNA COI genes as follows:

- L6154 5'-AYC ARC AYY TRT TYT GRT TCT-3'
- H6556 5'-TGR AAR TGI CGI ACW ACRTA-3' (Teletchea *et al*., 2006)

The PCR was conducted in a Peltier thermal cycler (MJ Research Waltham, MA, USA), with the following profile: pre-denaturation at 95°C for 5 min, 30 cycles of denaturation at 94°C, annealing at 50°C and extension at 70°C for 1 min each, followed by final extension at 72°C for 5 min. The PCR products were purified using Wizard® SV Gel and a PCR Clean-Up System by Promega (Promega Madison, WI). The

Table 1: Sample location and size (n) of *Channa striata* analyzed in this study

Location	Latitude	Longitude	n	Code
Amphoe Muang, Ayutthaya	14°24'0"	100°31'43"	9	AY
Amphoe Wiset Chai Chan, Angthong	14°34'40"	100°10'40"	10	AT
Amphoe Inburi, Singburi	14°55'31.96"	100°16'10.63"	10	SB
Amphoe Phattana Nikhom, Lopburi	14°52'26"	100°59'21"	10	LB
Amphoe Song Phi Nong, Suphanburi	14°11'20"	99°58'33"	10	SP
Amphoe Khlong Khlung, Kamphaeng Phet	16°20'00.01"	99°71'66.67"	8	KP
Amphoe Muang, Phichit	16°43'33.32"	100°36'66.69"	9	PJ

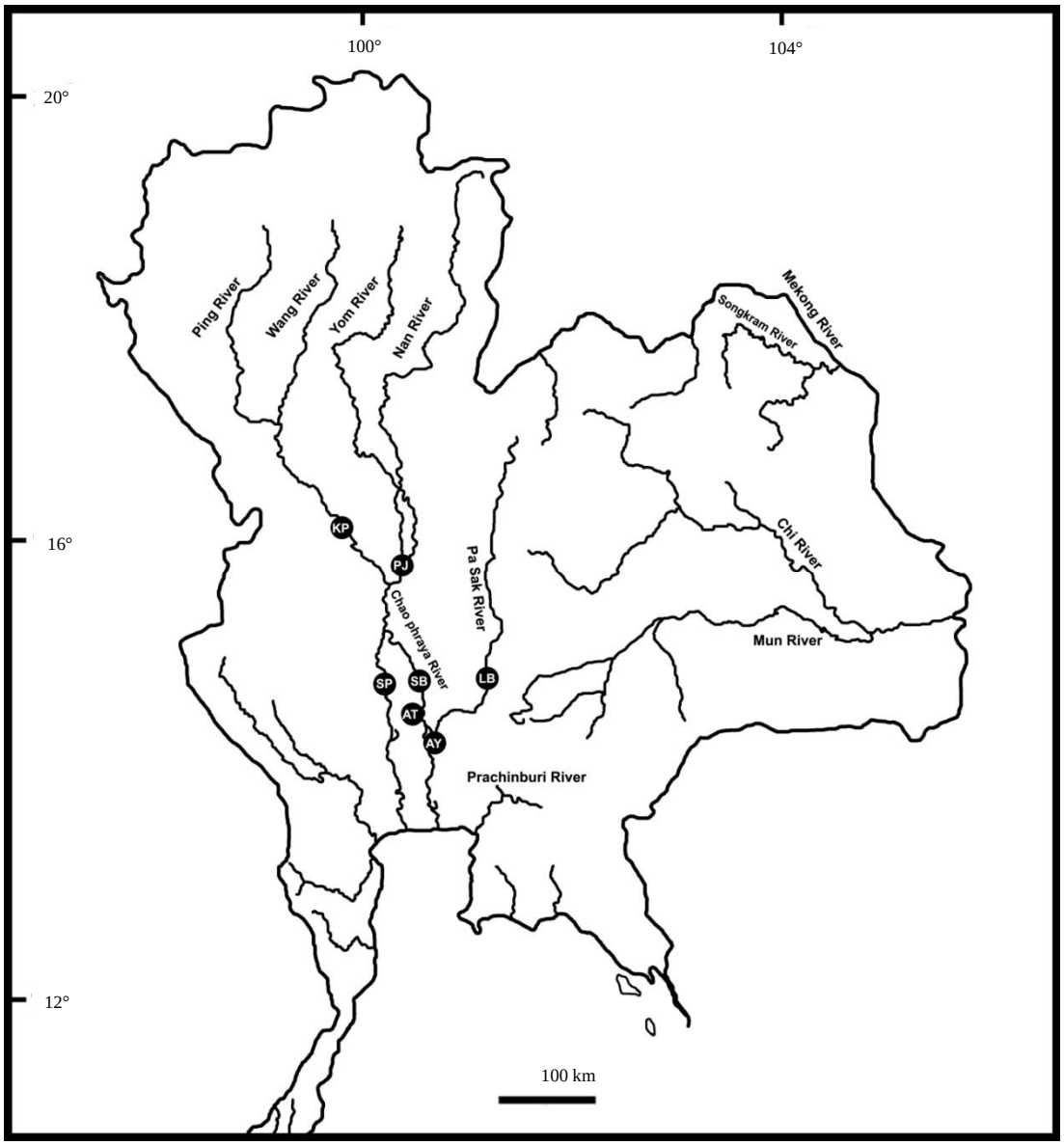

Fig. 1: Locations of sampling sites indicated by letters in black circle. Letters code correspond to locations listed in Table 1

purified PCR product was sequenced on an ABI3730XL genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Data analysis: Sequences were edited using MEGA 6.0v6.01 (Tamura *et al*., 2013) and aligned with Clustal W 1.6, implemented in the same software. Molecular diversity indices [number of haplotypes, transitions, transversions and haplotype diversity (h) and nucleotide diversities (π), besides a population comparison by pairwise F-statistics, were calculated by using Arlequin version 3.1 software (Schneider *et al*., 2000), in order to reveal the level of genetic variation and population structure. The program network (http://www.fluxus-technology.com) was used to construct a median-joining network.

RESULTS

A total of 396 bp of COI sequencealignments, with 66 (16.7%) variable nucleotide positionsand 59 (14.9%) parsimoniously informative sites, were obtained. Sixty of the base substitutions were transitions, whereas only six was a transversion. All the variable sites occurred at the third codon position. Altogether, 33 haplotypes were identified from all the 66 individuals sampled. The mean base composition (%)

Table 2: Population name, number of examined individuals, haplotype and nucleotide diversities and number of haplotypes in each population

Population name	Examined individuals	Haplotype diversity (h) (±SD)	Nucleotide diversity (π) (±SD)	Haplotypes
AY	9	1.000±0.052	0.02307±0.00231	9
AT	10	0.978±0.054	0.02166±0.00241	9
SB	11	0.982±0.046	0.01482±0.00223	10
LB	11	0.873±0.089	0.00442±0.00083	7
SP	10	0.956±0.059	0.02672±0.00216	8
KP	8	0.750±0.096	0.02441±0.00409	3
PJ	8	0.857±0.082	0.00433±0.00053	4

Table 3: Pairwise Fst between populations of *Channa striata*, based on the COI gene

	AY	AT	SB	LB	SP	KP	PJ
AY		-0.125	-0.0012	-0.02112	0.0165	0.12432	0.16588
AT			-0.0012	-0.02112	0.0165	0.12432	0.16588
SB				0.01172	0.12587	0.09219	-0.01951
LB					0.20376	0.39627*	0.05732
SP						0.40627*	0.09781
KP							0.27891*

*Significant F_{st} ($p< 0.05$) based on 10000 permutations of haplotype frequencies among samples

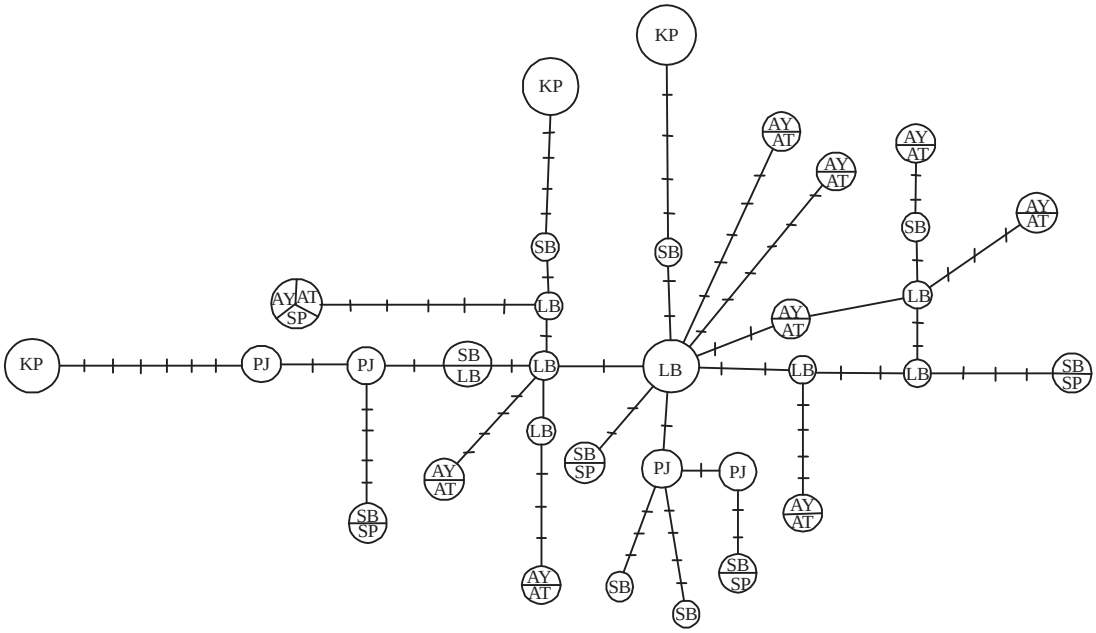

Fig. 2: Median-joining network for the 33 haplotypes of mtDNA COI (396 bp) with circle diameter proportional to sample sizes

was 30.5A:25.5G:23.3T:20.7C. Haplotypes in samples from Lopburi were common but not exclusive to the LB population. Fourteen haplotypes were shared by multiple populations and 19 haplotypes have been found to be population specific. Median-joining network demonstrated that haplotype clades did not correspond to the geographic distribution.

High genetic variations in each population were revealed from their haplotype and nucleotide diversity values (Table 2). Haplotype nucleotide diversities ranged from 0.750-1.000 and 0.00433-0.02672, respectively. As a whole, high mean population haplotype (0.913) and nucleotide (0.01707) diversities were observed. Genetic diversity was slightly higherin AY, AT, SB, SP and PJ regions (h: 0.857-1.000 and π: 0.00433-0.02672), than in KP populations (h: 0.750 and π: 0.02441). Population differentiation (F_{ST}), overlapping haplotype distribution, haplotype sharing and low nucleotide mutation among all the populations were apparent from the data. Nevertheless, genetic differentiation was observed among LB, SP, KP and PJ populations (F_{ST} ranging from 0.27891-0.40627) (Table 3). Figure 2 shows a Median-joining network for the 33 haplotypes of mtDNA COI (396 bp) as visualization of haplotypes relationships. The

network does not indicate a differential geographic distribution of haplotypes among populations.

DISCUSSION

The haplotype diversity values inferred from mtDNA COI gene sequencerevealed high genetic diversity of *C. striata* popuations investigated in this study. This was supported by previous studies exhibiting relatively high population haplotype diversity of *C. striata* populations mainly from the Mekong and Chao Phraya rivers (0.97) inferred from mtDNA cytochrome b gene sequence (Adamson, 2010), *C. striata* populations in Malaysia gene sequence and (0.90) inferred from mtDNA ND5 gene sequence (Tan *et al*., 2013) and *C. striata* populations in Malaysia gene sequence and (0.80) inferred from mtDNA COI gene sequence (Song *et al*., 2013). Similar high genetic diversities have been observed in other freshwater fish populations; the cyprinid, *Acrossocheilus paradoxus* (1.00) inferred from multiple mtDNA segments (Wang *et al*., 2000), four Hawaiian freshwater fishes, *Lentipes concolor*, *Stenogobius hawaiiensis*, *Sicyopterus stimpsoni* and *Awaous guamensis* (0.47-0.98) inferred from both coding and non-coding regions of mtDNA (Chubb *et al*., 1998). This relatively high haplotype diversity observed in most *C. striata* populations was often noted in freshwater fishes inhabiting non-glaciated regions (during the past glaciations era) or temperate regions (Bernatchez and Wilson, 1998; Roos, 2004). Based on F_{ST} analyses, there was no genetic differentiation observed among AY, AT, SB, LB and SP populations. This might result from high gene flow. However, genetic differentiation was observed among LB, SP, KP and PJ populations. These populations were incompletely divergent, evident by haplotype distribution, haplotype sharing and low nucleotide mutation. Similar haplotype sharing have been found in *C. striata* population between the East Peninsular Malaysia and Sumatra populations and this result revealed extraordinary migration ability of *C. striata* (>500 km) through ancient connectivity (Tan *et al*., 2013).

The low level of genetic divergence within geographical regions which is contradictory to the low migratory behavior of *C. striata* (Halls *et al*., 1998; Amilhat and Lorenzon, 2005), implies the existence of factors other than free gene flow. The possible alternative factors are either recent population expansion with insufficient time for coalescence (Wang *et al*., 2000) or interconnection of the areas studied. Furthermore, this region is also liable to high level of flooding, thereby facilitating lateral dispersal to flood plains, watersheds or between adjacent populations (Hurwood and Hughes, 1998; Wang *et al*., 2000).

Due to their high economic value, the conservation of *C. striata* species is of great concern. Based onFST analyses, our analysis demonstrated the suitability of partial COI genes for determining the genetic diversity in *C. striata* population. The newly obtained sequences provided relevant information for identification of *Channa* species in the future. Nevertheless, based on the limited genetic information, all inferences should be reconfirmed using further, faster evolving markers, such as microsatellites.

Based on its high genetic variability fish populations in the river basins of cental Thailand are the mostappropriate wild species candidate population to receive priority as a baseline stock for selective breeding. Information on stock structure is required for conservation management of native populations of *C. striata*. The information of stock structure is vital for planning management and conservation of natural resources, in addition to being useful in genetic improvement programs (Abdul Muneer *et al*., 2009).

Furthermore, habitat fragmentation potentially cause further losses in genetic diversity or an increase in inbreeding depression. Therefore, habitat protection from agricultural and industrial activities, including the development of surrounding areas which prevent population connectivity, must be carefully regulated. As an alternative, the introduction and improvement of *C. striata* aquaculture is a valuable option for ensuring the maintenance of this species. Nevertheless, hatchery facilities and operation, as well as brood-stock selection, must be systematically controlled, in order to prevent fugitive genetic contamination into the wild and other adverse genetic risks (Mahidol *et al*., 2007).

CONCLUSION

Haplotype and nucleotide diversity values of COI gene sequences in each populations demonstrated high genetic variation of striped snakehead fish (*Channa striata*) in river basins of central Thailand. The haplotype clades were not corresponding to the geographic distribution of this species. The high genetic variations of striped snakehead fish in this area are probably influenced by high gene flow between regional population due to fish dispersal by flood and aquaculture. Based on their high genetic variability and positive contribution towards genetic variation in this region, AY, AT, SB, LB and SP populations of wild species are the most appropriate candidates to receive priority as a baseline stock for selective breeding. Overall, this study provided highly relevant genetic information for planning management, conservation and ranching guidelines for *C. striata* in river basins of cental Thailand.

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