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*Original Article*

# Morphological and genetic differences between cultured and wild populations of *Channa striata* in Viet Nam and its phylogenetic relationship with other *Channa* species

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**Abstract**

The Channa genus includes important species for aquaculture and interesting targets for phylogenetic studies. In the Mekong Delta, Viet Nam, four species of this genus (*Channa striata*, *C. micropeltes*, *C. lucius*, and *C. gachua*) are naturally distributed and other phenotypes that look like *C. striata* have been observed in aquaculture conditions. The taxonomic status of newly-observed phenotypes including "triangle-head" snakehead (THS) and square-head snakehead (SHS) is still controversial. This study compared morphological characteristics and Cytochrome C oxidase subunit I (COI) sequences of different *C. striata*-like phenotypes and investigated the phylogenetic relationship of Channa species based on COI. Morphological results show that THS, SHS, and wild C. striata have similar ranges for meristic traits but differ in morphometric ratios, especially the shape of their head and length of their gut. Kimura-2P genetic distances among three phenotypes (0.0017-0.0062) are equivalent to those of *C. striata* samples from Mainland Southeast Asian countries. The results indicate that THS and SHS belong to *C. striata*, and this species exhibits within-species diversity in both morphology and COI sequences. The phylogenetic analysis indicates that *C. striata* individuals form a monophyletic group and are genetically distinct from other Channa species in the Vietnamese Mekong Delta. Congeneric distances of four species range from 0.1836 to 0.2436, indicating high divergence among Channa species.

**Keywords:** Channidae, species classification, DNA barcoding, phylogeny, morphology

## 1. Introduction

Cryptic species (two distinct species that are morphologically similar and classified as one species) in animals including fishes have been recognized for a long time but recently more and more examples have been discovered thanks to the advances of DNA sequence analyses (Bickford *et al.*, 2007). On the other hand, some species exhibit large within-species variation in morphological traits, especially morphometric traits relating to body depth, head, snout and mouth shape and caudal peduncle length (Elmer *et al.*, 2010). Large morphological variation among individuals of the same species can lead to misclassification if no evidence from DNA sequences is available. Therefore, incorporating morphological and molecular methods, such as DNA barcoding (Hebert and Gregory, 2005; Valentini *et al.*, 2009; Ward *et al.*, 2005), in species classification and taxonomic studies, can help to greatly improve our understanding of biodiversity (Will *et al.*, 2005).

The *Channa* genus, that has a high level of species diversity with 34 species occurring mostly in southern Asia (Fishbase.org), has been an interesting target for phylogenetic studies (Adamson *et al.*, 2010). Previous studies have focused on large scales of inter-specific genetic relationships (Adamson *et al.*, 2010; Lakra *et al.*, 2010; Li *et al.*, 2006) and regional intra-specific differentiation (Adamson *et al.*, 2010).

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Among *Channa* species, *C. striata,* collected from the wild in five locations in Southeast Asian countries, has the highest level of within-species divergence (Adamson *et al.*, 2010). On a smaller scale in Malaysia, however, Song *et al.* (2013) found that within-species genetic difference of *C. striata* was lower than that of other species and did not correlate with morphological variation. Whether this species displays similar patterns of variation in different environments, in other regions of their natural distribution, is still in need of further investigation.

*C. striata* is one of the important aquaculture species and favorite food of local people in Southeast Asian countries. In the Mekong Delta of Viet Nam, there are four species of *Channa*: *C. striata, C. micropeltes, C. lucius,* and *C. gachua* (Tran *et al.*, 2013). In addition, three snakehead phenotypes that look like *C. striata* have been found in aquaculture conditions and are called "triangle-head snakehead" (THS), "projected lip snakehead" (PLS), and "square-head snakehead" (SHS). Their local common names are based on the appearance of their head and mouth. They grow faster than *C. striata* in the same culture conditions (personal observation). Differences in growth rates and some morphological traits have raised questions of whether they are cryptic species or divergent phenotypes of the same species, i.e., *C. striata*. A previous study comparing morphological characteristics between PLS and *C. striata* showed that 18 of 20 measurable parameters were significantly different ($p<0.05$) (Nguyen and Lam, 2005). However, morphological comparision has not provided convincing or comprehensive answers.

This study investigated the taxonomic classification of different phenotypes of *Channa* species by incorporating morphology and DNA barcode comparisons. Cytochrome c oxidase subunite I (COI), a mitochondrial gene, was used in this study. In addition, COI sequences of *C. striata* phenotyes were also compared with the same species in Southeast Asian countries and with other *Channa* species. Previous phylogenetic studies have used other genes (e.g., mtDNA cytochrome b and nuclear DNA (nDNA) Recombination Activation Gene-1, RAG1 (Adamson *et al.*, 2010)). Results from our study provide additional information on the phylogenetic relationship of the diversified *Channa* genus and on a link between genetic divergence and morphological variation of *C. striata*.

## 2. Materials and Methods

### 2.1 Fish sampling

Wild fish of *C. striata* (n=30), *C. micropeltes* (n=5), *C. gachua* (n=5), and *C. lucius* (n=27) were collected from rice fields and canals; while cultured individuals of THS (n=34) and SHS (n=24) were collected at cultured ponds and local markets in Can Tho, Hau Giang, and Vinh Long provinces (central areas of the Mekong Delta, Viet Nam). Fish were kept alive or stored in ice and transferred to the fish genetic laboratory in Can Tho University. After morphological measurement, a small piece (~ $1cm^2$) of caudal fin from each sample was collected and stored in ethanol 96% for DNA analysis.

### 2.2 Morphological classification and measurement

External characteristics including color, body shape, head, tail and lateral line of fish individuals were observed. A total of 88 samples of *C. striata* individuals were collected, representing three morpho-types (wild, THS, and SHS), and morphological parameters were measured based on the guidance of Rainboth (1996) and Tran *et al.* (2013). Six meristic traits were counted, including lateral line scales, scales above and below the lateral line, and numbers of dorsal, pectoral, and anal fin rays (Table 1). Body weight, total length and standard length together with 15 other morphometric parameters (Table 2) were measured, seven of which were transformed into ratios to standard length (SL, body length from the head to the tail, excluding the caudal fin), and eight that were transformed relative to head length (HL). In addition, gut length was measured to test differences in relative gut length (RGL = Gut length/Standard length) among *C. striata* and new phenotypes.

### 2.3 DNA analysis methods

DNA was extracted from fish fins by using the Phenol-chloroform method (Taggart *et al.*, 1992). The quality of extracted DNA and PCR products was checked by agarose electrophoresis (1%). Good DNA samples with clear bands were amplified COI gene by using the primer pair of Fish F2-t1 (5'-TGTAAAACGACGGCCAG-TCGACTAATCATAAAGATATCGGCAC-3') and Fish R2-t1 (5'CAGGAAACAGCTATGACACTTCAGGGTGACCGAA-GAATCAGAA'3) (Ivanova *et al.*, 2007; Ward *et al.*, 2005). The final concentrations of PCR ingredients in 30 µL PCR volume included 1X buffer, 0.2 mM dNTP, 2.5 mM $MgCl_2$, and 2.5 pmol for each of primers Fish F2-t1 and Fish R2-t1, 1.25 U Taq polymerase (Fermentas), and 100 ng DNA. The temperature cycles of PCR reaction included one cycle at 95°C in two minutes, 35 cycles of amplification including 30 seconds at 94°C, 30 seconds at 52°C and one minute at 72°C, and one cycle of final extension at 72°C for 10 minutes (Ivanova *et al.*, 2007). Five good PCR products (checked on agarose gel 1.5%) from each fish species and morpho-type (except n=2 for SHS) were chosen for sequencing and analyzed using ABI 3100 by Nam Khoa Biotek Company (ISO 9001-2000, in Ho Chi Minh city, Viet Nam).

### 2.4 Data analyses

Meristic traits were reported as range, mode, and frequency of mode values. Morphometric data (not adjusted for body sizes because range sizes of fish among three groups were statistically similar, Table 1) were checked for outliers

and to confirm that they followed a normal distribution. Then, one way ANOVA and post-hoc analysis using Tukey HSD tests were used to test differences in the mean of morphological ratios among *C. striata* and two morpho-types. Morphometric ratios were log-transformed and submitted to principal component analyses. Statistical analyses were carried out using R (R Core Team, 2014).

DNA sequences were analyzed using programs Finch TV 1.4.0 (http://www.geospiza.com/), MEGA 6 (Tamura *et al.*, 2013), and BLAST (Basis Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Finch TV and MEGA 6 were used to view and check quality of DNA sequences between two-direction sequences. If forward and reverse sequences were mismatched in a nucleotide, the nucleotide with higher quality value was selected. Edited sequences within and among species were aligned and then the level of sequence similarity was compared with nucleotide databases in Genbank using the BLAST program. Genetic distances within and between species were estimated based on Kimura-2P method employed in MEGA 6. The phylogenetic relationships among the studied *Channa* species and within *C. striata* in other countries (downloaded from BOLD systems databases) were constructed using the maximum likelihood statistical method. The phylogenetic tree was computed based on bootstrapping method with 1,000 iterations employed in MEGA 6.

## 3. Results

### 3.1 Morphological comparison of *Channa* species

#### 3.1.1 Appearance and meristic parameters

Wild *C. striata*, THS, and SHS are similar in body shape and appearance; therefore, it is difficult to differentiate them by eye. They have dark brown dorsal colouration and whitish ventral colouration. Their lateral line breaks suddenly in one point and drops two scale rows. Six meristic parameters (Table 1) of wild *C. striata* are consistent with previous reports (Truong and Tran, 1993; Allen, 1991, cited by Fishbase.org), and are in similar ranges to those of THS and SHS. Modes of meristic traits were the same among three groups. Frequencies of modes vary from 23.5-73.5%, indicating high variation in meristic traits within groups.

#### 3.1.2 Ratios of morphometric parameters

All of the ratios of morphometric measurements are significantly different among the three groups of wild *C. striata,* THS, and SHS ($p<0.05$), especially in the shape of their head. Ratios of head length to standard length; small head width (head width before eyes), large head width (head width at the biggest position of the head) and eyes distance

Table 1. Range, mode and frequency of meristic parameters of wild *C. striata*, triangle-head snakehead (THS) and square-head snakehead (SHS)

| Countable parameters | | Wild *C. striata* (N=30) | THS (N=34) | SHS (N=24) |
|---|---|---|---|---|
| | Weight* (g) | 169 ± 27 | 179 ± 77 | 191 ± 45 |
| | Length range (cm) | 23-30 | 23-34 | 21-35 |
| Scales of lateral line | Range | 53-56 | 52-57 | 52-59 |
| | Mode (Frequency, %) | 54 (36.7) | 56 (37.5) | 54 (23.5) |
| Scales above lateral line | Range | 6.5-7.0 | 5.5 - 6.5 | 6.0-8.0 |
| | Mode (Frequency, %) | 7 (50.0) | 6 (54.2) | 7 (44.1) |
| Scales below lateral line | Range | 7.5-9.0 | 8.0-9.0 | 8.0-8.5 |
| | Mode (Frequency, %) | 8 (50.0) | 8 (41.7) | 8 (64.7) |
| Dorsal fin rays | Range | 41-42 | 38-42 | 40-42 |
| | Mode (Frequency, %) | 42 (53.3) | 42 (41.7) | 42 (52.9) |
| Pectoral fin rays | Range | 15-16 | 14-15 | 15-17 |
| | Mode (Frequency, %) | 16 (60.0) | 15 (50.0) | 17 (52.9) |
| Anal fin rays | Range | 26 -27 | 23-25 | 25-26 |
| | Mode (Frequency, %) | 27 (56.7) | 25 (50.0) | 26 (73.5) |

(*) Mean weight and length were not significantly different among three groups ($P>0.05$).

to head length describe different shapes of fish head. Being the same head length, wild *C. striata*'s head is the slimmest among three groups. The head of SHS is larger and shorter; therefore it looks like a square shape. Meanwhile, THS has the shortest ratio of head length/ standard length, the largest ratio of large head width, and the smallest ratio of head width before the eyes, creating the shape of a triangle. However, being the same standard length, for example SL=25 cm, the size and shape of wild *C. striata* and SHS are similar (SHW and LHW are 4.34 cm and 2.73 cm for wild *C. striata*; and 4.30 cm and 2.80 cm for SHS), which are larger than those of THS (4.20 cm and 2.48 cm, respectively) (calculated from ratios in Table 2, shown in Figure 1).

Differences in morphometric ratios among three groups are presented in a PCA plot (Figure 2). THS is different from the other two groups in PCA1 which explains 41.4% of the variation. Variation components explained by PCA2 and PCA3 are 18.3% and 9.1%, respectively. Each of the other PCAs contributes less than 1% of total explained variation. Morphometric ratios that are important in contributing to positive loading for the first three PCAs include measurements of head shape and mouth sizes.

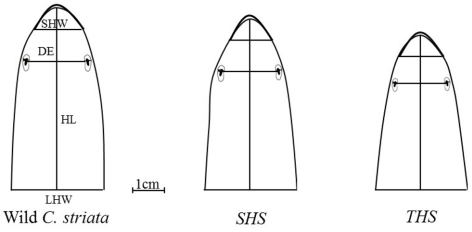


Figure 1. Schematic diagram of head measurements (Small head width, SHW; Large head width, LHW; Distance of two eyes, DE; Head length, HL) of wild *C. striata*, square-head snakehead (SHS) and triangle-head snakehead (THS) with the same standard length of 25 cm.

### 3.1.3 Relative gut length (RGL)

Relative gut length (RGL) varies among fish species depending on feeding ecology types. RGL of three *Channa* groups is lower than one (Figure 3), as expected because snakehead species are carnivores (Courtenay and Williams, 2004; Lee and Ng, 1994). THS and SHS have higher RGL compared to wild *C. striata* ($p<0.05$). In cultured conditions,

Table 2. Mean of morphometric measurements (%) of wild *C. striata*, triangle-head snakehead (THS) and square-head snakehead (SHS)

| Measurable parameters | Wild *C. striata* (N=30) | THS (N=34) | SHS (N=24) |
|---|---|---|---|
| Relative to standard length | | | |
| Head length (HL) | $33.1 \pm 1.1^{c}$ | $28.2 \pm 1.5^{a}$ | $31.6 \pm 0.8^{b}$ |
| Body depth (BD) | $14.3 \pm 0.5^{b}$ | $15.6 \pm 0.6^{c}$ | $13.4 \pm 0.8^{a}$ |
| Height of caudal fin (HCP) | $8.5 \pm 0.3^{b}$ | $8.8 \pm 0.5^{c}$ | $7.9 \pm 0.5^{a}$ |
| dfD* | $36.0 \pm 0.7^{b}$ | $33.1 \pm 1.4^{a}$ | $35.6 \pm 1.5^{b}$ |
| dfP* | $34.2 \pm 1.2^{b}$ | $31.3 \pm 1.3^{a}$ | $33.8 \pm 1.0^{b}$ |
| dfA* | $55.6 \pm 1.9^{b}$ | $54.0 \pm 2.6^{a}$ | $55.5 \pm 2.0^{b}$ |
| Dorsal fin length (DL) | $59.1 \pm 1.7^{b}$ | $61.0 \pm 2.7^{c}$ | $56.8 \pm 1.6^{a}$ |
| Relative to head length | | | |
| Head depth (HD) | $42.5 \pm 2.1^{a}$ | $51.4 \pm 2.4^{c}$ | $43.3 \pm 2.1^{a}$ |
| Small head width (SHW) | $33.0 \pm 1.7^{a}$ | $35.2 \pm 2.2^{b}$ | $35.5 \pm 1.8^{b}$ |
| Large head width (LHW) | $52.5 \pm 2.1^{a}$ | $59.6 \pm 2.6^{c}$ | $54.5 \pm 2.1^{b}$ |
| Distance of two eyes (DE) | $27.2 \pm 1.3^{a}$ | $30.5 \pm 1.7^{c}$ | $28.4 \pm 1.5^{b}$ |
| Eyes diameter (ED) | $11.6 \pm 0.7^{a}$ | $14.1 \pm 0.9^{b}$ | $11.7 \pm 1.0^{a}$ |
| Upper jaw length (UJ) | $38.3 \pm 1.5^{b}$ | $36.5 \pm 1.9^{a}$ | $39.1 \pm 1.9^{b}$ |
| Lower jaw length (LJ) | $41.6 \pm 1.9^{b}$ | $40.4 \pm 1.9^{a}$ | $44.1 \pm 1.6^{c}$ |
| Mouth width (MW) | $42.7 \pm 1.9^{ab}$ | $42.1 \pm 2.0^{a}$ | $43.5 \pm 1.6^{b}$ |
| Relative to large head width | | | |
| Small head width (SHW) | $63.0 \pm 2.9^{b}$ | 59.1 ± 3.4 a | $65.3 \pm 3.3^{c}$ |
| Relative to Lower jaw length | | | |
| Upper jaw length (UJ) | $92.1 \pm 3.8^{b}$ | $90.5 \pm 4.2^{ab}$ | $88.6 \pm 3.4^{a}$ |
| Mouth width (MW) | $102.7 \pm 1.6^{ab}$ | $104.3 \pm 5.2^{b}$ | $98.7 \pm 3.3^{a}$ |

(*) dfD, dfP, and dfA are predorsal, prepectoral and preanal distances, respectively.
The values in the same row with different characters are significantly different at $p<0.05$.

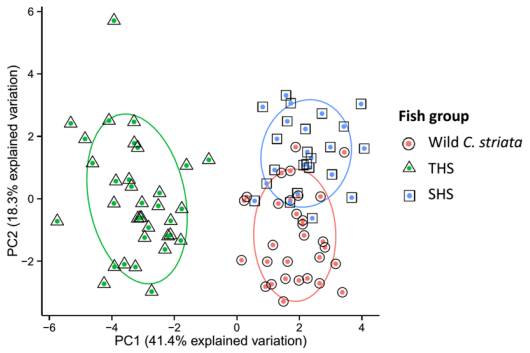


Figure 2. Two-dimension plot of principle component (PC) analysis using 18 log-transformed metric ratios (in Table 2) of three groups of wild *C. striata*, triange head (THS) and square head (SHS) snakehead. The ellipse circles indicate 68% probability for each fish group.

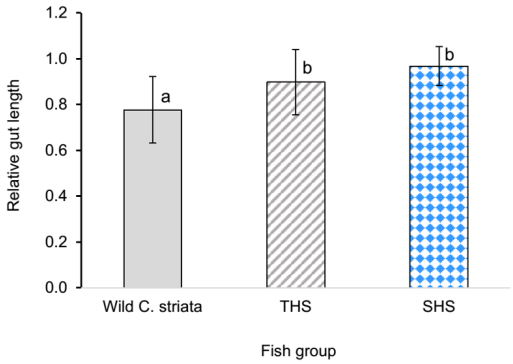


Figure 3. The relative gut length of wild *C. striata*, triangle-head snakehead (THS) and square-head snakehead (SHS). The error bars represent one standard deviation. Different letters next to the bars indicate significant difference among the means ($P<0.05$).

THS and SHS used to be fed trash fish; however, trash fish recently has been replaced by commercial feed in snakehead's diets. Changes in feed used and the large amount of feed fed in cultured conditions may result in an increase of their gut length.

### 3.2 Phylogeny relationship of *Channa* species based on COI sequences

COI sequences with the length of 652 base-pairs were compared among four *Channa* species and two new phenotypes (Genbank assession numbers: KT001931 - KT001938). Nucleotide composition and GC% of 1st and 3rd codon bases differ in four groups, corresponding to four species of *Channa* genus (Table 3). The two new phenotypes are in the same group of *C. striata*. On the other hand, *C. micropeltes* and *C. lucius* COI sequences differ greatly in GC content of 3rd base composition.

Comparing to the database in Genbank, COI sequences of four species *C. micropeltes*, *C. striata*, *C. gachua* and *C. lucius* have high levels (99-100%) of similarity to the same reported species. Additionally, two new phenotypes THS and SHS are highly similar (99%) to *C. striata*.

Within morpho-group genetic distances based on Kimura-2P nucleotide diversity are small for wild *C. striata* (0.0047±0.0019) and SHS (0.0029±0.0021), while all THS individuals are monomorphic at this locus. In contrast, inter-specific genetic distances among the four different species is much higher, ranging from 0.1836-0.2449 (Table 4). *C. micropeltes* and *C. lucius* are most similar to each other genetically and more genetically divergent from the other two species. Between-group genetic differences among THS, SHS, and wild *C. striata* (0.0017-0.0062) are as small as within-group distances.

The phylogenetic tree (Figure 4) using COI data in this study and COI sequences of *C. striata* from the BOLD systems database (www.boldsystems.org) shows that *C. striata* from across the species range and the two snakehead morpho-types (THS and SHS) form a monophyletic group, which is different from other *Channa* species (percent bootstrap support 100%). In the group of *C. striata*, THS and SHS cluster with the wild *C. striata* in the Mekong and other countries of the Mainland Southeast Asia and the Philippines (within cluster genetic distances 0.002-0.009). This group is very similar to *C. striata* in Indonesia, while in comparison the *C. striata* of Indian origin is genetically divergent, and distinct from all Southeast Asian individuals

Table 3. Percentage of nucleotide composition of *Channa* species and two morpho-types

| Species | T | C | A | G | GC#1 | GC#2 | GC#3 |
|---|---|---|---|---|---|---|---|
| *Channa micropeltes* | 26.6 | 31.0 | 23.8 | 18.6 | 57.0 | 42.5 | 49.1 |
| *Channa gachua* | 30.0 | 28.9 | 23.7 | 17.4 | 53.9 | 42.1 | 42.9±0.1 |
| *Channa lucius* | 30.0 | 27.8 | 24.3 | 18.0 | 56.6 | 42.5 | 38.2 |
| *Wild Channa striata* | 29.5 | 28.9 | 23.9 | 17.6 | 56.0±0.2 | 42.5 | 41.1±0.2 |
| THS | 29.5 | 28.8 | 24.0 | 17.7 | 56.1 | 42.5 | 40.8 |
| SHS | 29.5 | 28.9 | 23.9 | 17.8 | 56.1 | 42.5 | 41.2 |
| Average | 29.2 | 29.1 | 23.9 | 17.8 | 55.9±1.0 | 42.3±0.2 | 42.5±3.6 |

Note: THS: triangle-head snakehead, SHS: square-head snakehead

Table 4. Genetic distance between groups of *Channa* species and two morpho-types

| | *C.micropeltes* | *Wild C. striata* | *C. gachua* | *C. lucius* | *THS* | SHS |
|---|---|---|---|---|---|---|
| *C. micropeltes* | | 0.0175 | 0.0203 | 0.0173 | 0.0176 | 0.0175 |
| *Wild C. striata* | 0.1915 | | 0.0184 | 0.0192 | 0.0024 | 0.0014 |
| *C. gachua* | 0.2436 | 0.2075 | | 0.0203 | 0.0185 | 0.0184 |
| *C. lucius* | 0.1836 | 0.2354 | 0.2449 | | 0.0189 | 0.0191 |
| *THS* | 0.1946 | 0.0062 | 0.2082 | 0.2331 | | 0.0029 |
| *SHS* | 0.1926 | 0.0038 | 0.2082 | 0.2353 | 0.0017 | |

Note: Values of genetic distance in lower diagonal and standard error in upper diagonal.
TSH: triangle-head snakehead, SHS: square-head snakehead

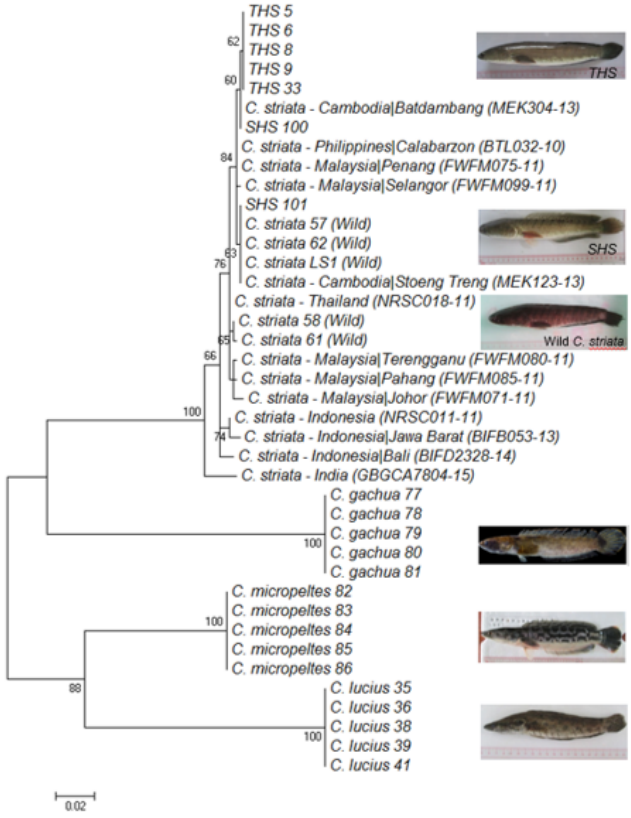


Figure 4. Phylogenetic tree of *Channa* species in the Mekong Delta, Viet Nam, and *C. striata* from across the species range. Sequences of COI that were downloaded from BOLD Systems are indicated with process ID in parentheses. The numbers on the nodes are maximum likelihood bootstrap values. The bar shows the relative branch length distance in nucleotide substitutions computed using the Kimura 2-parameter method. Photo of *C. gachua* from Tran Dac Dinh *et al.*, 2013.

(genetic distances 0.041-0.050) used in this analysis. Combining the genetic distances within and between groups of THS, SHS and wild *C. striata,* and the Genbank sequences, it can be concluded from the phylogenetic tree that THS and SHS are the same species as *C. striata*. These results indicate that *C. striata* is a morphologically diverse species with two new phenotypes but small genetic distances among morphotypes based on the COI gene do not support evidence of cryptic species.

## 4. Discussion

The most important finding of this study is that the two previously undefined phenotypes of *Channa* species (triangle-head, THS, and square-head snakehead, SHS) cultured in the Mekong Delta, Viet Nam, are morphologically different in comparison to wild type *C. striata* but, nonetheless, can still be considered to be members of the species *C. striata*. This result demonstrates that *C. striata* exhibits high within-species variation in morphology and diversity in COI sequences, even on a small geographic scale with different environmental conditions. The finding is consistent with results found in regional scales by Adamson *et al.* (2010). *C. striata* has been a target of aquaculture in Viet Nam for about 20 years. Differences in living environments and feeding types between cultured and wild conditions could be main factors affecting morphological variation among snakehead populations. Snakeheads are usually cultured in small ponds or hapa nets, where their movement is restricted to small areas. They are fed with trash fish, home-made or commercial pellets, and hence cultured fish require less locomotion to obtain adequate food in comparison to wild fish. The most variable characteristics affected by degrees of movement are the shape of their head and body. Similar findings have been reported in different fish species, such as topsmelt silverside *Atherinops affinis* (O'Reilly and Horn, 2004), African catfish *Clarias gariepinus* (Turan *et al.*, 2005), three-spined stickleback *Gasterosteus aculeatus* (Aguirre and Akinpelu, 2010), and blacktail shiner *Cyprinella venusta* (Haas *et al.*, 2010). In *C. venusta*, for example, populations from reservoirs are deep-bodied and have smaller heads compared to those inhabiting streams (Haas *et al.*, 2010). The length of the dorsal fin base, position of the dorsal fin and body depth also increases with increase in reservoir size. Haas *et al.* (2010) suggested that water impoundment drives evolutionary changes in morphology among blacktail shiner populations. Other environmental factors such as water flow and dissolved oxygen were found

to directly influence relative gill size, body shape and caudal fin shape of *Barbus neumayeri* (Langerhans *et al.*, 2007).

Larger RGL of cultured snakeheads (THS and SHS) compared to that of wild *C. striata* indicates that the digestive system of snakeaheads is also influenced by feed and feeding in cultured conditions. Longer intestine length is beneficial to cultured fish to store more food and increase digestive efficiency (Sibly 1981, cited by Wagner *et al.*, 2009). A review study based on 32 cichlid fish species revealed that diet is a determinant of intestine length at both intra- and inter-specific levels of fishes, suggesting their plastic response to the trade-off between nutritional needs and energetic costs (Wagner *et al.*, 2009). The change in intestine length can occur very fast, within 1-2 years as observed in silver carp (*Hypophthalmichthys molitrix*) exposed to different food resources (Ke *et al.*, 2008).

A previous study reported that high morphological divergence of *C. striata* populations in Malaysia did not correspond to molecular divergence based on the COI gene (Song *et al.*, 2013). However, we found the concordance between morphological and COI sequence variation of *C. striata*; while no intra-specific divergence was found in other species of *Channa* genus.

Genetic distance among *C. striata* morpho-types in the Mekong Delta, Viet Nam is equal to that of snakeheads distributed in different countries of Mainland Southeast Asia including Peninsular Malaysia, Cambodia, Thailand and Viet Nam (Figure 4). Genetic evidence based on cytochrome b also shows similar phylogenetic relationships of *C. striata* among Asia regions (Adamson *et al.*, 2010). This can be explained by current geographic distances (between Mainland Southeast Asia - India and - Indonesia) and historical biogeography. *C. striata* and other species of snakehead family originated from Himalayan region (Böhme, 2004). Ancestors of *C. striata* are then thought to have dispersed to Southeast Asia in the late Miocene, when warm and wet climate facilitated their overland movement to different regions (Adamson *et al.*, 2010).

The phylogenetic relationship among four *Channa* species based on COI sequences is similar to that based on a nuclear DNA (nDNA) recombination-activating gene I, RAG1 (Adamson *et al.*, 2010). Both studies show that *C. striata* and *C. gachua* are in the same clade, different from the clade of *C. micropeltes* and *C. lucius*. The concordant results between nDNA and mtDNA indicate the robustness of the phylogenetic tree. Genetic distances based on COI gene among four *Channa* species in this study (range 18.36-24.36%, Table 4) are also similar to those of the same species in Malaysia based on cytochrome b, ranging 21.70-31.40% (Abol-Munafi *et al.*, 2007). These congeneric distances are relatively high compared to those found in the *Channa* genus by Song *et al.* (2013). Using the same COI gene, Song *et al.* (2013) reported that congeneric distances varied from 1.7% - 10.10%, and *C. striata* was genetically closest to *C. lucius*. Disconcordance between our results and the findings by Song *et al.* (2013) can be atttributed by differences in the partial length of COI sequences (582 bp in Song *et al.* (2013) and 652 bp in our study), differences in taxa analyzed, or differences in phylogenetic analysis method.

High genetic distances between species versus low genetic distances within species of *Channa* genus provide more evidence that the COI barcoding gene is a powerful tool to complement to the traditional taxonomic method based on morphology.

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