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Genetic Variation Analysis between Wild and Cultured *Pangasianodon hypopthalmus* using COI and Cytochrome b Among Asian Countries

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ABSTRACT

One of the top species in the aquaculture sector, known as striped catfish or *Pangasianodon hypophthalmus*, is an important and valuable freshwater fish in many countries. Due to the high demand for this species, their number has declined to "threatened" levels. Hence, the purpose of this study is to analyse the genetic variation of wild and cultured striped catfish collected from five producers in Asian countries; Thailand, Vietnam, Indonesia, India, and Philippines, by using mitochondrial DNA partial region data sequence; CO1 and cytochrome b gene. Population analyses using 395 base pairs length for CO1 and 275 base pairs length of cytochrome b partial region nucleotide sequence have shown no significance difference between wild and cultured striped catfish. Vietnam species had shown a wide range of genetic distance of the intrapopulation compared with other countries in the range of 0.000-0.040 for CO1 gene and 0.003-0.008 for cytochrome b gene. The Neighbour-joining method has also been used to construct phylogenetic trees using CO1 gene; the tree formed few subclades with mixed populations, and the tree using cytochrome b showed only Vietnam species divided into a few sub-populations. For the other four countries, Thailand, Indonesia, India, and Philippines were in the same group. Hence, this study's findings may provide a reference for inter and intra-relationships of *P. hypophthalmus* that may help in the aquaculture activity of this striped catfish.

Keywords: *Pangasianodon hypopthalmus, aquaculture, genetic diversity, CO1, cytochrome b*

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INTRODUCTION

Pangasianodon hypophthalmus locally known as "Ikan patin sangkar" belongs to the family of *Pangasiidae*, and its common names are sutchi catfish, river catfish, and striped catfish. *P.hypophthalmus* is freshwater fish and inhabits rivers and lakes with a pH range from 6.5-7.5 and the temperature range of 22-26 °C. *Pangasianodon hypophthalmus* can migrate at a long distance as it is a migratory fish species [1]. It feeds on algae, zooplankton, and insects make it an omnivorous animal. Even for the adult, it feeds on fruit, crustaceans, and fish [2,3]. *Pangasianodon hypophthalmus* has gained economic importance, especially in many Asian countries, due to its tasty meat [4]. Their meat is highly valuable and has a high price market; for example, it is around RM12 to 15/kg in Malaysia. Over the past decades, due to the high demand for fisheries, a significant reduction in the world's fish stocks has occurred, and no longer capable of achieving optimum yield, so the aquaculture industry has played an important role in supplying seafood for human consumption [5]. The worldwide production of fisheries has reaching a peak at around 171 million tons in 2016, of which 90% of them are from Asia, and 47% of them are from the aquaculture sector [6].

Pangasianodon hypophthalmus is listed as one of the endangered species in the IUCN Red List of Threatened Species [7]. Thus, it is necessary to understand the life history, growth rate, and mortality of the *Pangasianodon hypophthalmus* in order to culture it and ensure the genetic of the culture is the same as in the wild so that the aquaculture industry can be identified as a successful way to protect and increase stocks for this species. Breeding is one of the options to meet the high demand for this species. However, several previous studies showed the decreases in genetic variability in the cultured species compared to the wild species causing worries and confusion to consumers. As reported in previous studies, *Liobagruss reinii* (Japanese torrent catfish) [8], *Clarias macrocephalus* (bighead catfish) [9], and *Catla catla* (Indian carp) [10] had shown differences in genetic composition when being cultured. Thus, conserving genetic structure to avoid declines in genetic variability in hatchery species is very important. The study on genetic differences between the wild and culture species is crucial as there are not many studies reported on genetic variation between the wild and culture, specifically for *Pangasianodon hypophthalmus* species by using mitochondrial DNA. This study can give further information to improve aquaculture and breeding programs, especially for the future species of *Pangasianodon hypophthalmus*.

DNA sequence data is proven to be the most reliable method in studying the motif of variation and relationship among species [11,12]. Many applications use genomic and mitochondrial DNA, COI and cytochrome b are the most used techniques for establishing phylogenetic relationships within and between populations [13,14]. The use of DNA markers helps observe and exploit genetic variation in the entire genome of the species [15]. In the study by Thuy Tran & Nguyen [16], they successfully used cytochrome b gene marker in constructing a

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phylogenetic tree of nine catfish species from the Mekong River. Hence, this study is performed to analyse the COI and cytochrome b gene marker sequences of wild and culture species of *P. hypophthalmus* from the five Asian countries: Thailand, Vietnam, Indonesia, India, and Philippines, and constructing the phylogenetic trees of this species.

EXPERIMENTAL

Data retrieval: COI and cytochrome b partial region of Pangasianodon hypopthalmus, Clarias batrachus and Tachysurus fulvidraco

A total of 28 individuals of *P.hypopthalmus*, one *Clarias batrachus* and one *Tachysurus fulvidraco* were retrieved from NCBI GenBank website for COI sequences (Table 1a), including four individuals from Thailand, seven individuals from Vietnam, four individuals from Indonesia and India, and the other nine individuals from Philippines. For cytochrome b a total of 38 individuals of were retrieved from a few previous studies according to its accession number and the NCBI GenBank website. A total of 36 individuals of *P.hypothalmus*, including 20 individuals from Thailand from the study of Phadphon et al., [17], India, six individuals from the study of Dwivedi et al., [18]. The other four individuals of Vietnam from Tran et al., [16], five individuals from Indonesia, and three individuals from Philippines were obtained from the NCBI GenBank database based on their accession number (Table 1b).

Table 1a: List of all 30 individuals for COI sequences used in this study

No.	Accession number	Species	Sample size	Type	Geographic area	Reference
1	KY118581	*P.hypophthalmus*	395	Wild	Thailand	NCBI
2	KY118582					
3	KY118583					
4	KR080263					
5	KY586002			Wild	Vietnam	NCBI
6	KY586005					
7	KY586006					
8	KY586000					
9	MN073457					

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10	MK777722					
11	MG981070					
12	KU692729			Culture	Indonesia	NCBI
13	KU692728					
14	KU692727					
15	KX685193					
16	MG230659			Wild	India	NCBI
17	MG230660					
18	KM232616					
19	HQ009497					
20	KF604667			Wild	Phillipines	NCBI
21	KF604668					
22	KF604669					
23	KF604670					
24	KF604671					
25	HQ682713			Culture	Phillipines	NCBI
26	HQ682714					
27	HQ682715					
28	HQ682716					
29	MG988399	*Clarias batrachus*	395	Outgroup	Bangladesh	NCBI
30	KP112368	*Tachysurus fulvidraco*	395	Outgroup	China	NCBI

Table 1b: List of all 40 individuals used in this study

No.	Accession number	Species	Sample size	Type	Geographic area	Reference
1	MN027132	*P.hypophthalmus*	275	Wild	Thailand	Phadphon et al., [17]
2	MN027133					
3	MN027134					
4	MN027135					
5	MN027136					
6	MN027137					
7	MN027138					
8	MN027139					
9	MN027140					

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10	MN027141					
11	MN027142					
12	MN027143					
13	MN027144					
14	MN027145					
15	MN027146					
16	MN027147					
17	MN027148					
18	MN027149					
19	MN027150					
20	MN027151					
21	KY586017			Wild	Vietnam	Tran et al,.[16]
22	KY586020					
23	KY586027					
24	KY586007					
25	KR007734			Culture	Indonesia	NCBI
26	KR007735					
27	KAB5587329					
28	ALD47542					
29	ALD47543					
30	KM434890			Culture	India	Dwivedi et al., [18]
31	KM434891					
32	KM434892					
33	KM434893					
34	KM434894					
35	KM434895					
36	AJF21329			Culture	Philippines	NCBI
37	KJ533254					
38	KJ533255					
39	AB822528	*Clarias batrachus*	275	Outgroup	Thailand	NCBI
40	AB015992	*Tachysurus fulvidraco*	275	Outgroup	Japan	NCBI

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Sampling Data Distribution

Figure 1 shows an Asia map, the origin of all the samples in this study. Five countries are involved in this study, including Thailand, Vietnam, Indonesia, India, and Philippines. All of these countries are involved in producing *P.hypopthalmus* species.

Figure 1: Sampling locations for *P.hypopthalmus* species situated in Asia

Data Analysis

ClustalW in MEGA X software was used to align the consensus COI and cytochrome b partial sequence of each sample. Kimura 2-Parameter method (K2P) was used to calculate the pairwise genetic distances within species, genus, and family, and also between species [19]. Neighbour-Joining trees at 500 pseudoreplicates [20] were constructed using the K2P model. K2P genetic distances and Neighbour Joining trees were produced using the MEGA version 10.1.8 software [21].

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DAMBE was used to check for the correct alignment of all the sequence data, and ABGD is another analyse online method that simple and fast which able to split the alignment sequence data set into species, that might complement with other evidence in an integrative phylogenic scientific method [22], which was performed to determine the number of species present in multiple sequences used in a graph form. The sequences were uploaded to the ABGD website. Kimura 2-parameter distance was selected when running the ABGD with the transition and transversion ratio equal to 2 and with a Fasta file input of the alignment [22]. The initial and recursive partitions showed from the graph indicated as a function of the prior limit between intra- and interspecies divergence.

RESULTS AND DISCUSSION

Multiple Sequence Alignment Analysis

In this study using MEGA X software, the length of aligned nucleotide sequences of *P.hypopthalmus* and the outgroups *Clarias batrachus* and *Tachysurus fulvidraco* were trimmed to 395 base pairs for COI sequences and 275 base pairs for cytochrome b sequences. Table 2a shows variable region (V) of COI sequences of *P.hypopthalmus* that showed 42.18%, the conserved region (C) was 68.36% and 13.09% for Parsimony informative region (Pi).

Whereas for cytochrome b sequences the variable region (V) showed 28.73% the conserved region (C) was 71.27% and 8.73% for Parsimony informative region (Pi). The conserved and variable regions are the regions that help to discover the similarities and differences among the sample species [23]. Parsimony informative region containing a minimum of two amino acids occurred with the lowest frequency of two.

Table 2a: The percentage of aligned partial sequences of COI gene

Regions	Percentage of the sequence data
C	68.36%
V	42.18%
Pi	13.09%

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Table 2b: The percentage of aligned partial sequences of cytochrome b gene

Regions	Percentage of the sequence data
C	71.27%
V	28.73%
Pi	8.73%

Model Test

In DNA sequences analysis, models of nucleotide substitution are frequently used, especially in estimating evolutionary parameters and building phylogenetic trees [24]. In this model test, the parameter used only Kimura-2-Parameter (K2+G) because Kimura is looking for the transversion and transition that occurred in the nucleotide sequence of each sample. The amount of transition and transversion is denoted in the percentage, and it seems to be higher than the transversion in this data.

Substitution Saturation Test

DAMBE software was used in this study to perform a substitution saturation test. This test was conducted to determine whether all the sequences selected that have undergone multiple alignment sequencing can construct a phylogenetic tree. The graph shows diverge data between the transitions to transversion, so it is good enough to build the phylogenetic tree to identify each sample's relationship [25].

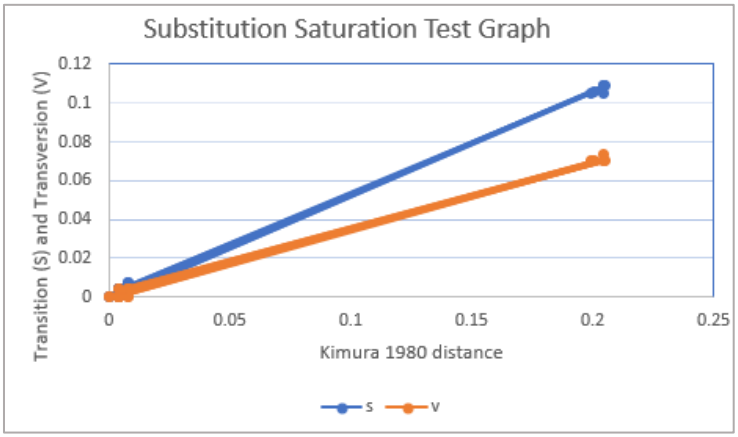

Figure 2: Diverged graph which indicated the data could proceed in constructing the phylogenetic trees

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Genetic distance table

The pairwise genetic distance analysis was used to produce genetic distance data, wherein this study are shown in Table 4a and Table 4b. The genetic distance shows an evolutionary divergence between the species which means the genetic difference value between species or population was measured to know how closely related those species. According to Sato & Miyazaki [26], data on amino acid or nucleotide sequences are used when comparing two distantly related species. If the value below 0.1% considered the sequences to be in the same species but with variation [26]. Thus, table 3a and 3b shows the genetic distance between *P.hypopthalmus* was below 0.1%, and Vietnam samples were observed to have shown low variation between other regions for both COI and cytochrome b gene marker which is around 0.003% to 0.040% and 0.003% to 0.008% respectively. As for the genetic distance between *P.hypothalmus* and the outgroups *Clarias batrachus* and *Tachysurus fulvidraco*, they were more than 0.1% which means they are not closely related.

Phylogenetic analysis

The phylogenetic tree for the sequences in this study was analysed using the Neighbour-joining method. The trees was built using trimmed nucleotide sequences [27] with the sample size of 395 base pairs for COI and 275 base pair for cytochrome b. The phylogenetic tree was built using aligned nucleotide sequences that only contain conserved regions from all individuals. The outgroups for the trees uses *Clarias batrachus* (Family: *Clariidae*) and *Tachysurus fulvidraco* (Family*: Bagridae*), the species categorized under the same order with *P.hypopthalmus*, which is Siluriformes order. The samples are from 5 Asian countries: Thailand, Vietnam, Indonesia, India, and the Philippines. The wild species are from 2 Asia countries: Thailand and Vietnam, whereas for the culture species, they are from India, Indonesia, and the Philippines.

Genetic variation is a significant feature of every species under domestication, since high additive genetic variance is more likely to occur for productive traits. The study's main objective was to compare the genetic variability within and between wild and culture stocks of *Pangasianodon hypopthalmus* and compare them between some Asian countries that also conduct aquaculture for this species which are Thailand, Vietnam, India, Indonesia and Philippines. This examines any genetic erosion due to inbreeding and genetic drift during broodstock management in the hatcheries. The distribution and genetic differentiation of species depend on biological, environmental, and historical factors. Sea level fluctuations and alternate seasons during the past have influenced freshwater fish populations [28-30].

The results in this in-silico study showed that no difference in genetic variation between wild and cultured species according to the wild species from two different countries Thailand and Vietnam, and cultured from three Asian countries; India, Indonesia and Philippines as shown in Table 3a and 3b. Furthermore, the close and no difference in genetic between wild and cultured

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species in Table 3a,3b and Figure 3a and 3b indicated the genetic changes in cultured species have not deteriorated. Contrary with the result in the study of Mohindra [31] that used cytochrome b marker. They successfully identified there was genetic diversity of *P.pangasius* species between the three different rivers in India. In the study of Vu, Ha, Thuy, Trang, and Nguyen [32], they used a new sequencing method known as DArT-seq and found that there were differences for Thailand species but no difference between wild and culture species in Cambodia and Vietnam. This study used the two most common mtDNA genetic markers: CO1 and cytochrome b, in addressing their phylogenetic interrelationships where both phylogenetic trees show *P. hypophthalmus* were in the same clade. But both have further divided into few other subclades, especially for the Vietnam species that showed a wide range of genetic distance and proven there were phylogenetic interrelationships within and between the other Asian populations. The Vietnam species result only showed a variation within the species in the range of 0.000-0.040 for CO1 gene and 0.003-0.008 for cytochrome b gene compared with the other Asian countries (Thailand, India, Indonesia and Philippines).

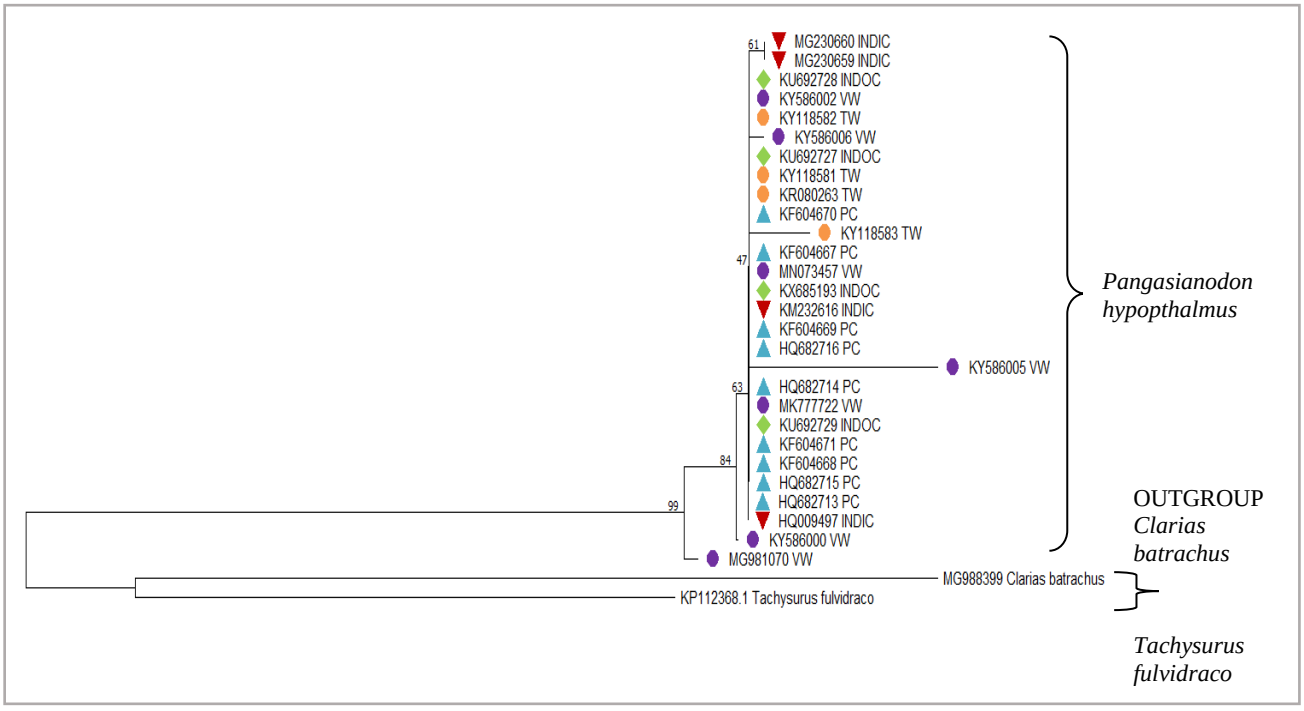

Figure 3a: A Neighbour-Joining tree of 30 COI sequences of *Pangasianodon hypopthalmus* together with outgroups *Clarias batrachus* and *Tachysurus fulvidraco* indicates ● TW as Wild species from Thailand, ● VW as Wild species from Vietnam, ▲PC as Culture species from Philippines, ◆INDOC as Culture species from Indonesia, ▼INDIC as Culture species from India.

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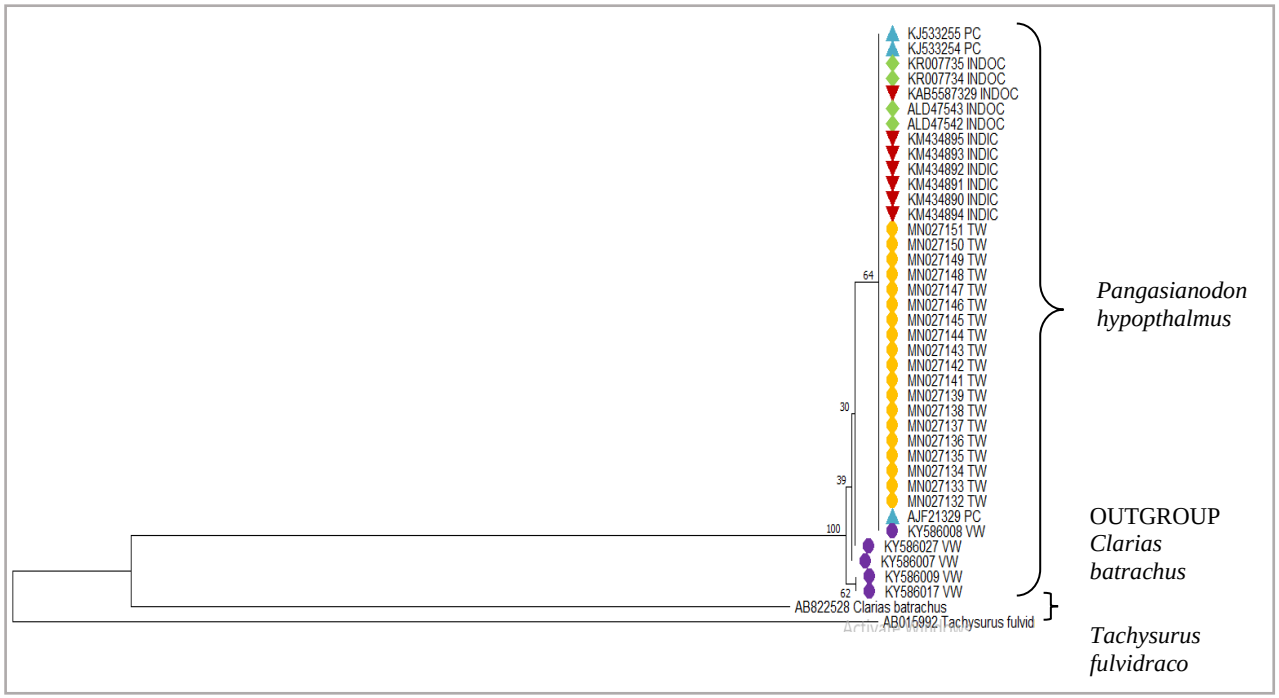

Figure 3b: A Neighbour-Joining tree of 40 cytochrome b sequences *Pangasianodon hypopthalmus* together with outgroups *Clarias batrachus* and *Tachysurus fulvidraco* indicates TW as Wild species from Thailand, VW as Wild species from Vietnam, PC as Culture species from Philippines, INDOC as Culture species from Indonesia, INDIC as Culture species from India.

For phylogenetic tree of CO1 gene in Figure 3a it shows few subclades consist of mixed population whereas for cytochrome b the population can be seen more clear division between the population in the clade so, cytochrome b gene more suitable and have better potential in determining the interrelationships between the population study than CO1 gene. As in the study of Chen *et al.*, (2019) they proved cytochrome b was better in showing intraspecific relationship in population genetic analysis than CO1 gene marker in their study.

In addition, the Mekong River is a huge river composed of a few countries in Southeast Asia. Due to geographical differences, it might cause the Vietnam species to have a wider genetic distance in their population and be divided into a few sub-populations, as shown in Figure 3a and 3b. According to the study of Kadar [29], the Mekong River populations were found to be the most diverse, but in this study, the result shows that they are still in the same genetic species. According to FAO [6], in captive breeding programs for terrestrial and aquatic animal species, aquaculture must maintain an inbreeding level of below 1% per generation to ensure breeding quality.

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On the other hand, the genetics of wild and cultured *P. hypophthalmus* in Thailand are identical to three other Asian aquaculture countries (India, Indonesia, and Philippines). It pointed out that they are genetically identical and can be concluded as a successful alternative for commercial production. The primary source of genetic diversity for aquaculture stocks is wild populations. Getting the breed from the wild and maintaining the genetics would help minimize overexploitation of striped catfish and thereby preserve genetic resources for this species, and the use of molecular data in a molecular analysis study can provide significant insight into the evolutionary relations that exist among different groups of organisms.

ABGD analysis as supporting result analysis

Automatic Barcode Gap Discovery (ABGD) is a simple, straightforward method for splitting a sequence alignment data set into potential species that would complement other evidence in an integrative taxonomic approach in the range of 1% to 3% [22]. The result for cytochrome b gene marker successfully identified three species which are *P.hypopthalmus, Clarias batracus* and *Tachysurus fulvidraco* but for COI gene marker there were 4 species where the Vietnam species with KY586005 accession number was identified as another species apart from other *P.hypopthalmus,* so it might need further identification.

Limitation and suggestions

The estimates or interpretation are potentially somewhat biased due to the low and imbalanced sample sizes and absent comparison between the wild and culture from the same population/countries in this analysis. The study by Kadar [29] proved that the combination of mitochondrial and microsatellite markers gives more detailed information on the genetic variation and population structure of *C.macrocephalus.* To improve in the further study, include additional markers like microsatellite can be one of the ways to get more promising results rather than using mitochondrial markers alone. While carrying out the selection programme for the aquaculture, such details will serve as additional input.

CONCLUSION

The low intra- and inter population of genetic variation between wild and culture of *P. hypophthalmus* with some Asian countries may give baseline information about the species' relationship and might also assist farmers in having effective restoration programmes in their aquaculture activity.

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AUTHOR'S CONTRIBUTION

Norfatimah Mohamed Yunus designed the research, supervised research progress, wrote and revised the article. Nik Azwarina R Azmi carried out the research, wrote and revised the article. Lyena Watty Zuraine Ahmad, Roziah Kambol and Nurul Aili Zakaria anchored the review, revisions and approved the article submission.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

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APPENDIX

I) Genetic distance table

Table 3a: Genetic distance table for COl gene marker

		1	2	3	4	5	6	7
1	*P.hypophthalmus* (Philippines - Culture) N:9	0.000-0.000						
2	*P.hypophthalmus* (Indonesia - Culture) N:4	0.000-0.000	0.000-0.000					
3	*P.hypophthalmus* (India- Culture) N:4	0.003-0.003	0.003-0.003	0.000-0.000				
4	*P.hypophthalmus* (Thailand - Wild) N:4	0.000-0.010	0.000-0.010	0.003-0.010	0.000-0.000			
5	*P.hypophthalmus* (Vietnam - Wild) N:7	0.000-0.030	0.000-0.010	0.000-0.003	0.000-0.040	0.000-0.000		
6	*Clarias batrachus* – Outgroup N:1	0.273-0.273	0.273-0.293	0.273-0.278	0.268-0.314	0.274-0.273	0.000-0.000	
7	*Tachysurus fulvidraco* - Outgroup N:1	0.229-0.229	0.229-0.229	0.229-0.232	0.229-0.247	0.234-0.280	0.225-0.225	0.000-0.000

Table 3b: Genetic distance table for cytochrome B gene marker

		1	2	3	4	5	6	7
1	*P.hypophthalmus* (Philippines - Culture) N:3	0.000-0.000						
2	*P.hypophthalmus* (Indonesia - Culture) N:5	0.000-0.000	0.000-0.000					
3	*P.hypophthalmus* (India- Culture) N:6	0.000-0.000	0.000-0.000	0.000-0.000				
4	*P.hypophthalmus* (Thailand - Wild) N:20	0.000-0.000	0.000-0.000	0.000-0.000	0.000-0.000			
5	*P.hypophthalmus* (Vietnam - Wild) N:4	0.003-0.007	0.004-0.007	0.004-0.007	0.004-0.008	0.000-0.000		
6	*Clarias batrachus* – Outgroup N:1	0.240-0.247	0.247-0.247	0.247-0.247	0.247-0.247	0.240-0.246	0.000-0.000	
7	*Tachysurus fulvidraco* – Outgroup N:1	0.304-0.304	0.304-0.304	0.304-0.304	0.304-0.304	0.304-0.304	0.304-0.304	0.000-0.000

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II) Kimura 2-Parameter model test

COI

Table. Maximum Likelihood fits of 24 different nucleotide substitution models

Model	Parameters	BIC	AICc	*lnL*	*(+I)*	*(+G)*	*R*	*f*(A)	*f*(T)	*f*(C)	*f*(G)	*r*(AT)	*r*(AC)	*r*(AG)	*r*(TA)	*r*(TC)	*r*(TG)	*r*(CA)	*r*(CT)	*r*(CG)	*r*(GA)	*r*(GT)	*r*(GC)
K2	58	2869.376	2441.912	-1162.666	n/a	n/a	1.18	0.250	0.250	0.250	0.250	0.057	0.057	0.136	0.057	0.136	0.057	0.057	0.136	0.057	0.136	0.057	0.057
T92	59	2870.717	2435.893	-1158.646	n/a	n/a	1.18	0.295	0.295	0.205	0.205	0.066	0.046	0.113	0.066	0.113	0.046	0.066	0.162	0.046	0.162	0.066	0.046
HKY	61	2873.176	2423.633	-1150.495	n/a	n/a	1.18	0.271	0.318	0.249	0.162	0.073	0.057	0.088	0.062	0.134	0.037	0.062	0.172	0.037	0.147	0.073	0.057
K2+I	59	2873.594	2438.770	-1160.085	0.34	n/a	1.24	0.250	0.250	0.250	0.250	0.056	0.056	0.138	0.056	0.138	0.056	0.056	0.138	0.056	0.138	0.056	0.056
T92+I	60	2875.108	2432.924	-1156.152	0.37	n/a	1.25	0.295	0.295	0.205	0.205	0.064	0.045	0.116	0.064	0.116	0.045	0.064	0.166	0.045	0.166	0.064	0.045
K2+G	59	2875.110	2440.285	-1160.842	n/a	1.25	1.25	0.250	0.250	0.250	0.250	0.056	0.056	0.139	0.056	0.139	0.056	0.056	0.139	0.056	0.139	0.056	0.056
T92+G	60	2875.490	2433.306	-1156.343	n/a	1.07	1.26	0.295	0.295	0.205	0.205	0.064	0.045	0.116	0.064	0.116	0.045	0.064	0.167	0.045	0.167	0.064	0.045
HKY+I	62	2876.001	2419.099	-1147.218	0.38	n/a	1.26	0.271	0.318	0.249	0.162	0.071	0.055	0.090	0.060	0.138	0.036	0.060	0.177	0.036	0.151	0.071	0.055
HKY+G	62	2877.479	2420.576	-1147.957	n/a	1.00	1.27	0.271	0.318	0.249	0.162	0.070	0.055	0.091	0.060	0.139	0.036	0.060	0.178	0.036	0.151	0.070	0.055
JC	57	2880.961	2460.857	-1173.148	n/a	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
TN93	62	2881.565	2424.663	-1150.000	n/a	n/a	1.18	0.271	0.318	0.249	0.162	0.074	0.058	0.074	0.063	0.148	0.038	0.063	0.190	0.038	0.124	0.074	0.058
K2+G+I	60	2882.979	2440.795	-1160.087	0.34	200.00	1.24	0.250	0.250	0.250	0.250	0.056	0.056	0.138	0.056	0.138	0.056	0.056	0.138	0.056	0.138	0.056	0.056
T92+G+I	61	2883.315	2433.772	-1155.565	0.37	200.00	1.25	0.295	0.295	0.205	0.205	0.064	0.045	0.116	0.064	0.116	0.045	0.064	0.166	0.045	0.166	0.064	0.045
TN93+I	63	2884.414	2420.152	-1146.734	0.38	n/a	1.27	0.271	0.318	0.249	0.162	0.071	0.055	0.075	0.060	0.154	0.036	0.060	0.198	0.036	0.126	0.071	0.055
HKY+G+I	63	2885.417	2421.156	-1147.236	0.38	200.00	1.27	0.271	0.318	0.249	0.162	0.070	0.055	0.091	0.060	0.139	0.036	0.060	0.178	0.036	0.151	0.070	0.055
TN93+G	63	2885.799	2421.538	-1147.427	n/a	1.01	1.28	0.271	0.318	0.249	0.162	0.071	0.055	0.075	0.060	0.156	0.036	0.060	0.200	0.036	0.125	0.071	0.055
JC+I	58	2885.886	2458.422	-1170.921	0.31	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC+G	58	2887.528	2460.064	-1171.742	n/a	1.48	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
TN93+G+I	64	2893.797	2422.177	-1146.736	0.37	200.00	1.27	0.271	0.318	0.249	0.162	0.071	0.055	0.075	0.060	0.155	0.036	0.060	0.198	0.036	0.126	0.071	0.055
JC+G+I	59	2895.274	2460.449	-1170.924	0.31	200.00	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
GTR	65	2907.139	2428.162	-1148.717	n/a	n/a	1.19	0.271	0.318	0.249	0.162	0.082	0.072	0.075	0.070	0.150	0.026	0.078	0.192	0.031	0.126	0.050	0.048
GTR+I	66	2910.699	2424.364	-1145.807	0.36	n/a	1.27	0.271	0.318	0.249	0.162	0.076	0.070	0.076	0.065	0.157	0.026	0.076	0.200	0.031	0.126	0.050	0.047
GTR+G	66	2912.115	2425.780	-1146.515	n/a	1.11	1.28	0.271	0.318	0.249	0.162	0.076	0.069	0.075	0.065	0.158	0.026	0.076	0.202	0.030	0.126	0.050	0.047
GTR+G+I	67	2920.274	2426.582	-1145.904	0.16	1.99	1.28	0.271	0.318	0.249	0.162	0.076	0.069	0.075	0.065	0.158	0.026	0.075	0.202	0.031	0.126	0.050	0.047

Cytochrome b

Table. Maximum Likelihood fits of 24 different nucleotide substitution models

Model	Parameters	BIC	AICc	*lnL*	*(+I)*	*(+G)*	*R*	*f*(A)	*f*(T)	*f*(C)	*f*(G)	*r*(AT)	*r*(AC)	*r*(AG)	*r*(TA)	*r*(TC)	*r*(TG)	*r*(CA)	*r*(CT)	*r*(CG)	*r*(GA)	*r*(GT)	*r*(GC)
TN93	82	2185.091	1587.274	-711.014	n/a	n/a	1.89	0.280	0.326	0.241	0.153	0.059	0.043	0.043	0.051	0.220	0.028	0.051	0.299	0.028	0.078	0.059	0.043
T92	79	2185.656	1609.667	-725.255	n/a	n/a	1.79	0.303	0.303	0.197	0.197	0.053	0.034	0.128	0.053	0.128	0.034	0.053	0.198	0.034	0.198	0.053	0.034
HKY	81	2187.449	1596.908	-716.846	n/a	n/a	1.81	0.280	0.326	0.241	0.153	0.058	0.043	0.099	0.050	0.155	0.027	0.050	0.211	0.027	0.181	0.058	0.043
GTR	85	2188.155	1568.514	-698.587	n/a	n/a	1.87	0.280	0.326	0.241	0.153	0.055	0.105	0.045	0.048	0.225	0.000	0.122	0.305	0.004	0.083	0.000	0.007
K2	78	2192.601	1623.888	-733.380	n/a	n/a	1.79	0.250	0.250	0.250	0.250	0.045	0.045	0.161	0.045	0.161	0.045	0.045	0.161	0.045	0.161	0.045	0.045
TN93+G	83	2194.281	1589.189	-710.956	n/a	4.52	1.96	0.280	0.326	0.241	0.153	0.057	0.042	0.042	0.049	0.224	0.027	0.049	0.304	0.027	0.077	0.057	0.042
TN93+I	83	2194.297	1589.205	-710.964	0.11	n/a	1.95	0.280	0.326	0.241	0.153	0.058	0.043	0.042	0.050	0.223	0.027	0.050	0.303	0.027	0.077	0.058	0.043
T92+I	80	2194.770	1611.505	-725.159	0.16	n/a	1.85	0.303	0.303	0.197	0.197	0.052	0.033	0.130	0.052	0.130	0.033	0.052	0.200	0.033	0.200	0.052	0.033
T92+G	80	2194.779	1611.514	-725.163	n/a	3.81	1.85	0.303	0.303	0.197	0.197	0.052	0.034	0.130	0.052	0.130	0.034	0.052	0.200	0.034	0.200	0.052	0.034
HKY+G	82	2196.503	1598.687	-716.720	n/a	3.14	1.86	0.280	0.326	0.241	0.153	0.057	0.042	0.100	0.049	0.157	0.027	0.049	0.213	0.027	0.183	0.057	0.042
HKY+I	82	2196.505	1598.688	-716.721	0.17	n/a	1.86	0.280	0.326	0.241	0.153	0.057	0.042	0.100	0.049	0.157	0.027	0.049	0.213	0.027	0.183	0.057	0.042
GTR+I	86	2197.447	1570.533	-698.581	0.04	n/a	1.89	0.280	0.326	0.241	0.153	0.055	0.105	0.045	0.047	0.226	0.000	0.122	0.307	0.004	0.082	0.000	0.007
GTR+G	86	2198.451	1571.536	-699.082	n/a	3.38	1.96	0.280	0.326	0.241	0.153	0.051	0.108	0.047	0.044	0.228	0.000	0.126	0.309	0.000	0.086	0.000	0.000
K2+I	79	2201.665	1625.676	-733.259	0.17	n/a	1.85	0.250	0.250	0.250	0.250	0.044	0.044	0.162	0.044	0.162	0.044	0.044	0.162	0.044	0.162	0.044	0.044
K2+G	79	2201.670	1625.681	-733.262	n/a	3.28	1.86	0.250	0.250	0.250	0.250	0.044	0.044	0.163	0.044	0.163	0.044	0.044	0.163	0.044	0.163	0.044	0.044
TN93+G+I	84	2203.586	1591.220	-710.956	0.00	4.61	1.96	0.280	0.326	0.241	0.153	0.058	0.042	0.042	0.049	0.224	0.027	0.049	0.304	0.027	0.077	0.058	0.042
T92+G+I	81	2204.085	1613.544	-725.163	0.00	3.82	1.85	0.303	0.303	0.197	0.197	0.052	0.034	0.130	0.052	0.130	0.034	0.052	0.200	0.034	0.200	0.052	0.034
HKY+G+I	83	2205.809	1600.717	-716.720	0.00	3.14	1.86	0.280	0.326	0.241	0.153	0.057	0.042	0.100	0.049	0.157	0.027	0.049	0.213	0.027	0.183	0.057	0.042
GTR+G+I	87	2206.749	1572.561	-698.579	0.00	12.23	1.89	0.280	0.326	0.241	0.153	0.054	0.105	0.045	0.047	0.227	0.000	0.122	0.307	0.004	0.082	0.000	0.007
K2+G+I	80	2210.975	1627.710	-733.262	0.00	3.28	1.86	0.250	0.250	0.250	0.250	0.044	0.044	0.163	0.044	0.163	0.044	0.044	0.163	0.044	0.163	0.044	0.044
JC	77	2212.454	1651.018	-747.959	n/a	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC+I	78	2221.759	1653.047	-747.959	0.01	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC+G	78	2221.759	1653.047	-747.959	n/a	200.00	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC+G+I	79	2231.065	1655.076	-747.959	0.00	200.00	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083