

Phylogenetics and population genetics of *Plotosus canius* (Siluriformes: Plotosidae) from Malaysian coastal waters

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ABSTRACT

Plotosus canius (Hamilton, 1822) is a significant marine species in Malaysia from nutritional and commercial perspectives. Despite numerous fundamental research on biological characteristics of *P. canius*, there are various concerns on the level of population differentiation, genomic structure, and the level of genetic variability among their populations due to deficiency of genetic-based studies. Deficiency on basic contexts such as stock identification, phylogenetic relationship and population genetic structure would negatively impact their sustainable conservation. Hence, this study was conducted to characterize the genetic structure of *P. canius* for the first time through the application of mitochondrial Cytochrome Oxidase I (COI) gene, cross amplification of *Tandanus tandanus* microsatellites, and a total of 117 collected specimens across five selected populations of Malaysia. The experimental results of the mitochondrial analysis revealed that the haplotype diversity and nucleotide diversity varied from 0.395–0.771 and 0.033–0.65 respectively. Moreover, the statistical analysis of microsatellites addressed a considerable heterozygote insufficiency in all populations, with average observed heterozygosity (H_o) value of 0.2168, which was lower than the standard heterozygosity in marine populations ($H_o = 0.79$). This alongside the high F_{is} values estimation, high pairwise differentiation among populations and low within population variations are supposed to be associated with small sample size, and inbreeding system. Besides, the significant finding of this study was the sharing of common haplotype [KR086940](#), which reflects a historical genetic connectivity between Peninsular Malaysia and Borneo populations due to the geological history of Southeast Asia during Pleistocene era. Demographic analyses showed that all populations were in an equilibrium state with no significant evidence of population expansion. To put it briefly, the current study has managed to provide an initial genomic database toward understanding of the genetic characterization, phylogenetic, molecular diversification and population structure in *P. canius*, and should be necessary highlighted for appropriate management and conservation of species. Further studies must be carried out involving more geographical and sampling sites, larger population size per site, and utilization of species specific microsatellites loci.

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INTRODUCTION

Plotosus canius (Hamilton, 1822) that is known as grey eel-catfish, black eel-tail catfish, canine catfish or Indian catfish (Khan et al., 2002; Riede, 2004; Usman et al., 2013; Prithiviraj, 2014), were diagnosed as a member of genus *Plotosus*, family Plotosidae (Froese & Pauly, 2015). They are being mainly distributed in estuaries, freshwater rivers, lagoons, and shallow waters of Australia and Southeast Asia (Carpenter, 1999; Prithiviraj & Annadurai, 2012). The species is an amphidromous and demersal bony fish that can live in marine, brackish and freshwater habitats. Their relocation in about 100 km range were described as cyclical and frequent horizontal movement on which could not be categorized as breeding migration (Riede, 2004). However, the species might be recently endured genetic destruction mostly due to overexploitation similar to other fish species (Pauly et al., 2002; Collette et al., 2011; Usman et al., 2013), their population structure could be considered as the reliable indicator in detection of sustainable and healthy marine environments (Thomsen et al., 2012; Bourlat et al., 2013).

Population structure is the direct consequence of biogeography (Leffler et al., 2012), which provides invaluable statistics on patterns of species dynamics, colonization, and isolation (Costello et al., 2003). As species become accustomed to new habitats, the effective size of population extends through its dispersal, resulting in intensification of genetic variation (Charlesworth & Willis, 2009). However, deterioration of environmental equations alongside with ecological fluctuations such as recent re-treatment of Pleistocene era have changed species extensive genetic patterns (Krishnamurthy & Francis, 2012). Adding to complication, the accuracy of associated conservation strategies can be successively restrained by deficiency of reliable knowledge on biodiversity, conservation resolution, and extent of biological destruction among taxonomic levels (Wright, Tregenza & Hosken, 2008; Butchart et al., 2010; Magurran et al., 2010; Pereira et al., 2010; Hoffmann et al., 2011). Such scarcities confidently offered a viable incentive to regulate the sustainable species variation (Primack, 2002; Duvernell et al., 2008; Appeltans et al., 2012; Bourlat et al., 2013; Leray & Knowlton, 2015) through advancing genomic protocols to challenge the genetic intimidations such as distraction of local traits, genetic drift and inbreeding effects (Tallmon, Luikart & Waples, 2004).

The same conservation obstacle is hypothetically threatening *P. canius* populations in Malaysia, since there is not any comprehensive documentation nor a single initial research on their genetic characterization. Indeed, regarding to their regional significance in Oceania and Southeast Asia (Usman et al., 2013), a few regional studies have been merely carried out on basic biological perceptions of *Plotosus canius* including their morphology and fisheries (Kumar, 2012; Usman et al., 2013), fecundity (Khan et al., 2002; Usman et al., 2013), feeding behaviour (Leh, Sasekumar & Chew, 2012), protein structure (Prithiviraj & Annadurai, 2012) and pharmacology (Prithiviraj, 2014). Such deficiencies have raised some severe concerns on the level of population structure, genetic variation and the

consequences of genetic differentiation among populations of *P. canius* especially in Malaysia. Thus, this study was performed to genetically characterize *P. canius* through the utilization of the mitochondrial Cytochrome Oxidase I (COI) gene and *Tandanus tandanus* microsatellites, in order to examine the accuracy of employed markers in phylogenetic study, genetic variation assignment, and population genetic structure of *P. canius* in Malaysia.

MATERIALS AND METHODS

Sample collection and DNA isolation

Total of 130 catfish samples demonstrating two species of family Plotosidae were directly collected, including of 117 samples of *Plotosus canius* and 13 samples of *Plotosus lineatus*. Sample collection of *P. canius* were performed in five various districts throughout Malaysia including: Negeri Sembilan (NSN), Selangor (SGR), Johor (JHR), Sarawak (SWK) and Sabah (SBH) (Fig. 1) from May–December 2014, while *P. lineatus* samples were only collected from Selangor. Sampling was carried out directly from fishermen in commercial fishing docks of Port Dickson (Negeri Sembilan), Kuala Selangor (Selangor), Kukup (Johor), Bintulu (Sarawak) and Putatan (Sabah). DNA extraction protocol was performed upon sample collection in laboratory via the Wizard[®] SV Genomic DNA Purification System (Promega, Madison, WI, USA), according to manufacturer's protocol instructions by using roughly 20 mg of specimens.

PCR amplification and sequencing of mitochondrial DNA

To accurately amplify a 655 bp fragment of mitochondrial DNA, PCR amplification were performed by using C_Fish1 primer set (Ward *et al.*, 2005) on the 5' end of COI gene. The amplification protocol were conducted in an overall volume of 25 μ l at which contained 0.6 μ l of each deoxynucleotide triphosphate (dNTPs), 0.7 μ l Taq polymerase, 1.2 μ l MgCl₂, 0.25 μ l of each primers, 2 μ l of concentrated genomic DNA, 2.5 μ l of Taq buffer and 18 μ l of distilled H₂O as stated by Ward *et al.* (2005), with slight modification. The PCR reaction was carried out using an Eppendorf Mastercycler based on the following thermal regime: 2 min of 95 °C initial denaturation step; 35 cycles of 94 °C denaturation step for 30 s, a 54 °C annealing temperature for 45 s and a 72 °C extension period of 1 min; followed by 72 °C final extension step for 10 min and a routine 4 °C final hold (Ward *et al.*, 2005). In order to confirm that the PCR reaction generated sufficient amplicon proportions, PCR amplification products were visualized using a 2.0% laboratory grade agarose gel containing 5 μ l GelRed stain. Amplified products were subsequently isolated and purified upon their visualization and documentation. DNA purification from gel was commonly carried out using the Wizard[®] SV Gel and PCR Clean-up System (Promega, Madison, WI, USA). Purified DNA samples were finally sent to private sector institution (1st Base laboratories Sdn Bhd) for sequencing to generate associated trace files and continuous read lengths intended for genetic and statistical analysis of mitochondrial DNA.

Statistical analysis of mitochondrial DNA

Trace files were manually end-trimmed using BioEdit software 7.2.5 (Hall, 1999) regarding to their homologous section. Afterwards, ClustalX 2.1 (Thompson *et al.*, 1997)

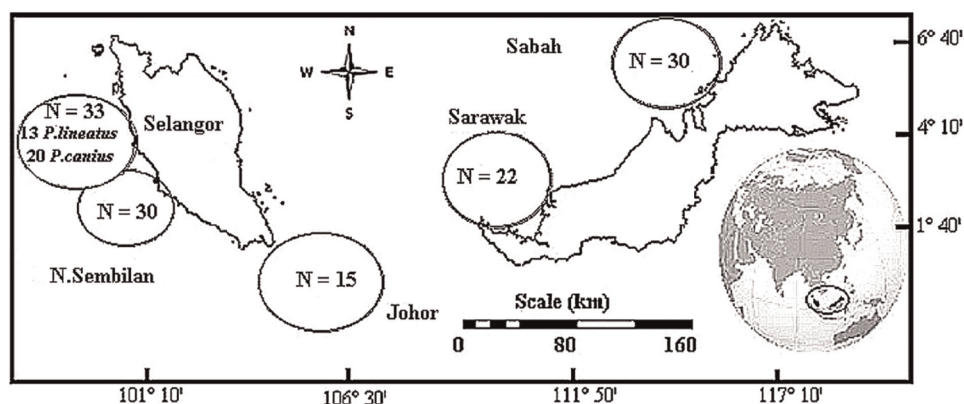


Figure 1 Sampling sites and sample size (N) diagram of *P. canius* and *P. lineatus* in Malaysia.

was applied to progressively manipulate, align and analyze the DNA sequences. Finally, haplotypes were detected with DnaSP software 5.10.01 (Librado & Rozas, 2009) and deposited into BOLDSYSTEM (International Barcode of Life) and GenBank.

To comparatively analyze mitochondrial DNA sequences, MEGA 6 program (Tamura et al., 2013) were used intending to understand the phylogenetic outlines of COI gene and species, generate sequence alignment and perform evolutionary analysis. Calculation of the pairwise distance was obtained through 1,000 bootstrap variance estimation and Tamura-Nei model (Tamura & Nei, 1993). Moreover, overall mean nucleotide distance of sequences was computed using same configuration at each codon positions separately. Subsequently, construction of phylogenetic tree from the highest grade aligned sequences of *P. canius* and *P. lineatus* was prompted in comparison to one haplotype of African sharp tooth catfish *Clarias gariepinus* (ANGBF8254-12) from Thailand as an outgroup through Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods using a mutual 1,000 replication bootstrap. Next, Minimum Spanning Network (MSN) was computed using PopART (Bandelt, Forster & Röhl, 1994) application among obtained sequences of *P. canius*.

Extraction of genetic features from assembly of sequences based on some rudimentary implemented analytical tests was performed through Arlequin software 3.5 (Excoffier, Laval & Schneider, 2005). As the most crucial objective was to compute the genetic structure, hierarchical analysis of molecular variance (AMOVA) and pairwise F_{ST} values of chi square test, population differentiation was successively calculated among five populations of *P. canius* in Malaysia. Analysis of molecular variance was carried out using 1,000 permutation to compute distance matrix of sequences, while the same structure were implemented for comparison of all available pair samples and populations through calculation of F_{ST} with 0.05 significance level.

Demographic history was estimated using Tajima's D test (Tajima, 1989) and Fu's F_s test (Fu, 1997) to test the hypothesis of neutrality of the COI gene. A negative Tajima's D-values might indicate bottleneck, selection or population expansion (Tajima, 1989). Mismatch distributions test was also computed to evaluate the hypothesis of recent population expansion or growth (Rogers & Harpending, 1992). The distribution is

commonly unimodal in populations that have undergone a recent demographic expansion, and is multimodal in stable or equilibrium populations. All analyses were carried out using ARLEQUIN, while associated mismatch graphs were obtained from DnaSP.

Microsatellite genotyping

To run measured PCR protocol, optimization of PCR composition were performed at which made positive outcomes based on initial PCR regime using the main protocol for the amplification of *Tandanus tandanus* (Rourke et al., 2010) with minor regulation in the amount and concentration of primers, Taq DNA polymerase, MgCl₂ and dNTPs to enhance the accuracy of the protocol. However, the optimum annealing temperature were practically calculated 55 °C similar as original species. To consciously amplify DNA fragments, PCR amplification were performed by using five cross-amplified microsatellites of *T. tandanus* (Rourke et al., 2010; Rourke & Gilligan, 2010) on the 5' end, presented in Table 5. The feasible amplification protocol were conducted in a total volume of 25 µl solution inclosing 0.6 µl of each deoxynucleotide triphosphate (dNTPs), 0.7 µl Taq DNA polymerase, 1.2 µl MgCl₂, 0.25 µl of each primers, 2 µl of concentrated genomic DNA, 2.5 µl of Taq buffer and 18 µl of distilled H₂O as stated by Rourke & Gilligan (2010) with slight modification.

The PCR reaction was carried out using a gradient Eppendorf Mastercycler based on the following thermal adjusted protocol: 2 min of 95 °C initial denaturation step; 35 cycles of 95 °C denaturation step for 30 s, a 55 °C annealing temperature for 45 s and a 72 °C extension period of 1 min; followed by a 72 °C final extension (elongation) step for 10 min and a routine 4 °C final hold (Rourke et al., 2010; Rourke & Gilligan, 2010). Afterwards, PCR amplification products were visualized using a 4.0% MetaPhor agarose gel containing 5 µl GelRed staining solution. Subsequently, gel images were subjected to microsatellite screening and approximately 15 µl of the florescent label products were packed and sent to First Base Laboratories (private institution) for fragments analysis.

Genetic analysis of microsatellite markers

In order to verify any null alleles and scoring error, MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004) were applied using diploid data obtained from CONVERT software (Glaubitz, 2004). GENEPOP 4.2 (Rousset, 2008) was employed in order to evaluate the conformity to the “Hardy-Weinberg expectations” (HWE) with 10,000 permutations for test of exact probability. Observed heterozygosity (H_o) was estimated using GENALEX 6.5 (Peakall & Smouse, 2012). Successively, FSTAT 2.9.3.2 (Goudet, 1995) was applied to calculate the expected heterozygosity (H_e), with 15,000 permutation and MolKin 3.0 (Gutiérrez et al., 2005) to validate the genetic analysis among genetic dataset using Polymorphism Information Content (PIC). Afterwards, ARLEQUIN 3.0 (Excoffier, Laval & Schneider, 2005) were used to analyze the genetic configuration, hierarchical AMOVA and pairwise F_{ST} estimations among all five involved populations, using reflection of 95% significance level and 10,000 permutations. Assignment of each individual to their genetic groups (K) employing admixture model and its associated

frequency of allelic data was carried out using the STRUCTURE program 2.1 (Pritchard, Stephens & Donnelly, 2000). Next, GENECLASS2 2.0 (Piry et al., 2004) was implemented to conduct assignment of individuals into the most plausible inheritance group. Finally, probability of current bottleneck was tested using BOTTLENECK software 1.2.02 (Piry, Luikart & Cornuet, 1999).

RESULTS

Phylogenetic and population analysis inferred from mitochondrial DNA

Among overall number of 130 studied specimens using one COI gene, a full mass of 118 reliable sequences (approximately 91%) were identified. Once the sequencing was completed, the C_Fish1 accordingly confirmed as suitable primer set amplifying in *P. canius* and *P. lineatus* DNA samples. Nevertheless, in some cases there were some uncertain base calls, there was no observation of any stop codons nor any instances of insertion or deletion in sequences. Deficiency of structural stop codon more likely supposed to be associated with every amplified mitochondrial sequences, and all that, alongside with the read length of amplified sequences implies that nuclear sequences initiated from vertebrate mitochondrial DNA are not sequenced. Such occasions are based on the fact that nuclear sequences have typically read lengths less than 600 bp (Zhang & Hewitt, 1996). Therefore, the selected COI gene alone was considered for phylogenetic and population structure analysis of *P. canius* and *P. lineatus* in advance.

Preliminary evaluation of verified sequences was mainly generated 20 haplotypes in five populations of *P. canius* and 3 haplotypes in one population of *P. lineatus* (Table 1). Based on the presented data of obtained haplotypes, it can be evidently seen that in *P. canius* samples, KR086940 was found as the most common haplotype in the entire populations from Malaysia, however it was not found in the Negeri Sembilan and the Sabah populations. Moreover, KR086939 was detected as the second common haplotype in *P. canius* populations. The N. Sembilan and the Selangor populations had the most unique haplotypes, while the Johor population had just two shared haplotypes each. In other words, the N. Sembilan and the Selangor population possessed the highest number of identified haplotypes ($n = 6$), while the N. Sembilan and the Sabah populations had five and four haplotypes respectively.

For the 655 available COI nucleotides, 509 sites (roughly 78%) were detected as conserved sites, 146 (22%) as variable sites and 136 (21%) identified as parsim-informative sites. The average nucleotide composition in *P. canius* was 29% T, 27.6% C, 25.2% A and 18.3% G, while the average C + G content of selected positions was calculated as 45.9%, in *P. lineatus* though, calculation was 28, 28.6, 25, 18.4 and 47% respectively. Translation of all 23 haplotypes for conserved 655 bp fragment was produced 165 amino acids, which presented no signal of pseudogene in their structure. However, the sample size noticeably varied, ranging from 13–30 in collected samples of *P. lineatus* and *P. canius* from five different districts in Malaysia; in regard to the fact that original sample sizes were moderate and some sequences were failed, the actual sample collection

Table 1 Overview of haplotypes, their sampling sites and accession numbers.

Species	BOLDSYSTEM index	GenBank accession number	Sampling site
<i>Plotosus lineatus</i>	NUPM017-14	KP258659	Selangor
	NUPM016-14	KP258657	
	NUPM015-14	KP258658	
<i>Plotosus canius</i>	NUPM001-14	KP258648	Negeri Sembilan
	NUPM002-14	KP258651	
	NUPM006-14	KP258655	
	NUPM023-15	KR086935	Sabah
	NUPM003-14	KP258650	
	NUPM004-14	KP258649	
	NUPM005-14	KP258656	
	NUPM022-15	KR086936	
	NUPM007-14	KP258654	Selangor
	NUPM008-14	KP258653	
	NUPM009-14	KP258652	
	NUPM020-15	KR086938	Sarawak
	NUPM021-15	KR086937	
	NUPM010-14	KP221601	
	NUPM011-14	KP221602	
	NUPM012-14	KP221603	
	NUPM013-14	KP221604	Selangor
	NUPM014-14	KP221605	
	NUPM018-15	KR086940	Sarawak
NUPM019-15	KR086939	Johor	
		Johor	
		Negeri Sembilan	

is in the desired range recommended by *Zhang et al. (2010)*. Basically, in order to detect approximately 80% genetic variation, a collection of 31.9–617.8 samples could be needed, although it is far from real laboratory and field work measures; hence, it is suggested that at least 10 sample might be desirably sufficient to accurately identify the genetic variability in real phylogenetic studies (*Zhang et al., 2010*). An overview of most crucial outcomes in polymorphism analysis (*Table 2*) indicated that the number of variable sites are moderately fluctuating from 2 in *P. lineatus* to 54 in *P. canius* samples from Sarawak, While the degree of nucleotide diversity was relatively low (0.00067–0.0391) and as its consequence, the level of polymorphism and genetic variation in populations reasonably presented small portion. Besides, the degree of haplotypes diversity waved from 0.395 (Sabah) to 0.771 (Sarawak).

The Tamura-Nei pairwise distance matrix (*Table 3*) indicated a comparatively high overall interspecies pairwise divergence of 25.2%, while the least interspecific distance

Table 2 Summary of 23 observed mitochondrial DNA haplotypes and their distribution, nucleotide diversity, number of haplotypes, haplotype diversity and number of polymorphic sites.

Haplotype GenBank accession number	<i>P. canius</i>					<i>P. lineatus</i>
	Selangor <i>n</i> = 20	Negeri Sembilan <i>n</i> = 18	Johor <i>n</i> = 15	Sabah <i>n</i> = 30	Sarawak <i>n</i> = 22	Selangor <i>n</i> = 13
KP258659						7.8*
KP258657						15.3*
KP258658						76.9*
KP258648		5.6*				
KP258651		16.6*				
KP258655		11.1*				
KR086935		5.6*				
KP258650				3.3*		
KP258649				3.3*		
KP258656				76.6*		
KR086936				16.8*		
KP258654	5*					
KP258653	10*					
KP258652	5*					
KR086938	5*					
KR086937	10*					
KP221601					4.5*	
KP221602					31.8*	
KP221603					13.6*	
KP221604					4.5*	
KP221605					9*	
KR086940	65*		60*		36.6*	
KR086939		61.1*	40*			
Nucleotide diversity (Pi JC)	0.00457	0.00184	0.00306	0.00134	0.0391	0.00067
Number of haplotypes	6	5	2	4	6	3
Haplotype diversity (Hd)	0.642	0.614	0.667	0.395	0.771	0.410
Number of polymorphic site	14	5	3	4	57	2

Note:

* Haplotype frequencies in each population are presented as percentage.

was 0.2%. The Tamura-Nei intraspecific distance however, ranged from 0.2–9.7% between *P. canius* from Sarawak and Selangor. Nevertheless, the majority of *P. canius* pairwise distances displayed low levels of conspecific divergence roughly around 1%. The greatest genetic differences was observed between the Selangor and Sarawak (KR086937–KP221604) samples (9.7%), which is moderately reasonable due to their geographical distance. The next significantly high variances was detected between the Sabah-Sarawak

Table 3 Pairwise Tamura-Nei genetic distance in 23 employed haplotypes of *P. canius* and *P. lineatus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
1	KP221604																							
2	KP221603	0.002																						
3	KP221605	0.003	0.002																					
4	KP258655	0.091	0.089	0.087																				
5	KP258654	0.093	0.091	0.089	0.002																			
6	KR086940	0.091	0.089	0.087	0.089	0.002																		
7	KP258653	0.090	0.088	0.086	0.002	0.003	0.002																	
8	KR086937	0.097	0.094	0.092	0.005	0.006	0.005	0.006																
9	KP258652	0.096	0.094	0.092	0.006	0.008	0.006	0.008	0.005															
10	KR086938	0.089	0.087	0.084	0.002	0.003	0.002	0.003	0.006	0.006														
11	KP221601	0.091	0.089	0.087	0.003	0.005	0.003	0.005	0.008	0.009	0.005													
12	KR086936	0.089	0.087	0.084	0.005	0.006	0.005	0.006	0.009	0.011	0.006	0.008												
13	KP258650	0.090	0.088	0.086	0.006	0.008	0.006	0.008	0.011	0.012	0.008	0.009	0.002											
14	KP258649	0.091	0.089	0.087	0.006	0.008	0.006	0.008	0.011	0.012	0.008	0.009	0.002	0.003										
15	KP258656	0.093	0.091	0.089	0.005	0.006	0.005	0.006	0.009	0.011	0.006	0.008	0.003	0.005	0.005									
16	KP258648	0.087	0.084	0.082	0.006	0.008	0.006	0.008	0.011	0.012	0.008	0.009	0.005	0.006	0.006	0.005								
17	KR086935	0.087	0.084	0.082	0.006	0.008	0.006	0.008	0.011	0.012	0.008	0.009	0.005	0.006	0.006	0.005	0.000							
18	KP258651	0.089	0.087	0.084	0.005	0.006	0.005	0.006	0.009	0.011	0.006	0.008	0.003	0.005	0.005	0.003	0.002	0.002						
19	KR086939	0.089	0.087	0.084	0.005	0.006	0.005	0.006	0.009	0.011	0.006	0.008	0.003	0.005	0.005	0.003	0.002	0.000						
20	KP221602	0.078	0.076	0.078	0.028	0.029	0.028	0.029	0.032	0.032	0.026	0.028	0.026	0.027	0.027	0.026	0.024	0.024	0.022	0.022				
21	KP258657	0.241	0.239	0.235	0.238	0.240	0.238	0.240	0.246	0.246	0.235	0.245	0.240	0.242	0.237	0.240	0.237	0.237	0.241	0.241	0.252			
22	KP258658	0.237	0.235	0.231	0.235	0.237	0.235	0.237	0.243	0.243	0.232	0.241	0.236	0.239	0.234	0.236	0.234	0.234	0.237	0.237	0.248	0.002		
23	KP258659	0.237	0.235	0.231	0.235	0.237	0.235	0.237	0.243	0.243	0.232	0.241	0.236	0.239	0.234	0.236	0.234	0.234	0.237	0.237	0.248	0.003	0.002	

pair (KP258656–KP221604) and Negeri Sembilan–Sarawak (KP258654–KP221604), although in later occasion, distance between Sarawak–Sabah sites is extraordinarily closer than Sarawak–Negeri Sembilan.

Phylogenetic analysis using the 23 haplotypes of the genus *Plotosus* and one haplotype of *C. gariiepinus* showed monophyletic status between *P. canius* and *P. lineatus* via the Neighbour-Joining method (Fig. 2). As stated, the three haplotypes from Sarawak population formed a basal clade for *P. canius* using Neighbour-Joining algorithm. Moreover, constructed topology precisely proved the pairwise genetic distances of shared haplotypes, highlighting that KR086940 (SGR, SWK, JHR) have the lowest distance to KP258654 (SGR) and highest to KP221604 (SWK), while KR086939 (JHR, NSN) have the greatest divergence from KP221604 (SWK) and smallest amount from KP258648 (NSN), as their subdivision clades illustrated.

Similarly, application of Maximum Likelihood algorithm (Fig. 3) have been subsequently confirmed the calculation of pairwise genetic distances among sequences of *P. canius* and *P. lineatus* with exception of some negligible changes in topology of clades and branches.

The MSN of 20 haplotypes of *P. canius* (Fig. 4) in Malaysia presented more haplotype variability in Sarawak and Negeri Sembilan populations with six and five haplotypes respectively. Indeed, the Sarawak and Negeri Sembilan sequences illustrated a fairly high diversity, while the two haplotypes of Johor possessed the lowest variability. However, the phylogram revealed two relatively irrefutable geographical clades (Sarawak and Negeri Sembilan), occurrence of mix haplotypes with other clades indicating that no accurate geographical genetic structure have been certainly detected in studied populations of *P. canius*. Analysis of potential geographical clades have been similarly suggested that all populations are moderately mixed except in Sarawak and Negeri Sembilan. Although there are some possible clades, it was not precisely feasible to clustering the population based on their geographical divisions due to existence of exclusively one connecting mutational steps for most sequences. Hence, analysis was not capable of showing precise separation of geographical clades.

Analysis of population differentiation inferred from pairwise distance of F_{ST} and Chi-square among studied populations of *P. canius* is displayed in Table 4. Significant genetic variations were detected in all assessments within *P. canius* sequences and two considered species of eel-tail catfishes ($P < 0.001$). However, there were significant distance diversity in genetic variations of almost entire evaluations among populations of Malaysia especially between the Sabah and the Negeri Sembilan populations. As expected, the most diversity were identified between *P. canius* populations from the Negeri Sembilan and the Sabah with the rate of 0.62504, which basically means they are nearly genetically divided due to their distance and subsequent decrease in gene flow. However, the lowest genetic distance was detected between Selangor cluster and the Johor clade by the F_{ST} values of 0.05417. Hence, it was considered that maximum sharing of genetic material occurred between Johor and Selangor Populations, while the minimum genetic similarity identified among Sabah and Negeri Sembilan sequences.

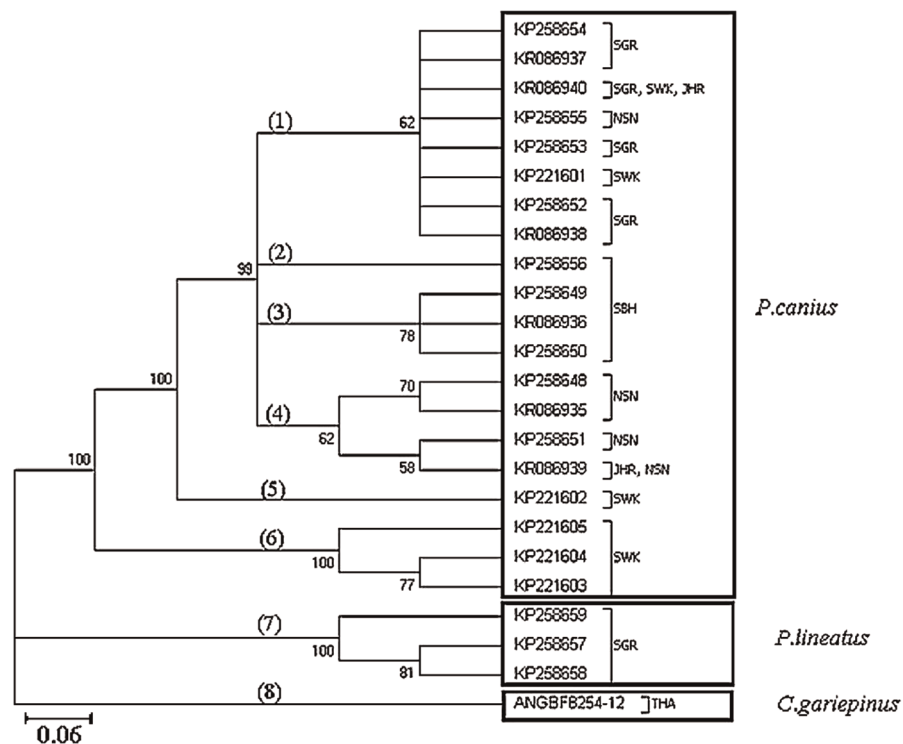


Figure 2 Summary of Neighbour-Joining relationship in 24 employed sequences of *P. canius*, *P. lineatus* and *C. gariepinus* (clades have been indicated by bold numbers in round brackets).

Hierarchical statistics of AMOVA (Table 5) was clearly suggested that roughly 36% of experimental deviations were inter-population variations, while within population variations were merely responsible for approximately 64% of overall differentiation.

The illustration of mismatch distribution (Fig. 5) have shown multimodal pattern for *P. canius* populations in Malaysia. Moreover, Tajima's *D* test was negative for Selangor, Sabah and N. Sembilan populations, but not significant (Table 6). Besides, Fu's test showed negative signal for Selangor population, which wasn't similarly significant. These statistics suggest that populations of *P. canius* didn't possibly experience demographic expansion for a long period of time in Malaysia. However, comparison of θ_1 and θ_0 parameters for all populations indicates a relatively slow growth rate in female populations except in Sabah.

Population genetic results inferred from genotyping analysis

Fragment analysis were estimated DNA band sizes in *P. canius* that are illustrated alongside with size range in original species (*Tandanus tandanus*), the sequence of each primers and associate annealing temperatures in Table 7.

Genotyping results did not found any signal of null allele nor large allele failure, hence nor scoring inaccuracies due to stuttering. All the five microsatellites loci showed accurate and successful amplification in all populations, although heterozygous alleles were not found in all populations. One microsatellite loci (Tan 3-27), was evidently monomorphic in all populations while Tan 1-2, Tan 1-10, Tan 1-7 and Tan 3-28 were polymorphic in

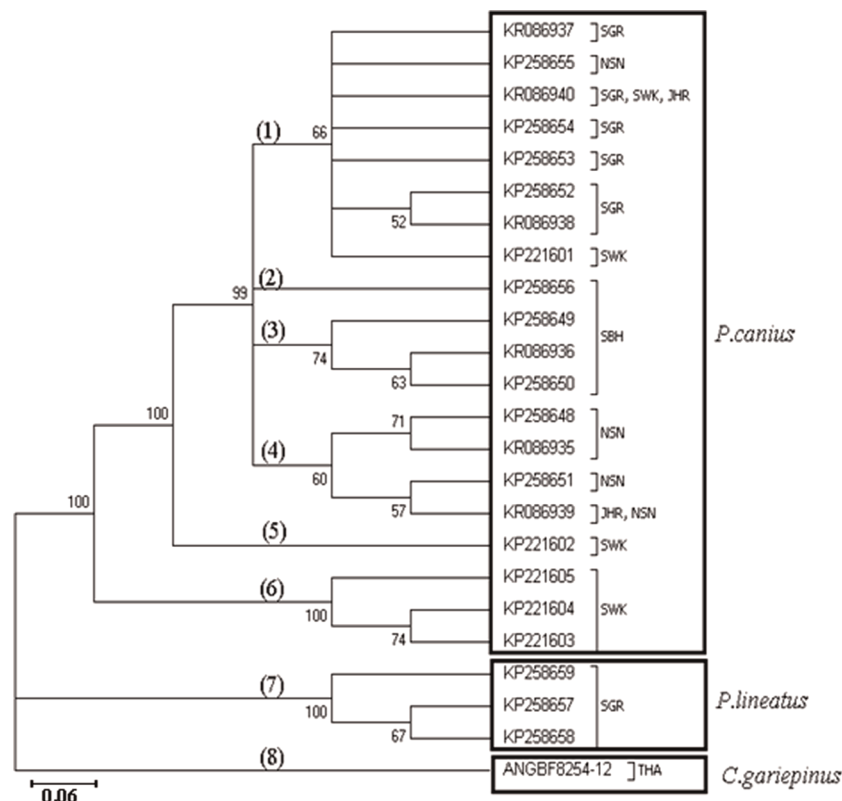


Figure 3 Summary of maximum likelihood relationship in 24 employed sequences of *P. canius*, *P. lineatus* and *C. gariepinus* (clades have been indicated by bold numbers in round brackets).

at least one population. After implementing the sequential Bonferroni adjustment (Rice, 1989), only four out of the 50 (8%) loci pairs were significant for linkage disequilibrium ($P < 0.05$). Thus, all the five microsatellites loci were considered useful for genetic applications based on the absence of consistent linkage disequilibrium in locus pairs among the studied populations.

Furthermore, after Bonferroni adjustment, nine out of the 25 (36%) microsatellite loci still showed deviation from Hardy-Weinberg Equilibrium (HWE), which might be owing to heterozygote deficiency effects (Table 8). Heterozygote deficiency could be caused by population structuring, null alleles or inbreeding (Brook et al., 2002).

Data from Table 8 showed that six out of nine deviations were related to Tan 1-7 and Tan 3-28 among all the five *P. canius* populations. The fact that the two loci did not show any signal of deviation from HWE in three populations may imply the probability that the estimated deviations could have been originated from either the occurrence of uncertain structure or inbreeding among these three population divisions (Pritchard, Stephens & Donnelly, 2000). Fairly high level of consistency was detected toward inclusion or exclusion of Tan 1-7 and Tan 3-28, accordingly these loci have been retained for further analysis. *Fis* ($P < 0.05$) estimations have been considerably diverse from zero, except in locus Tan 1-2 from Sabah. This alongside with substantial departure from HWE indicates the damaging effect of heterozygote deficiency within associated populations.

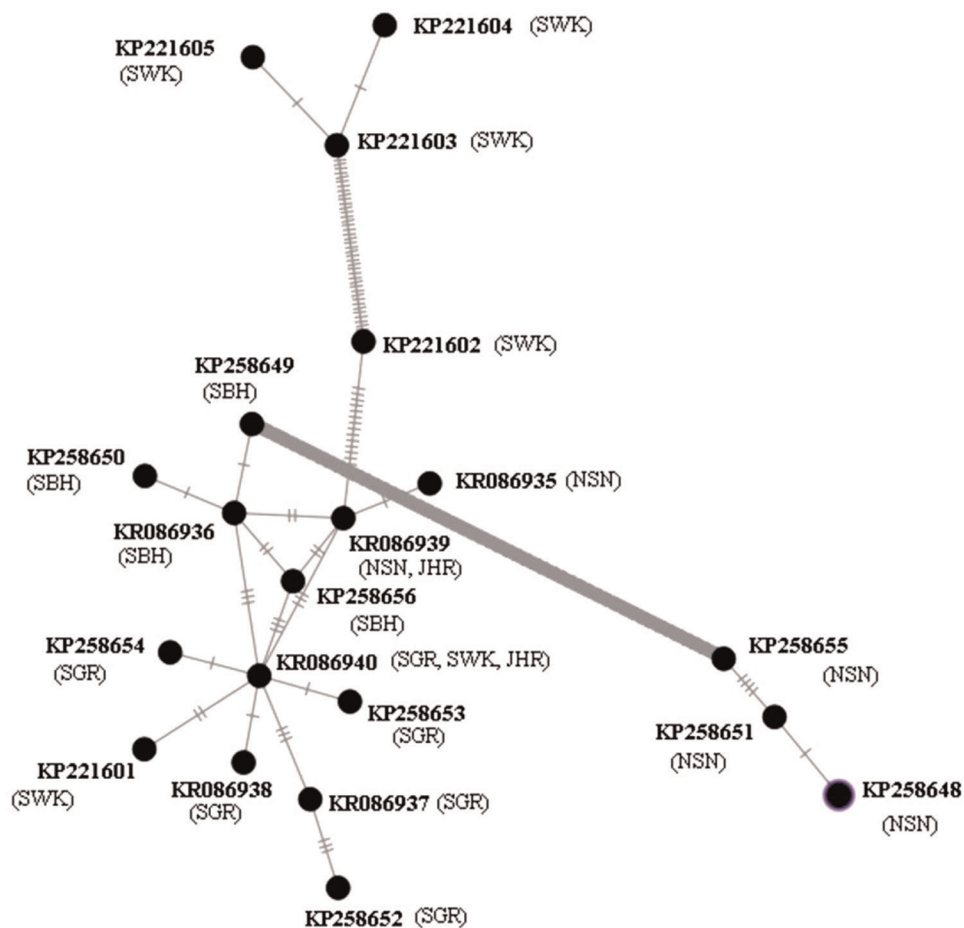


Figure 4 MSN of 20 haplotypes of *P. canius*.

Table 4 Population pairwise (F_{ST}) values of chi square test for population variation originated with 1,000 permutations.

	<i>P. canius</i> SBH	<i>P. canius</i> JHR	<i>P. canius</i> SGR	<i>P. canius</i> NSN	<i>P. canius</i> SWK
<i>P. canius</i> SBH	0.00000				
<i>P. canius</i> JHR	0.60156	0.00000			
<i>P. canius</i> SGR	0.43390	0.05417	0.00000		
<i>P. canius</i> NSN	0.62504	0.44097	0.09806	0.00000	
<i>P. canius</i> SWK	0.41533	0.27777	0.29359	0.31333	0.00000

Table 5 Hierarchical AMOVA in *P. canius*.

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation
Among populations	4	77.873	3.09472	35.55
Between populations	18	100.969	5.60941	64.45

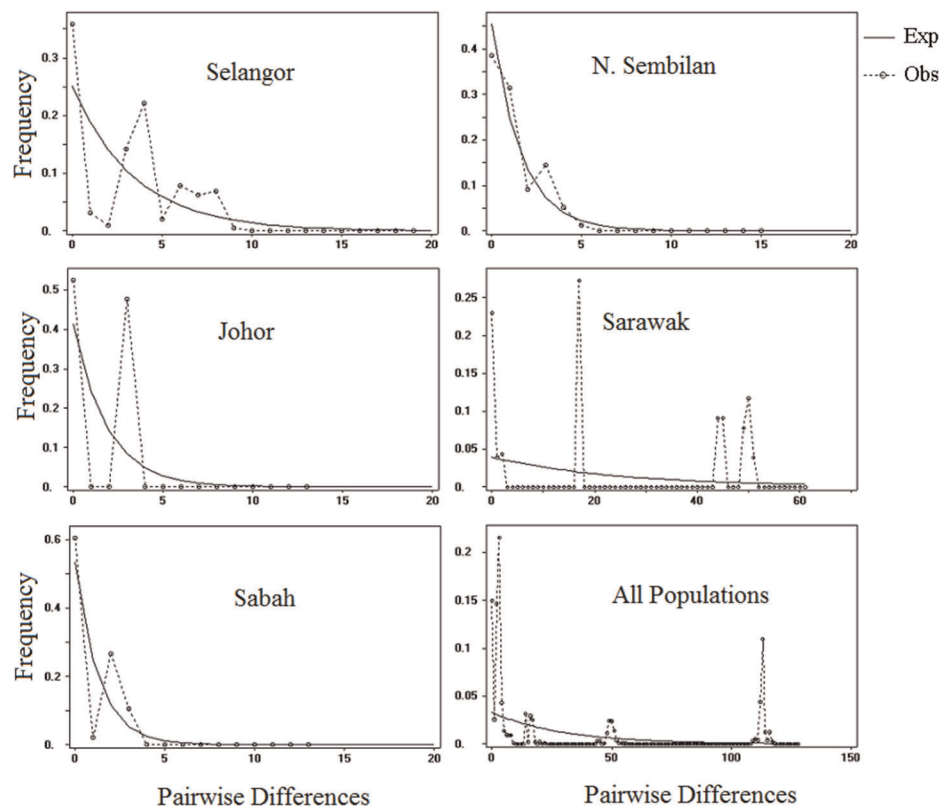


Figure 5 Mismatch distribution of *P. canius* for different geographical regions and whole population.

Table 6 Tajima's D and Fu's F_s , corresponding P -value, and mismatch distribution parameter estimates (significance level: $P < 0.01$).

Species	Tajima's D		Fu's F_s		Mismatch distribution		
	D	P	F_s	P	τ (95% CI)	θ_0	θ_1
Selangor	-0.89	0.20	-0.01	0.53	10.98	1.64	3.44
N. Sembilan	-0.84	0.23	0.72	0.61	4.18	0.334	0.62
Johor	1.63	0.96	3.63	0.94	26.17	0.99	1.51
Sabah	-0.35	0.42	0.08	0.49	0.74	0.001	99,783
Sarawak	2.24	0.99	15.14	1.00	58.39	26.44	53.03
All populations	1.42	0.38	6.08	0.33	22.39	8.74	51,632

However, the positive calculated estimations could be translated as decrease in heterozygous levels among offspring in population, mostly owing to non-random mating and its subsequent inbreeding. On the other hand, negative F_s estimates might be indication of increasing in heterozygosity level, which could usually occur as a result of random mating system, hence genes should be probably more different (Pritchard, Stephens & Donnelly, 2000).

Analysis of population genetic inferred from molecular coancestry information (Table 9) revealed that PIC of the applied microsatellites varied from 19.86–73.99.

Table 7 Five engaged primer sets and their associated size and temperature in *T. tandanus* and *P. canius*.

Primer	Sequence	Size	Size	Annealing temperature
		<i>T. tandanus</i>	<i>P. canius</i>	
Tan 1-2	F: 5'CCGACTGTCAGTGAAAAGGAG3' R: 5'AGGGTCTGGGAGTGAATGAG3'	216–244	349–385	55 °C
Tan 1-7	F: 5'TGTATGGAGCTACTAACAAAACAGG3' R: 5'TACTCCAGCCCTGAAGGTG3'	181–227	114–125	55 °C
Tan 1-10	F: 5'CCTGATTTCTCTCCCAAGG3' R: 5'AGAAAGGTGGTGCATGTGTG3'	298–310	91–97	55 °C
Tan 3-27	F: 5'TGTGGAAGGTTGGGGTTATG3' R: 5'CGTGATCATGCAAAACAGATG3'	215–269	167–168	55 °C
Tan 3-28	F: 5'CCCCATTTGCTTTTTTCTCTG3' R: 5'TGTTGAAAGCGGCATGTTAG3'	289–301	280–299	55 °C

However, loci with numerous allele numbers and a PIC value of 1 are considered as highly polymorphic and thus most desirable, lowest rates are also slightly informative (if and only if $PIC > 0$) (Botstein et al., 1980). Besides, the arithmetic values of heterozygosity (0.2235–0.8357) confirmed the consistent application of all the five microsatellite loci in population genetic study of *P. canius* in Malaysia.

Hierarchical results of microsatellites showed that approximately 64% of experimental variations were originated from inter-population variations, while within individuals variations were only accountable for roughly 28.5% of overall differentiation (Table 10).

F_{ST} plot estimations of all involved microsatellites noticeably illustrated that all pairwise calculations presented a fairly high differentiations among populations ranging from 0.29711 between populations of Selangor and Negeri Sembilan to 0.80500 between populations of Sarawak and Johor (Table 11). The current microsatellite experiment showed that the highest genetic differentiation was between the Johor population and the other populations from Peninsular Malaysia and Borneo. Indeed, the Johor population showed strong deviation from other collected populations, while displayed relatively low intra-population genetic variation (Table 11), suggesting that the Johor population was less connected to the others during a sizeable period of its evolutionary phase. Likewise, the Sarawak population in the Southwest region of the South China Sea had high F_{ST} values between other populations, signifying the restricted gene flow between the Sarawak population and the other populations. The differentiation level among populations of Negeri Sembilan and Selangor ($F_{ST} = 0.29711$, $P < 0.05$) showed relatively lower values, even in comparison with their neighbouring populations, theoretically demonstrating the populations that endured inbreeding or genetic drift since their isolation from other populations. Similarly, the Negeri Sembilan and Sabah ($F_{ST} = 0.39244$, $P < 0.05$) populations also showed small but significant variances in relation to their close neighbour populations.

Levels of genetic variations seem to be widely fluctuated between *P. canius* populations owing to H_e and Ar oscillation on which H_e extending from 0.0000–0.6769 (Selangor) and Ar varied from 1–3 (Negeri Sembilan and Selangor). Obviously, two significant

Table 8 Genetic variation at 5 microsatellite as of five populations of *P. caninus* in Malaysia.

Locus	N. Sembilan	Sabah	Selangor	Sarawak	Johor	Total* Mean
<i>N</i>	30	30	20	22	15	117
Tan 1-2						
<i>N_a</i>	1	2	1	1	2	4
<i>Ar</i>	1.000	2.000	1.000	1.000	2.000	3.714
<i>H_o</i>	0.0000	0.0000	0.0000	0.0000	0.6000	0.0769
<i>H_e</i>	0.0000	0.4994	0.0000	0.0000	0.4345	0.5974
<i>F_{is}</i>	–	0.000	–	–	–0.400	
HW	–	1.0000	–	–	0.1791	
Tan 1-7						
<i>N_a</i>	2	2	3	2	1	6
<i>Ar</i>	2.000	2.000	3.000	2.000	1.000	5.844
<i>H_o</i>	1.000	0.0000	0.6000	0.0000	0.0000	0.3590
<i>H_e</i>	0.5085	0.4994	0.6769	0.4947	0.0000	0.7727
<i>F_{is}</i>	–1.000	1.000	0.116	1.000	–	
HW	0.0000	1.0000	0.5856	1.0000	–	
Tan 1-10						
<i>N_a</i>	1	1	1	1	2	3
<i>Ar</i>	1.000	1.000	1.000	1.000	2.000	2.564
<i>H_o</i>	0.0000	0.0000	0.0000	0.0000	0.4000	0.0513
<i>H_e</i>	0.0000	0.0000	0.0000	0.0000	0.3310	0.3472
<i>F_{is}</i>	–	–	–	–	–0.217	
HW	–	–	–	–	0.5395	
Tan 3-27						
<i>N_a</i>	1	1	1	1	1	2
<i>Ar</i>	1.000	1.000	1.000	1.000	1.000	1.988
<i>H_o</i>	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<i>H_e</i>	0.0000	0.0000	0.0000	0.0000	0.0000	0.2242
<i>F_{is}</i>	–	–	–	–	–	
HW	–	–	–	–	–	
Tan 3-28						
<i>N_a</i>	3	2	3	2	1	8
<i>Ar</i>	3.000	2.000	3.000	2.000	1.000	7.592
<i>H_o</i>	0.4667	1.0000	0.3000	0.0000	0.0000	0.4274
<i>H_e</i>	0.6169	0.5885	0.4769	0.4947	0.0000	0.8393
<i>F_{is}</i>	0.247	–1.000	0.377	1.000	–	
HW	0.5862	0.0000	0.5771	1.0000	–	

Note:

Sample size (*N*), Number of alleles (*N_a*), allele richness (*Ar*), (*H_o*), (*H_e*), inbreeding coefficient (*F_{is}*) ($P < 0.05$ symbolic accustomed nominal level (5%) 0.000042, and Hardy-Weinberg expectation (disequilibrium) (HW).

clusters could be seen among established populations of *P. caninus* in this study, one cluster with low allelic richness and *H_e* estimations (Johor samples using locus Tan 1-2 and locus Tan 1-10), and another cluster with relatively acceptable *H_e* and allelic richness (the other four populations using locus Tan 3-28 and locus Tan 1-7). However, the genetic variation

Table 9 Analysis of population genetic using molecular coancestry information.

Microsatellite	N_a	Heterozygosity*	PIC (%)	Effective allele no.
Tan 1-2	4	0.5949	54.12	2.47
Tan 1-7	6	0.7694	73.99	4.34
Tan 1-10	3	0.3457	30.11	1.53
Tan 3-27	2	0.2235	19.86	1.29
Tan 3-28	8	0.8357	81.71	6.09

Notes:

Number of alleles (N_a) and PIC.

* Heterozygosity was estimated as arithmetic mean of expected and H_e .

Table 10 Hierarchical AMOVA in *P. canius*.

Source of variation	Sum of squares	Variance components	Variation %
Among populations	191.456	1.02366	63.77361
Among individuals within populations	79.039	0.12422	7.73898
Within individuals	53.500	0.45726	28.48741

in the current study was highly reliant on microsatellites and their sequences as the engaged loci did not specifically develop for *P. canius*. Moreover, allelic frequencies among virtually each combination of population pairs showed highly significant differentiation ($F_{ST} < 0.05$) (Table 11), implying that gene flow might be highly restricted among studied populations.

Sampled populations of *P. canius* were basically distributed into five minor clusters using Bayesian analysis. Consequently, the initial highest membership value (q) of the studied populations including Negeri Sembilan, Sabah, Selangor, Sarawak, Johor was estimated as 0.941, 0.983, 0.968, 0.988, and 0.986 respectively (Table 12). The application of STRUCTURE program subsequently illustrated 4 major K (isolated clusters) (Fig. 6). Regarding the fact that assessing the expected value of K might not be straightforward (Evanno, Regnaut & Goudet, 2005), Bayesian structure analysis of the current study revealed the highest probability of K for *P. canius* in $K = 2$. The four estimated clusters were included Cluster 1: Johor, Cluster 2: N. Sembilan and Selangor, Cluster 3: Sabah, and Cluster 4: Sarawak (Fig. 7).

Population assignment outcomes evidently revealed that almost all individuals were assigned to their original populations with the probability rate of $P > 0.05$ (Table 13). However, the estimation of individual assignment to their populations with the same probabilities revealed the closer rates in comparison levels to other sampling sites. For instance, the Negeri Sembilan population had a relatively closer assignment ratio to the Selangor population.

Analysis of population bottleneck did not identified substantiating signals of recent population drop in all populations studied using the two phase model (T.P.M) estimations (Table 14). Furthermore, calculation of the infinite-allele model (I.A.M) comprehensively implied that there was no bottleneck evidence among the studied

Table 11 Pairwise F_{ST} estimations through *P. canius* populations generated from five microsatellites loci and inclusion of five populations. All calculations were fairly significant ($P < 0.05$) using 10,000 permutations.

		N. Sembilan	Sabah	Selangor	Sarawak	Johor
1	N. Sembilan	0.00000				
2	Sabah	0.39244	0.00000			
3	Selangor	0.29711	0.41039	0.00000		
4	Sarawak	0.71934	0.68086	0.72437	0.00000	
5	Johor	0.73211	0.68561	0.74834	0.80500	0.00000

Table 12 Membership ratio estimated for each population of *P. canius*.

Populations	Cluster membership				
	1	2	3	4	5
N. Sembilan	0.005	0.041	0.941	0.010	0.004
Sabah	0.005	0.004	0.005	0.983	0.003
Selangor	0.005	0.968	0.019	0.005	0.004
Sarawak	0.003	0.003	0.003	0.003	0.988
Johor	0.985	0.004	0.004	0.004	0.003

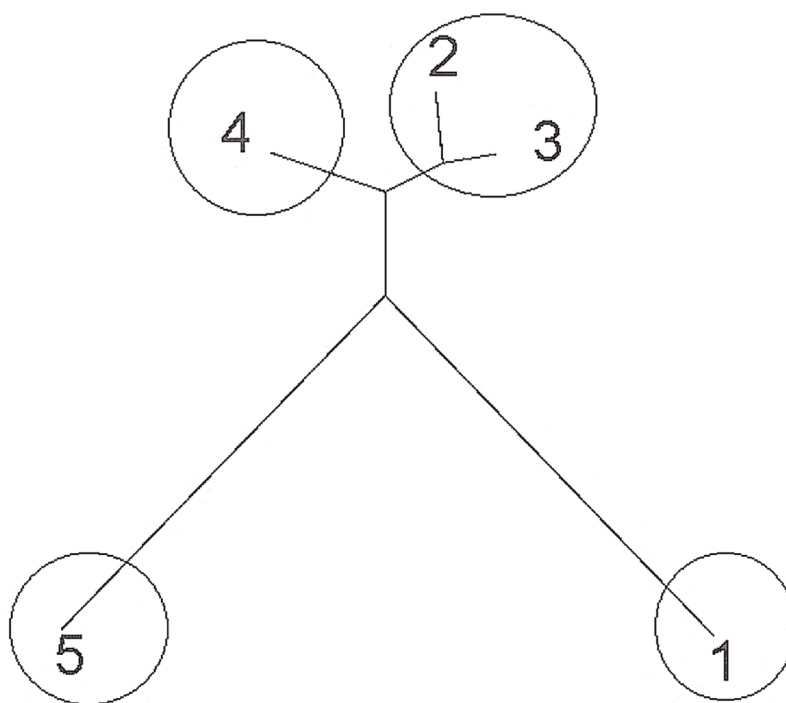


Figure 6 Tree plot scheme of five engaged populations of *P. canius*.

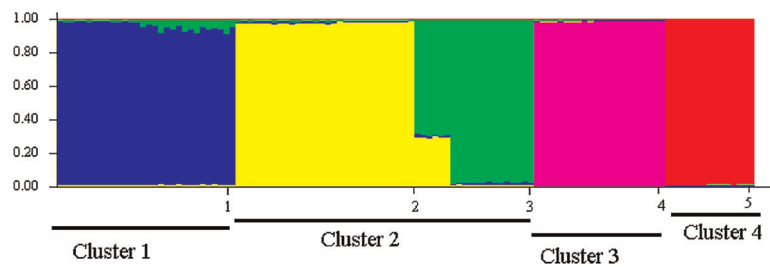


Figure 7 Population structure of five *P. caninus* populations in Malaysia.

Table 13 Population assignment based upon five microsatellite loci frequencies in *P. caninus*.

Assigned population	CA%	Original location				
		N. Sembilan (<i>n</i> = 30)	Sabah (<i>n</i> = 30)	Selangor (<i>n</i> = 20)	Sarawak (<i>n</i> = 22)	Johor (<i>n</i> = 15)
N. Sembilan	100	4.219	70.330	35.873	117.792	92.839
Sabah	100	70.143	4.338	58.286	132.093	96.994
Selangor	100	35.937	58.537	4.715	101.049	83.156
Sarawak	100	115.433	129.921	98.626	3.133	101.070
Johor	100	88.554	92.895	78.807	99.143	2.761

Note:

CA, correct assignment.

Table 14 *P* values originated from bottleneck analysis within five populations of *P. caninus*.

	I.A.M	T.P.M			S.M.M	Mode Shift
		60	70	80		
N. Sembilan	0.22672	0.24435	0.26030	0.26596	0.28595	Y
Sabah	0.07656	0.09247	0.09295	0.09683	0.10790	Y
Selangor	0.28119	0.31416	0.34221	0.32656	0.64363	Y
Sarawak	0.17976	0.21249	0.21558	0.20907	0.24057	Y
Johor	0.18105	0.21600	0.24444	0.26510	0.22513	Y

Notes:

I.A.M, infinite allele model; T.P.M, two phase model; S.M.M, stepwise mutational model, estimation indicate the mutation in stepwise mutational model; Y, yes; N, no; Significant values $P < 0.05$.

populations, while the parallel statement was assumed using the stepwise mutation model (S.M.M). Moreover, the shift-mode estimation of allele frequencies was perceived in all five populations, while altogether none of the mutation models were broadly illustrated consistent signals of bottleneck in engaged populations. Therefore, the current experiment could not detect any signals of bottleneck within the *P. caninus* populations based on three applied models. Nevertheless, outcomes of mutational models consistently suggested the extension in populations due to absence of genetic drift and/or an invasive allele originating from different populations (Piry, Luikart & Cornuet, 1999).

DISCUSSION

Phylogeography and evolutionary history

This study has confirmed the efficiency of the COI barcode in identification of eel-tail catfish species. Barcoding fragment has been effectively sequenced mitochondrial DNA isolated from two species of family Plotosidae. Current study provided the first sequence database of *P. canius* to be submitted into barcoding data set. The first and the most common outcome of the undergone experiment could be the involvement of two common haplotype. While the most common sequence was KR086940 between populations of Selangor ($n = 7$), Sarawak ($n = 6$), and Johor ($n = 9$), the KR086939 identified as second common haplotype sharing between Johor ($n = 6$) and Negeri Sembilan ($n = 4$). Nevertheless, the most significant finding should be the occurrence of shared haplotype between Selangor, Johor (Peninsular Malaysia) populations and the Sarawak (Borneo) population. The haplotype sharing and their consequent gene flow could practically happen due to several reasons such as breeding migration, mutation, pelagic larvae, and sharing of common ancestors (Frankham, 1996).

Migration is a common behaviour in nearly 3% of all extant fish species (Binder, Cooke & Hinch, 2011). However, there is practically no record on migration and migratory behaviour of family Plotosidae, thus the first assumption of dispersal via migration and ocean current might be highly unlikely since majority of the catfish species cannot endure long distance swimming (more than 500 km) due to their body shape and structure (Jónsson, 1982). In addition, marine dispersal of eggs, larvae and even juveniles of *P. canius* between two separate ocean currents comprising Straits of Malacca (Selangor and Johor) and South China Sea currents (Sarawak) might also be questionable. In the Strait of Melaka, circulation of currents (particularly in surface) are due to effects of tidal patterns and wind, while the route of both surface and deep layer currents are shown to be relatively the same and toward northwest (Rizal et al., 2010). In Johor, however, currents are highly depend on strong winds during monsoon seasons. Indeed, if the monsoon is in its northeast route, the current streams toward South along the coastal region of Malaysia, otherwise, the current will be northward (Mohd Akhir & Chuen, 2011). Finally, pattern of ocean currents in western South China Sea are largely influenced by season. The circulation in South segment of western current tends to be stable and northward where after separation from coastal region, it forms a northeastern pattern in summer. In fall, however, it strongly flows toward southwest (Fang et al., 2012). Therefore, general patterns of aquatic circulation in Strait of Melaka, Johor maritime territory and western area of South China Sea might not strongly implies the probable distribution of grey eel-tail catfish eggs, larvae and juveniles and its consequent gene flow and genetic connectivity.

Considering all possible expectation on genetic variability and gene flow of *P. canius* in Peninsular Malaysia, the second hypothesis of sharing common ancestor due to historical geographic events may reflect the most plausible explanation. Southeast Asia is believed to have endured simultaneous glaciation and consequent deglaciation along with its associated decreasing and increasing of marine levels during the Pleistocene

period, which greatly influenced continental and oceanic configuration (Voris, 2000). During such variations, some regions might be preserved their stable environmental conditions that is nowadays called a refugium on which can greatly affect the gene flow and genetic variability particularly in endemic species (Hobbs et al., 2013). Moreover, geographical information proposed that the Pacific and Indian ocean were initially connected directly before the formation of Sundaland (nowadays submerged forming shallow ocean of most Southeast Asia with less than 100 m depth) during the Triassic up to the Pleistocene period (Esa et al., 2008), hence made such gene flow possible between these comparatively distant locations.

Demographic history analyses did not found any evidence of recent population expansion in all *P. canius* populations. Thus, the evolutionary history estimation (the point of expansion) could not be estimated but the *P. canius* populations were regarded as being in a stable state, possibly for a long period of time. Nevertheless, comparison of θ_1 and θ_0 parameters for all populations indicates a relatively slow growth rate in female populations of *P. canius* except in the Sabah population.

Hardy-Weinberg equilibrium and genetic diversity

Overall allelic richness revealed quite lower rates using the cross amplified primers ranging from 2–8 among the sampled populations in comparison with original species (Rourke et al., 2010). Tan 3-28 demonstrated the highest overall allelic rate fluctuating from 1–3 among five populations of *P. canius*, while the lowest level was detected in Tan 3-27. Moreover, the Selangor population showed maximum number of alleles (9), whereas the Johor and Sarawak populations exhibited the lowest (6). Similar instance of low allelic variation have been described in *Bolbometopon muricatum* (Priest et al., 2014), *Schizothorax biddulphi* (Palti et al., 2012) and *Prosopium cylindraceum* (Mccracken et al., 2014). A possible reason for the occurring of low levels of allelic richness might be due to the small employed population size. Hale, Burg & Steeves (2012) pointed out the positive effects of sampling size between 25–30 individuals per population, however they also mentioned the necessity of 5–100 samples per collection to avoid rare uninformative alleles. Marine vertebrates are believed to present greater allele difference at their microsatellite primers comparing to freshwater populations, which is mostly consistent with their higher population evolutionary size (Neff & Gross, 2001). Their research later revealed that the difference in microsatellite polymorphism among classes and species could be highly dependent upon changes in life history and population biology and moderately to differences happening to microsatellite functions during natural selection. Therefore, the fewer number of allele found in *P. canius* might be due to variation in its biology and historic traits, however, the correlation of allelic richness and sample size should not be overlooked.

The average value of H_o estimated in the five tested microsatellites in *P. canius* were as low as 0.2168, which showed high difference levels in comparison to standard heterozygosity in marine populations ($H_o = 0.79$) and anadromous fish species ($H_o = 0.68$) (DeWoody & Avise, 2000). In fact, considerable heterozygote deficiencies were observed in the engaged populations with the exception of the Tan 1-7 and Tan 3-28 loci. Similar

temporal pattern of low genetic diversity have been reported for *Pleuronectes platessa* in Island (Hoarau et al., 2005) and *Clarias macrocephalous* (Na-Nakorn et al., 1999), while in most catfish species higher levels of heterozygosity have been documented as in *Mystus nemurus* ($H_o = 0.44-0.57$) (Usmani et al., 2003). Several decisive issues might influence the genetic variability of marine species through the variation of Hardy-Weinberg including migration, genetic drift, sample size, over-exploitation, effective size of population and patterns of mating (DeWoody & Avise, 2000). Certainly, *P. canius* should not be presumably considered as long distance migratory marine fish species due to its body structure (Riede, 2004). Alternatively, a possibility of genetic drift in the current study is also suspicious as it basically happens only in small effective size populations that experiencing a period of bottleneck (DeWoody & Avise, 2000) at which is completely invalidated in marine species studies like current research.

Small sample size of collected populations might also be measured as a major parameter in detection of low heterozygosity variation because of the failure to accurately detect the entire extant alleles of the selected populations, hence, deficiency in heterozygote identification (Na-Nakorn et al., 1999). Indeed, the current collection size for *P. canius* used for population genetic analysis purposes should be quite small based on Kalinowski (2005); therefore, the hypothesis of deficiency in heterozygote detection due to the low level of sampled specimen could be accepted. The last cause of a low heterozygosity levels and its consequent genetic variation is non-random system of mating behaviour among populations (Brook et al., 2002; Balloux, Amos & Coulson, 2004). Estimation of HWD for the current study however, showed considerable deviation for approximately 36% of the primer/population pairs, which might be due to heterozygote deficiency effects. However, Balloux, Amos & Coulson (2004) highlighted that the positive correlation of inbreeding and heterozygosity needs to be examined through application of more polymorphic markers on which demonstrates greater proportion of linkage disequilibrium. Alternatively, the correlation of *Fis* values and inbreeding have been practically assessed and documented in many studies (Balloux, Amos & Coulson, 2004; Abdul-Muneer, 2005; O'Leary et al., 2013). The positive calculated estimations could be translated as a decrease in heterozygous levels among offspring in a population, mostly due to absence of random mating and its subsequent inbreeding. The current study showed considerable significance levels ($P < 0.05$) of *Fis* estimations. This alongside with substantial departure from HWE would indicate the damaging effect of heterozygosity deficit within the populations.

Analysis of population structure

A remarkably high levels of genetic structure were detected among populations of *P. canius* ranging from 0.05417–0.62504, showing significantly high structuring among studied populations except differences between Johor–Selangor samples ($F_{ST} = 0.05417$) and Selangor–Negeri Sembilan ($F_{ST} = 0.09806$). Moreover, AMOVA statistics evidently revealed that approximately 64% of genetic variations were due to within population variations. Hence, the fairly high F_{ST} rates, significant hierarchical molecular results and consequent higher genetic variances among *P. canius* populations in Peninsular Malaysia

and their relatives in Borneo, in addition to the detection of only one sharing haplotype (KR086940), would suggest the absence of contemporary gene flow among them most probably due to the geological modification, consequential rise in marine water levels and historical continental separation during the Pleistocene era (Esa et al., 2008; Song, 2012). However, exceptional cases between Selangor–Negeri Sembilan and Selangor–Johor might be inversely interpreted as occurrence of gene flow or migration regarding to fairly close distances rather than extraordinary distance between Borneo and Peninsular Malaysia. The sequential genetic diversity presented in this study could be caused by high haplotype frequencies among the five populations of *P. canius* in Malaysia, in addition to identification of unique sequences in each population (except in Johor). The present patterns of differentiation among catchments is believed to be significantly as a consequence of the Pleistocene associated historical and geological continental and sea level distraction and its subsequent isolation of lands and populations (Esa et al., 2008).

The calculated F_{ST} values of five microsatellites in *P. canius* showed significant estimation, indicating substantial genetic structure and differentiation among the studied populations. All populations also showed significantly high assignment rates, followed by a low membership recorded for other population clusters. High rates of proper assignment might indicate strong population differentiation among the studied populations (Paetkau et al., 2004). Although the Sabah population demonstrated a close pairwise distance with the Selangor and Negeri Sembilan populations, the Negeri Sembilan and Selangor populations showed the lowest differentiation level (0.29711), and also the highest cluster membership in comparison with other populations. Surprisingly, the highest level of pairwise F_{ST} differentiation has been estimated between the Johor population and the other four populations, in contrast to the closer geographical distance between the Johor and the Negeri Sembilan populations. Indeed, microsatellite analysis made a relatively counter-outcome in comparison with mitochondrial results, where F_{ST} estimation of former populations was estimated as the lowest among the *P. canius* samples. Discrepancies between genetic differentiation detection through microsatellite loci and mitochondrial DNA is believed to be related to three factors: (1) high sensibility of mitochondrial COI gene in detection of variation (Shaw, Arkhipkin & Al-Khairulla, 2004), (2) weaker nuclear-based subpopulation detection (Cano, Mäkinen & Merilä, 2008) and (3) technical complications of microsatellite like homoplasy (Estoup, Jarne & Cornuet, 2002).

One of the most common practical problems, which is believed to be mostly associated with microsatellite primers (due to higher mutation rate) is well-known as homoplasy (Balloux & Lugon-Moulin, 2002). Homoplasy might diminish the microsatellite-based population differentiation signals. The existence of homoplasy is highly dependent on the occurrence of different identical locus copies, while such identical character is not consequent of mutual ancestor. In fact, the occurrence of homoplasy might be correlated with combination effects of high rates of mutation, and oversized population together with strong restriction in allele size (Estoup, Jarne & Cornuet, 2002). However, the effective number of alleles on which presented in Tables 8 and 9 showed a low level of allele size frequency, the current population size of *P. canius* used for population genetic analysis is

ostensibly quite small based on *Kalinowski (2005)* rather than being oversized. Hence, the later effective cause of homoplasy is somehow nullified in this study. Furthermore, several microsatellite based studies have pointed out the significance of S.M.M on possibility of homoplasy in different taxa (*Angers & Bernatchez, 1997; Culver, Menotti-Raymond & O'Brien, 2001; Estoup, Jarne & Cornuet, 2002; Anmarkrud et al., 2008*), which was invalidated by provided statistics on bottleneck analysis of recent study in *Table 14*.

O'Reilly et al. (2004) later pointed out that implications of homoplasy in identification of population structure using microsatellite loci are supposedly common in marine species. Nevertheless, further researches have been implied that implications of genetic drift and gene migration might have considerably greater effects on population differentiation analysis in comparison with homoplasy (*Estoup, Jarne & Cornuet, 2002*). Basically, marine vertebrates supposed to have the higher population effective size (N_e) comparing to freshwater species (*Hauser & Carvalho, 2008*). Besides, genetic drift and effective size are believed to be greatly correlated, hence it is highly probable that neighbouring geographical populations demonstrate the imperceptible population differentiation and structures especially using neutral primers like microsatellites (*Larmuseau et al., 2010*).

CONCLUSION

The current genetic characterization of *P. canius* provided some basic results on their phylogeny and population structure. The phylogenetic and phylogeographic analysis of *P. canius* noticeably constructed accurate clusters in the five population of Malaysia, demonstrating the capability of the chosen mitochondrial COI barcoding gene to potentially assign the genus *Plotosus* into their biological taxa. Indeed, COI analysis resolved the phylogenetic relationships between *P. lineatus* and *P. canius* populations, supporting their taxonomic status as different species. A low mitochondrial differentiation was found among *P. canius* populations with some indication of endemism (haplotype restricted only to a particular population) as observed in the Sabah population. Nevertheless, the COI gene revealed sufficient informative interpretation of relationships among the five populations, supporting by reasonable bootstrapping values (> 85%). The sharing of haplotypes between a few samples from Peninsular Malaysia and their Sarawak counterpart of Borneo showed the sensitivity of the COI marker to infer the biogeographical history of *P. canius* and potentially other marine taxa in the region.

Microsatellites analyses on the population structure of *P. canius* showed slightly different patterns of structuring in comparison with the COI findings. Nevertheless, both markers detected higher level of among population differentiations than within population variability. In addition, four main clusters or genetic stocks of *P. canius* were identified using the cross species amplification study of *T. tandanus* microsatellites.

Finally, the results from this study has provided valuable understandings on the genetic characterization, molecular phylogeny, evolutionary kinship, and population structuring of *P. canius*, in particular, and the genus *Plotosus*, in general. However, further studies must be conducted using more geographical and sampling sites, larger population sizes

per site. Furthermore, designing species specific hypervariable nuclear markers such as microsatellite for *P. canius* must be considered in order to accurately analyze the population structure and genetic diversity of *P. canius* before implementation of advanced fisheries and conservation strategies.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Nima Khalili Samani conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables.
- Yuzine Esa conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- S.M. Nurul Amin contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Natrah Fatin Mohd Ikhsan reviewed drafts of the paper.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Fish species that has employed in this study is not under endangered list. Besides, there is no institute in Malaysia to issue any kind of approval letter for catchment or approval of research on involved species.

Data Deposition

The following information was supplied regarding data availability:

Relevant accession numbers, raw data and other associated details will be publicly accessible on GenBank and BOLDSYSTEM after publication of manuscript. Accession numbers are listed below:

BOLDSYSTEM Index GenBank Accession

NUPM017-14 [KP258659](#), NUPM016-14 [KP258657](#), NUPM015-14 [KP258658](#), NUPM001-14 [KP258648](#), NUPM002-14 [KP258651](#), NUPM006-14 [KP258655](#), NUPM023-15 [KR086935](#), NUPM003-14 [KP258650](#), NUPM004-14 [KP258649](#), NUPM005-14 [KP258656](#), NUPM022-15 [KR086936](#), NUPM007-14 [KP258654](#), NUPM008-14 [KP258653](#), NUPM009-14 [KP258652](#), NUPM020-15 [KR086938](#), NUPM021-15 [KR086937](#), NUPM010-14 [KP221601](#), NUPM011-14 [KP221602](#), NUPM012-14 [KP221603](#), NUPM013-14 [KP221604](#), NUPM014-14 [KP221605](#), NUPM018-15 [KR086940](#), NUPM019-15 [KR086939](#).

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REFERENCES

- Abdul-Muneer PM. 2005.** *Molecular Genetic Characterization of Endemic Yellow Catfish, Horabagrus brachysoma (Gunther)*. Cochin: Cochin University of Science and Technology. 238 pp.
- Angers B, Bernatchez L. 1997.** Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. *Molecular Biology and Evolution* **14**(3):230–238 DOI [10.1093/oxfordjournals.molbev.a025759](https://doi.org/10.1093/oxfordjournals.molbev.a025759).
- Anmarkrud JA, Kleven O, Bachmann L, Lifjeld JT. 2008.** Microsatellite evolution: mutations, sequence variation, and homoplasy in the hypervariable avian microsatellite locus HrU10. *BMC Evolutionary Biology* **8**(1):1–10 DOI [10.1186/1471-2148-8-138](https://doi.org/10.1186/1471-2148-8-138).
- Appeltans W, Ah Yong ST, Anderson G, Angel MV, Artois T, Bailly N, Bamber R, Barber A, Bartsch I, Berta A, Błażewicz-Paszkowycz M, Bock P, Boxshall G, Boyko CB, Brandão SN, Bray RA, Bruce NL, Cairns SD, Chan T-Y, Cheng L, Collins AG, Cribb T, Curini-Galletti M, Dahdouh-Guebas F, Davie PJF, Dawson MN, De Clerck O, Decock W, De Grave S, de Voogd NJ, Domning DP, Emig CC, Erséus C, Eschmeyer W, Fauchald K, Fautin DG, Feist SW, Franssen CHJM, Furuya H, Garcia-Alvarez O, Gerken S, Gibson D, Gittenberger A, Gofas S, Gómez-Daglio L, Gordon DP, Guiry MD, Hernandez F, Hoeksema BW, Hopcroft RR, Jaume D, Kirk P, Koedam N, Koenemann S, Kolb JB, Kristensen RM, Kroh A, Lambert G, Lazarus DB, Lemaitre R, Longshaw M, Lowry J, Macpherson E, Madin LP, Mah C, Mapstone G, McLaughlin PA, Mees J, Meland K, Messing CG, Mills CE, Molodtsova TN, Mooi R, Neuhaus B, Ng PKL, Nielsen C, Norenburg J, Opresko DM, Osawa M, Paulay G, Perrin W, Pilger JF, Poore GCB, Pugh P, Read GB, Reimer JD, Rius M, Rocha RM, Saiz-Salinas JI, Scarabino V, Schierwater B, Schmidt-Rhaesa A, Schnabel KE, Schotte M, Schuchert P, Schwabe E, Segers H, Self-Sullivan C, Shenkar N, Siegel V, Sterrer W, Stöhr S, Swalla B, Tasker ML, Thuesen EV, Timm T, Antonio Todaro M, Turon X, Tyler S, Uetz P, van der Land J, Vanhoorne B, van Ofwegen LP, van Soest RWM, Vanaverbeke J, Walker-Smith G, Chad Walter T, Warren A, Williams GC, Wilson SP, Costello MJ. 2012.** The magnitude of global marine species diversity. *Current Biology* **22**(23):2189–2202 DOI [10.1016/j.cub.2012.09.036](https://doi.org/10.1016/j.cub.2012.09.036).
- Balloux F, Amos W, Coulson T. 2004.** Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology* **13**(10):3021–3031 DOI [10.1111/j.1365-294X.2004.02318.x](https://doi.org/10.1111/j.1365-294X.2004.02318.x).
- Balloux F, Lugon-Moulin N. 2002.** The estimation of population differentiation with microsatellite markers. *Molecular Ecology* **11**(2):155–165 DOI [10.1046/j.0962-1083.2001.01436.x](https://doi.org/10.1046/j.0962-1083.2001.01436.x).
- Bandelt HJ, Forster P, Röhl A. 1994.** Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**(1):37–48 DOI [10.1093/oxfordjournals.molbev.a026036](https://doi.org/10.1093/oxfordjournals.molbev.a026036).
- Binder TR, Cooke SJ, Hinch SG. 2011.** The biology of fish migration. *Encyclopedia of Fish Physiology: From Genome to Environment*. San Diego: Academic Press, 1921–1927.
- Botstein D, White RL, Skolnick M, Davis RW. 1980.** Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* **32**(3):317–341.

- Bourlat SJ, Borja A, Gilbert J, Taylor MI, Davies N, Weisberg SB, Griffith JF, Lettieri T, Field D, Benzie J, Oliver Glöckner F, Rodríguez-Ezpeleta N, Faith DP, Bean TP, Obst M. 2013. Genomics in marine monitoring: new opportunities for assessing marine health status. *Marine Pollution Bulletin* 74(1):19–31 DOI 10.1016/j.marpolbul.2013.05.042.
- Brook BW, Tonkyn DW, O’Grady JJ, Frankham R. 2002. Contribution of inbreeding to extinction risk in threatened species. *Conservation Ecology* 6(1):16.
- Butchart SHM, Walpole M, Collen B, van Strien A, Scharlemann JPW, Almond REA, Baillie JEM, Bomhard B, Brown C, Bruno J, Carpenter KE, Carr GM, Chanson J, Chenery AM, Csirke J, Davidson NC, Dentener F, Foster M, Galli A, Galloway JN, Genovesi P, Gregory RD, Hockings M, Kapos V, Lamarque J-F, Leverington F, Loh J, McGeoch MA, McRae L, Minasyan A, Morcillo MH, Oldfield TEE, Pauly D, Quader S, Revenga C, Sauer JR, Skolnik B, Spear D, Stanwell-Smith D, Stuart SN, Symes A, Tierney M, Tyrrell TD, Vié J-C, Watson R. 2010. Global biodiversity: indicators of recent declines. *Science* 328(5982):1164–1168 DOI 10.1126/science.1187512.
- Cano JM, Mäkinen HS, Merilä J. 2008. Genetic evidence for male-biased dispersal in the three-spined stickleback (*Gasterosteus aculeatus*). *Methods in Ecology* 17(14):3234–3242 DOI 10.1111/j.1365-294x.2008.03837.x.
- Carpenter KE. 1999. In: Carpenter KE, Niem VH, eds. *The Living Marine Resource of Western Central Pacific. Volume 4: Bony Fishes (Mugilidae–Carangidae)*, vol. 3. Rome: FAO.
- Charlesworth D, Willis JH. 2009. The genetics of inbreeding depression. *Nature Reviews Genetics* 10(11):783–796 DOI 10.1038/nrg2664.
- Collette BB, Carpenter KE, Polidoro BA, Juan-Jordá MJ, Boustany A, Die DJ, Elfes C, Fox W, Graves J, Harrison LR, McManus R, Minte-Vera CV, Nelson R, Restrepo V, Schratwieser J, Sun C-L, Amorim A, Brick Peres M, Canales C, Cardenas G, Chang S-K, Chiang W-C, de Oliveira Leite N Jr, Harwell H, Lessa R, Fredou FL, Oxenford HA, Serra R, Shao K-T, Sumaila R, Wang S-P, Watson R, Yáñez E. 2011. High value and long life–double jeopardy for tunas and billfishes. *Science* 333(6040):291–292 DOI 10.1126/science.1208730.
- Costello AB, Down TE, Pollard SM, Pacas CJ, Taylor EB. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). *Evolution* 57(2):328–344 DOI 10.1111/j.0014-3820.2003.tb00267.x.
- Culver M, Menotti-Raymond MA, O’Brien SJ. 2001. Patterns of size homoplasy at 10 microsatellite loci in Pumas (*Puma concolor*). *Molecular Biology and Evolution* 18(6):1151–1156 DOI 10.1093/oxfordjournals.molbev.a003889.
- DeWoody JA, Avise JC. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology* 56(3):461–473 DOI 10.1111/j.1095-8649.2000.tb00748.x.
- Duvernell DD, Lindmeier JB, Faust KE, Whitehead A. 2008. Relative influences of historical and contemporary forces shaping the distribution of genetic variation in the Atlantic killifish, *Fundulus heteroclitus*. *Molecular Ecology* 17(5):1344–1360 DOI 10.1111/j.1365-294X.2007.03648.x.
- Esa Y, Siraj SS, Daud SK, A. Rahim KA, Japning JRR, Soon GT. 2008. Mitochondrial DNA diversity of *Tor tambroides Valenciennes* (Cyprinidae) from five natural populations in Malaysia. *Zoological Studies* 47(3):360–367.
- Estoup A, Jarne P, Cornuet J-M. 2002. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology* 11(9):1591–1604 DOI 10.1046/j.1365-294X.2002.01576.x.

- Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14(8):2611–2620 DOI 10.1111/j.1365-294X.2005.02553.x.
- Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1:47–50.
- Fang G, Wang G, Fang Y, Fang W. 2012. A review on the South China Sea western boundary current. *Acta Oceanologica Sinica* 31(5):1–10 DOI 10.1007/s13131-012-0231-y.
- Frankham R. 1996. Relationship of genetic variation to population size in wildlife. *Conservation Biology* 10(6):1500–1508 DOI 10.1046/j.1523-1739.1996.10061500.x.
- Froese R, Pauly D. 2015. FishBase. Available at www.fishbase.org.
- Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147(2):915–925.
- Glaubitz JC. 2004. CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes* 4(2):309–310 DOI 10.1111/j.1471-8286.2004.00597.x.
- Goudet J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86(6):485–486.
- Gutiérrez JP, Royo LJ, Álvarez I, Goyache F. 2005. MolKin v2.0: a computer program for genetic analysis of populations using molecular coancestry information. *Journal of Heredity* 96(6):718–721 DOI 10.1093/jhered/esi118.
- Hale ML, Burg TM, Steeves TE. 2012. Sampling for microsatellite-based population genetic studies: 25–30 individuals per population is enough to accurately estimate allele frequencies. *PLoS ONE* 7(9):e45170 DOI 10.1371/journal.pone.0045170.
- Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.
- Hauser L, Carvalho GR. 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. *Fish and Fisheries* 9(4):333–362 DOI 10.1111/j.1467-2979.2008.00299.x.
- Hoarau G, Boon E, Jongma DN, Ferber S, Pálsson J, Van der Veer HW, Rijnsdorp AD, Stam WT, Olsen JL. 2005. Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proceedings of the Royal Society B: Biological Sciences* 272(1562):497–503 DOI 10.1098/rspb.2004.2963.
- Hobbs JPA, Herwerden V, Jerry DR, Jones GP, Munday PL. 2013. High genetic diversity in geographically remote populations of endemic and widespread coral reef angelfishes (genus: *Centropyge*). *Diversity* 5(1):39–50 DOI 10.3390/d5010039.
- Hoffmann M, Hilton-Taylor C, Angulo A, Böhm M, Brooks TM, Butchart SHM, Carpenter KE, Chanson J, Collen B, Cox NA, Darwall WRT, Dulvy NK, Harrison LR, Katariya V, Pollock CM, Quader S, Richman NI, Rodrigues ASL, Tognelli MF, Vié J-C, Aguiar JM, Allen DJ, Allen GR, Amori G, Ananjeva NB, Andreone F, Andrew P, Aquino Ortiz AL, Baillie JEM, Baldi R, Bell BD, Biju SD, Bird JP, Black-Decima P, Julian Blanc J, Bolaños F, Bolivar-G W, Burfield IJ, Burton JA, Capper DR, Castro F, Catullo G, Cavanagh RD, Channing A, Chao NL, Chenery AM, Chiozza F, Clausnitzer V, Collar NJ, Collett LC, Collette BB, Cortez Fernandez CF, Craig MT, Crosby MJ, Cumberlidge N, Cuttelod A, Derocher AE, Diesmos AC, Donaldson JS, Duckworth JW, Dutson G, Dutta SK, Emslie RH, Farjon A, Fowler S, Freyhof J, Garshelis DL, Gerlach J, Gower DJ, Grant TD, Hammerson GA, Harris RB, Heaney LR, Blair Hedges S, Hero J-M, Hughes B, Hussain SA, Javier Icochea M, Inger RF, Ishii N, Iskandar DT, Jenkins RKB, Kaneko Y, Kottelat M, Kovacs KM, Kuzmin SL, La Marca E, Lamoreux JF, Lau MWN, Lavilla EO, Leus K,

- Lewis RL, Lichtenstein G, Livingstone SR, Lukoschek V, Mallon DP, McGowan PJK, McIvor A, Moehlman PD, Molur S, Muñoz Alonso A, Musick JA, Nowell K, Nussbaum RA, Olech W, Orlov NL, Papenfuss TJ, Parra-Olea G, Perrin WF, Polidoro BA, Pourkazemi M, Racey PA, Ragle JS, Ram M, Rathbun G, Reynolds RP, Rhodin AGJ, Richards SJ, Rodríguez LO, Ron SR, Rondinini C, Rylands AB, de Mitcheson YS, Sanciangco JC, Sanders KL, Santos-Barrera G, Schipper J, Self-Sullivan C, Shi Y, Shoemaker A, Short FT, Sillero-Zubiri C, Silvano DL, Smith KG, Smith AT, Snoeks J, Stattersfield AJ, Symes AJ, Taber AB, Talukdar BK, Temple HJ, Timmins R, Tobias JA, Tsytsulina K, Tweddle D, Ubeda C, Valenti SV, van Dijk PP, Veiga LM, Veloso A, Wege DC, Wilkinson M, Williamson EA, Xie F, Young BE, Resit Akçakaya H, Bennun L, Blackburn TM, Boitani L, Dublin HT, da Fonseca GAB, Gascon C, Lacher TE Jr, Mace GM, Mainka SA, McNeely JA, Mittermeier RA, McGregor Reid G, Rodriguez JP, Rosenberg AA, Samways MJ, Smart J, Stein BA, Stuart SN. 2011. The impact of conservation on the status of the world's vertebrates. *Science* 330(6010):1503–1509 DOI 10.1126/science.1194442.
- Jónsson G. 1982. Contribution to the biology of Catfish (*Anarhichas lupus*) at Iceland. *Journal of Marine Research Institute* 6(4):3–26.
- Kalinowski ST. 2005. Do polymorphic loci require large sample sizes to estimate genetic distances? *Journal of Heredity* 94(1):33–36 DOI 10.1038/sj.hdy.6800548.
- Khan MSA, Alam MJ, Rheman S, Mondal S, Rahman MM. 2002. Study on the Fecundity and GIS of Brackish Catfish *Plotosus canius*. *Online Journal of Biological Sciences* 2(4):232–234.
- Krishnamurthy PK, Francis RA. 2012. A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodiversity and Conservation* 21(8):1901–1919 DOI 10.1007/s10531-012-0306-2.
- Kumar B. 2012. Finding of (Plotosidae: Siluriforms) and its abundance from Parangipettai Coastal Area—a review. *International Journal of Recent Scientific Research* 3(6):482–485.
- Larmuseau MHD, Raeymaekers JAM, Hellemans B, Van Houdt JKJ, Volckaert FAM. 2010. Mito-nuclear discordance in the degree of population differentiation in a marine goby. *Journal of Heredity* 105(6):532–542 DOI 10.1038/hdy.2010.9.
- Leffler EM, Bullaughey K, Matute DR, Meyer WK, Séguérel L, Venkat A, Andolfatto P, Przeworski M. 2012. Revisiting an old riddle: what determines genetic diversity levels within species? *PLoS Biology* 10(9):e1001388 DOI 10.1371/journal.pbio.1001388.
- Leh MUC, Sasekumar A, Chew L-L. 2012. Feeding biology of Eel Catfish *Plotosus Canius* Hamilton in a Malaysian Mangrove Estuary and Mudflat. *Raffles Bulletin of Zoology* 60(2):551–557.
- Leray M, Knowlton N. 2015. DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences of the United States of America* 112(7):2076–2081 DOI 10.1073/pnas.1424997112.
- Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25(11):1451–1452 DOI 10.1093/bioinformatics/btp187.
- Mccracken GR, Wilson KL, Brewis HT, McBride MC, Paterson I, Perry R, Keefe D, Ruzzante DE. 2014. Development of 26 novel microsatellite makers for the round whitefish (*Prosopium cylindraceum*) and successful polymorphic cross-specific amplification of seven previously developed salmonid markers. *Conservation Genetic Resources* 6(4):1023–1026 DOI 10.1007/s12686-014-0276-7.
- Magurran AE, Baillie SR, Buckland ST, Dick JM, Elston DA, Scottm EM, Wattm AD. 2010. Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. *Trends in Ecology and Evolution* 25(10):574–582 DOI 10.1016/j.tree.2010.06.016.

- Mohd Akhir ME, Chuen YJ. 2011. Seasonal variation of water characteristics during inter-monsoon along the East Coast of Johor. *Journal of Sustainability Science and Management* 6(2):206–214.
- Na-Nakorn U, Taniguchi N, Nugroho E, Seki S, Kamonrat W. 1999. Isolation and characterization of microsatellite loci of *Clarias macrocephalus* and their application to genetic diversity study. *Fisheries Science* 65(4):520–526.
- Neff BD, Gross MR. 2001. Microsatellite evolution in vertebrates: inference from AC dinucleotide repeats. *Evolution* 55(9):1717–1733 DOI 10.1111/j.0014-3820.2001.tb00822.x.
- O’Leary SJ, Hice LA, Feldheim KA, Frisk MG, McElroy AE, Fast MD, Chapman DD. 2013. Severe inbreeding and small effective number of breeders in a formerly abundant marine fish. *PLoS ONE* 8(6):e66126 DOI 10.1371/journal.pone.0066126.
- O’Reilly PT, Canino MF, Bailey KM, Bentzen P. 2004. Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. *Molecular Ecology* 13(7):1799–1814 DOI 10.1111/j.1365-294X.2004.02214.x.
- Paetkau D, Slade R, Burden M, Estoup A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* 13(1):55–65 DOI 10.1046/j.1365-294X.2004.02008.x.
- Palti Y, Genet C, Gao G, Hu Y, You FM, Boussaha M, Rexroad CE III, Luo M-C. 2012. A second generation integrated map of the rainbow trout (*Oncorhynchus mykiss*) genome: analysis of conserved synteny with model fish genomes. *Marine Biotechnology* 14(3):343–357 DOI 10.1007/s10126-011-9418-z.
- Pauly D, Christensen V, Guénette S, Pitcher TJ, Sumaila UR, Walters CJ, Watson R, Zeller D. 2002. Towards sustainability in world fisheries. *Nature* 418(6898):689–695 DOI 10.1038/nature01017.
- Peakall R, Smouse PE. 2012. GenALEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28(19):2537–2539 DOI 10.1093/bioinformatics/bts460.
- Pereira HM, Leadley PW, Proença V, Alkemade R, Scharlemann JPW, Fernandez-Manjarrés JF, Araújo MB, Balvanera P, Biggs R, Cheung WWL, Chini L, Cooper HD, Gilman EL, Guénette S, Hurtt GC, Huntington HP, Mace GM, Oberdorff T, Revenga C, Rodrigues P, Scholes RJ, Sumaila UR, Walpole M. 2010. Scenarios for global biodiversity in the 21st century. *Science* 330(6010):1496–1501 DOI 10.1126/science.1196624.
- Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity* 95(6):536–539 DOI 10.1093/jhered/esh074.
- Piry S, Luikart G, Cornuet J-M. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity* 90(4):502–503 DOI 10.1093/jhered/90.4.502.
- Priest MA, Almany GR, Braun CD, Hamilton RJ, Lozano-Cortés DF, Saenz-agudelo P, Berumen ML. 2014. Isolation and characterization of 29 microsatellite markers for the bumphead parrotfish, *Bolbometopon muricatum*, and cross amplification in 12 related species. *Marine Biodiversity* 44(4):1–6.
- Primack R. 2002. *Essential of Conservation Genetics*. 3rd ed. Sunderland, MA: Sinauer Association Inc., 603 pp.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155(2):945–959.

- Prithiviraj N. 2014.** Studies on biological properties and in silico approach of *Plotosus canius*, from Parangipettai coastal waters. *International Journal of Modern Research Reviews* **1(6)**:24–37.
- Prithiviraj N, Annadurai D. 2012.** Studies on bioactive properties of the Catfish *Plotosus canius* (Hamilton, 1822). *International Journal of Recent Scientific Research* **3(6)**:467–473.
- Rice WR. 1989.** Analyzing tables of statistical tests. *Evolution* **43(1)**:223–225
DOI [10.2307/2409177](https://doi.org/10.2307/2409177).
- Riede K. 2004.** *Global Register of Migratory Species—from Global to Regional Scales. Final Report of the R&D Project 808 05 081*. Bonn: Federal Agency for Nature Conservation.
- Rizal S, Setiawan I, Iskandar T, Ilhamsyam Y, Musman M, Whahid M. 2010.** Currents simulation in the malacca straits by using three-dimensional numerical model. *Sains Malaysiana* **39(4)**:519–524.
- Rogers AR, Harpending H. 1992.** Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9(3)**:552–569.
- Rourke M, Gilligan D. 2010.** *Population Genetic Structure of Freshwater Catfish (Tandanus tandanus) in the Murray-Darling Basin and Coastal Catchments of New South Wales: Implications for Future Re-stocking, Final Report to the NSW Recreational Freshwater Fishing Trust for Project No. DPIFT48. Industry & Investment—Fisheries Final Report Series No. 123*. 73 pp.
- Rourke ML, Teske PR, Attard CRM, Gilligan DM, Beheregaray LB. 2010.** Isolation and characterisation of microsatellite loci in the Australian freshwater catfish (*Tandanus tandanus*) Murray–Darling Basin. *Conservation Genetic Resources* **2(1)**:245–248
DOI [10.1007/s12686-009-9161-1](https://doi.org/10.1007/s12686-009-9161-1).
- Rousset F. 2008.** GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8(1)**:103–106
DOI [10.1111/j.1471-8286.2007.01931.x](https://doi.org/10.1111/j.1471-8286.2007.01931.x).
- Shaw PW, Arkhipkin AI, Al-Khairulla H. 2004.** Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep-water troughs as barriers to genetic exchange. *Molecular Ecology* **13(11)**:3293–3303
DOI [10.1111/j.1365-294X.2004.02327.x](https://doi.org/10.1111/j.1365-294X.2004.02327.x).
- Song LM. 2012.** Understanding divergence in *Channa satiata*: a genetic and morphological approach. Master Thesis. University of Malaya, Kuala Lumpur, Malaysia.
- Tajima F. 1989.** Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123(3)**:585–595.
- Tallmon DA, Luikart G, Waples RS. 2004.** The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology and Evolution* **19(9)**:489–496
DOI [10.1016/j.tree.2004.07.003](https://doi.org/10.1016/j.tree.2004.07.003).
- Tamura K, Nei M. 1993.** Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10(3)**:512–526.
- Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S. 2013.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30(12)**:2725–2729
DOI [10.1093/molbev/mst197](https://doi.org/10.1093/molbev/mst197).
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997.** The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25(24)**:4876–4882 DOI [10.1093/nar/25.24.4876](https://doi.org/10.1093/nar/25.24.4876).

- Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M, Willerslev E. 2012.** Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS ONE* **7(8)**:e41732 DOI [10.1371/journal.pone.0041732](https://doi.org/10.1371/journal.pone.0041732).
- Usman BI, Amin SMN, Arshad A, Rahman MA. 2013.** Review of some biological aspects and fisheries of Grey-Eel Catfish *Plotosus canius* (Hamilton, 1822). *Asian Journal of Animal and Veterinary Advances* **2(8)**:154–167 DOI [10.3923/ajava.2013.154.167](https://doi.org/10.3923/ajava.2013.154.167).
- Usmani S, Tan SG, Siraj SS, Yusoff K. 2003.** Population structure of the Southeast Asian river catfish *Mystus nemurus*. *Animal Genetics* **34(6)**:462–464 DOI [10.1046/j.0268-9146.2003.01064.x](https://doi.org/10.1046/j.0268-9146.2003.01064.x).
- Van Oosterhout C, Hutchinson WE, Wills DPM, Shipley P. 2004.** MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4(3)**:535–538 DOI [10.1111/j.1471-8286.2004.00684.x](https://doi.org/10.1111/j.1471-8286.2004.00684.x).
- Voris HK. 2000.** Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography* **27(5)**:1153–1167 DOI [10.1046/j.1365-2699.2000.00489.x](https://doi.org/10.1046/j.1365-2699.2000.00489.x).
- Ward RD, Zemplak TS, Innes BH, Last PR, Hebert PDN. 2005.** DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **360(1462)**:1847–1857 DOI [10.1098/rstb.2005.1716](https://doi.org/10.1098/rstb.2005.1716).
- Wright LI, Tregenza T, Hosken DJ. 2008.** Inbreeding, inbreeding depression and extinction. *Conservation Genetics* **9(4)**:833–843 DOI [10.1007/s10592-007-9405-0](https://doi.org/10.1007/s10592-007-9405-0).
- Zhang AB, He LJ, Crozier RH, Muster C, Zhu C-D. 2010.** Estimating sample sizes for DNA barcoding. *Molecular Phylogenetic and Evolution* **54(3)**:1035–1039 DOI [10.1016/j.ympev.2009.09.014](https://doi.org/10.1016/j.ympev.2009.09.014).
- Zhang D-X, Hewitt GM. 1996.** Nuclear integrations: challenges for mitochondrial DNA markers. *Trends Ecology & Evolution* **11(6)**:247–251 DOI [10.1016/0169-5347\(96\)10031-8](https://doi.org/10.1016/0169-5347(96)10031-8).