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Genetic Relationships Between *Poecilia latipinna* and Selected Live-bearer Fishes in Malaysia

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ABSTRACT

Population study focusing the genetic diversity had been widely exploited for wild species but unfortunately little attempt was being made to understand the dynamics of genetic diversity on cultured aquarium species. This study was carried out to understand the dynamics of genetic diversity of cultured live-bearer fish in Malaysia. Taxonomic groupings of both *Xiphophorus* and *Poecilia* spp was validated using molecular markers. One hundred and thirty one samples of locally cultured live-bearer fishes which comprised of *Xiphophorus maculatus*, *Xiphophorus helleri*, *Poecilia reticulata* and *Poecilia latipinna* were sampled. The live-bearer fish samples were genotyped with 24 microsatellite (SSRs) primer pairs. Fifteen out of 24 SSRs primer pairs were able to amplify among the live-bearer fishes and were polymorphic. The average number of alleles was low (1 to 8). Differences between mean observed (H_O) and expected (H_e) heterozygosity for the four species live-bearer fishes were small ranging from 0.02 to 0.06 suggesting no drastic reduction of heterozygosity in cultured fish. The pair-wise comparisons of F_{ST} between these four species of live-bearer in general was high (F_{ST}>0.25). Such observation was expected as the four tested species of live-bearer belong to different taxonomic groups. A clear distinction between the species of *Xiphophorus* and *Poecilia* as revealed by SAHN-clustering tree plot seems to be in accordance with their taxonomy. The data generated from this study provide useful information in understanding the levels of genetic variation in cultured aquarium fish for resource management and conservation.

Key words: *Xiphophorus maculatus, Xiphophorus helleri, Poecilia reticulata, Poecilia latipinna, microsatellite, Genetic differentiation*

INTRODUCTION

Molly belongs to the group of live-bearer fish from the genus *Poecilia* and subgenus *Mollienesia* (Miller, 1975) which was originated from North America and Central America and distributed to various countries including Malaysia. It was first introduced into Malaysia fifteen years ago and had been locally cultured at Ulu Tiram, Johore for trading. According to a recent report by Department of Fisheries Malaysia (DOF, 2010), this species of live-bearer is one of the major export fish species in the country which consists of *Poecilia latipinna*, *Poecilia velifera* and *Poecilia sphenops*. However, there is no report on the presence of wild species in nature to date in the country.

A wide range of studies had been carried out to understand the genetic diversity dynamics on wild aquatic species such as in planktonic shrimps, prawns, pikeperch and sturgeon (Aziz *et al*., 2010; Gharibkhani *et al*., 2009; Norouzi *et al*., 2008). However, limited studies were attempted on cultured aquarium species. Intensive captive culture practices for a long period of time might have an effect on the overall genetic diversity in any fish species. Thus, it will be beneficial to understand the dynamics of genetic diversity in cultured aquarium species as such information could greatly facilitate choices of selection in any breeding program or in other downstream activities/studies particularly in development of mapping population.

Other fish species that belong to the group of live-bearer include platy (*Xiphophorus maculatus*), swordtail (*Xiphophorus helleri*) and guppy (*Poecilia reticulata*). Taxonomy had assigned molly in the same genus as guppy (both belongs to the genus of *Poecilia*). Studies by Meyer *et al*. (1994) had validated taxonomy grouping of both the *Xiphophorus* species with *P. reticulata*. Specifically, phylogenetic tree revealed that both *X. maculatus* and *X. helleri* were closely related and shared the same cluster while *P. reticulata* appeared to be an outgroup taxon. On the other hand, a separate study had been carried out by Ptacek and Breden (1998) on several *Poecilia* species within the subgenus *Mollienesia*. The sailfin molly (*P. latipinna*) was found to be closely related to the shortfin molly groups (*P. sphenops* and *P. mexicana*). They also observed that guppy (*P. reticulata*) showed high divergence and was not included within the *Mollienesia* taxon. Molecular validation on the taxonomic relationships between sailfin molly (*P. latipinna*) and the other three species of the live-bearer had not been studied so far.

Cross amplification of microsatellite (SSRs) markers between closely related species is common and had been widely used in phylogeny and population genetics (Chistiakov *et al*., 2006). Although, the use of microsatellite markers in phylogenic studies is not as informative as other markers however, data generated from these markers are increasing (Goldstein *et al*., 1999; Heath *et al*., 2001; Reusch *et al*., 2001). In the study, of population dynamics, SSRs had been widely applied due to their codominant character, small length, extensive genome coverage, relative abundance and high mutation rate. Since SSRs evolved fast, they are powerful tool for analyzing recent and contemporary event (Ellegren, 2000).

Hence, this study was carried out to understand the dynamics of genetic diversity in cultured aquarium species and to validate taxonomic groupings of both the *Xiphophorus* and *Poecilia* species by applying SSRs markers.

MATERIALS AND METHODS

Fish collection: Fish were collected from Aquatic International, Subang Jaya, Malaysia in the middle of October 2009 and maintained at Aquaculture Research Centre, Universiti Putra Malaysia, Puchong. These fishes originated from USA and had been locally cultured at Ulu Tiram, Johore, Malaysia. A total of 31 samples of red platy (*X. maculatus*), 32 samples of green swordtail (*X. helleri*), 30 samples of cultured assorted guppy (*P. reticulata*) and 38 samples of white sailfin molly (*P. latipinna*) were randomly selected. All the samples used were adults of approximately more than three months old with size range between 4.5 to 5.5 cm in both the platy and swordtail, 3.0 to 4.0 cm in guppy and 4.5 to 6.5 cm in molly. Each species of the live-bearer fish was designated as 'species group' throughout the text.

DNA extraction and microsatellite genotyping: Genomic DNA was extracted from fresh caudal fin clips according to kit protocols from Genomic Wizard Extraction Kit (Promega) with

Table 1: Characteristics of fifteen microsatellite prime pairs developed for *Xiphophorus maculatus* (Walter *et al.*, 2004) tested on the four live-bearer species

Locus	Forward 5' to 3'	Reverse 5' to 3'	Temperature (°C)	Repeat motif
Msa120	TTGCTGCTCCTGCAAATCAA	CATGAAGCTCCCTGCAAAGG	55	CA
Msb036	TGTGTATGCCGGAGGACGT	CGGAGCAGCGTTTTAGTTCG	55	ATG
Msb012	CAAAAGCATCTCATTGCAGGG	ACTGGATTGGACGAGGCATC	55	ATG
Msa018	CCACATTTAATGCTGTCAAGCC	GACAAGGTTTCCACTTTCAGAATTC	55	CA
Msa090	GGTGACCCACATGAGCTGAA	TGCTCTTCTGCTGCGCACT	55	CA
Msa045	TTTTGAGGTCGCTATAAGTCCTCA	GGTCGGCTTCAGACCTAGCAG	55	CA
Msc029	TCTGCAAAAGAATGTGCCAAA	TCGATTACTAAATTCGCTGATGTCTATT	55	TACA
Msa035	TGTGGGAATTCGAGAGCAGTT	TCCATCTGCCGAGTTCACAA	55	CA
Msa061	CCTACTCTGGTGAGCTGAGTGTTGTCA	GCCAGCTCTTCCAGGAGACTT	55	CA
Msb077	CTTTGGACTGGGTTTGGTTCC	CCAGCAGCATCTTCATCCTGT	55	ATG
Msa108	AAGGTTGCTGTTGGCAATGC	CGTTCCCTGACATGGTGTCTC	55	CA
Msb023	CTGCTGTCTCCATTCCTGAAGG	GCGAGCTGAGGCAGAAGATC	55	ATG
Msa026	CATTTCACAGTAAAAGCAGGTGGA	CCCACAGGCTGTATCCTAATGC	55	CA
Msa069	ATGTCAGAGGTGCAGTGGAACAT	TCTGCACATTTTAAATGGTCTACGTC	55	CA
Msa032	CCTCCGCCGACCAGC	CGGATCCCCGTGATCATC	55	CA

minor modification. A total of 26 microsatellite (SSRs) primer pairs designed by Walter *et al.* (2004) for *X. maculatus* were downloaded from online database (http://www.xiphophorus.txstate.edu /research/xiphbase/microsat.html) (Table 1). These primer pairs had been tested and successfully amplified on *Poecilia* species (Walter *et al.*, 2004). A Polymerase Chain Reaction (PCR) amplification was carried out using Eppendorf (Mastercycler gradient) and the PCR reaction volumes was reduced to 10 μL and thermal cycling conditions were carried according to the description by Promega product information (Catalog No. M8295). The amplified PCR products were electrophoresed on 4% metaphor gel (1X TBE buffer, 0.5 μg mL^{-1} gel red). Scoring of alleles was estimated using Promega ladder catalog number (No. G4511).

Data analysis: Microsatellite genotypes were submitted to MICROCHECKER (Van Oosterhout *et al.*, 2004) for null allele detection. Species group with null alleles were adjusted accordingly. Adjusted genotype frequency were analyzed with GENEPOP version-4 (Raymond and Rousset, 1995) for estimation of genetic diversity which comprised of the number of alleles and the observed and expected heterozygosity (H_O and H_E) at each locus. Polymorphic Information Content (PIC) of each microsatellite loci was calculated based on formula by Botstein *et al.* (1980) using Excel Microsatellite Tool kit (Park, 2001). Conformation to Hardy Weinberg Equilibrium (HWE) was carried out by locus and by population using POPGENE32 (Yeh and Boyle, 1997). The pair-wise genetic differentiation which determines the presence of species group structuring was evaluated through F-statistics (F_{IS}, F_{IT} and F_{ST}) (Weir and Cockerham, 1984). F-STAT (Goudet, 1995) was used to estimate F_{ST} (Weir and Cockerham, 1984) and R_{ST} (Slatkin, 1995) across species groups. The genetic disequilibrium between each pair of loci was estimated using GENEPOP version 4 (Raymond and Rousset, 1995). The genetic distance which measures the amount of variation between pairs of species group was calculated through isolation by distance between groups fitting in the equation of $D\sigma^2 = F_{ST}^{(1\text{-}FST)}$ (Rousset, 1997). The NTSYS version-2.1 (Rohlf, 1998) was subsequently used to construct a tree plot employing SAHN-clustering calculation.

RESULTS

Genetic diversity and population structuring: Fifteen out of 26 SSRs primer pairs were able to amplify within and among species groups. Null alleles were present in all the loci even after genotypes adjustment with the tendency towards homozygote excess. However, there was no scoring error due to stuttering and no evidence of large allele dropout in all the loci evaluated. Three out of 15 SSRs primer pairs namely Msa018, Msa035 and Msa032 could not amplified on *P. reticulata* species. A total number of 170 alleles were detected. The highest total number of allele was found in *P. latipinna* (58 alleles) and the lowest was found in *X. maculatus* (30 alleles) (Table 2).

The *P. latipinna* showed highest number of primer pairs which were informative having Polymorphic Information Content (PIC) values more than 0.5 as compared with other species groups. Seven out of 15 primer pairs fell in informative category namely Msa120 (PIC = 0.74), Msb036 (0.51), Msa090 (0.62), Msa061 (0.67), Msa077 (0.77), Msa108 (0.59) and Msb023 (0.58). Majority of PIC values in *X. maculatus* fell in less informative category (PIC<0.5) with an exception in primer pair Msb023. In *X. helleri,* only primer pairs Msa069 and Msa032 were categorized as informative with the PIC values of 0.53 and 0.57, respectively. Similarly, two out of the 15 primer pairs in *P. reticulata* fell in the informative categories which were Msb023 and Msa026 having PIC values of 0.60 and 0.51, respectively (Table 2).

Departures from HWE was significant ($p<0.05$) in most of the primer pairs even after genotype adjustment. In *X. maculatus,* only three primer pairs confirmed to HWE. In *X. helleri,* six primer pairs were in HWE. The *P. reticulata* recorded the highest number of primer pairs that confirmed to HWE with a total of eight primer pairs while in *P. latipinna,* only four primer pairs were in HWE. On average, the HWE results were not in equilibrium in the four species groups evaluated (Table 2).

The average observed heterozygosity (H_O) ranged from 0.05 to 0.27 while, the average expected heterozygosity (H_e) ranged from 0.07 to 0.27. The *P. latipinna* had the highest average H_O among the four species groups while the lowest was found in *X. maculatus*. Overall, both *X. helleri* and *P. reticulata* showed mean H_O higher than H_E. The F_{IS} values on both species were negative, indicating heterozygote excess. On the other hand, *X. maculatus* showed lower mean H_O compared to the H_E. A check on the F_{IS} value was positive, indicating heterozygote deficiency. The values of H_O and H_E were similar in *P. latipinna* (Table 3).

In population genetics, a test for Linkage Disequilibrium (LD) investigates genotypic disequilibrium at different set of primer pairs. In other words, LD is to test association of alleles by different sets of primer pairs. Overall, LD was observed between thirty-five out of 105 possible combinations (approximately 33%). However, only primer pair Msc029 did not show linkage disequilibrium with all the other primer pairs with an exception with primer pair Msb023 (Table 4).

The average estimator of genetic differentiation using R_{ST} was higher than of estimated from F_{ST} across the species group (Table 5). Pair wise comparisons in F_{ST} were high ($F_{ST}>0.25$) ranging from 0.33 to 0.86. The highest pairwise F_{ST} value was found between *X. maculatus* and *P. latipinna* and the lowest was found between *P. reticulata* and *X. helleri* (Table 6). The largest difference in genetic distance was found between *X. maculatus* and *P. latipinna* and the smallest was found between *P. reticulata* and *P. latipinna* (Table 6). The SAHN-clustering tree plot generated by utilizing genetic distance values revealed two clusters. The first cluster comprised of *X. maculatus* and *X. helleri* while the second cluster was shared by both *P. reticulata* and *P. latipinna* (Fig. 1). These four species of live-bearer groups seem to be clustered according to their respective genus that corroborates their taxonomy.

Table 2: Microsatellite markers, number of alleles (Na), polymorphic information content (PIC) and probability in Hardy-Weinberg equilibrium (HWE)

	Na				PIC				HWE			
Locus	*X. maculatus*	*X. helleri*	*P. reticulata*	*P. latipinna*	*X. maculatus*	*X. helleri*	*P. reticulata*	*P. latipinna*	*X. maculatus*	*X. helleri*	*P. reticulata*	*P. latipinna*
Msa120	2	2	3	5	0.11	0.06	0.43	0.74	0.000	**0.861**	0.000	0.000
Msb036	1	3	1	3	0.00	0.31	0.00	0.51	0	0.001	0	0.000
Msb012	2	1	4	3	0.37	0.00	0.44	0.27	0.000	0	**0.723**	**0.154**
Msa018	2	4	-	4	0.14	0.61	-	0.29	0.000	0.021	-	0.008
Msa090	1	2	4	5	0.00	0.21	0.42	0.62	0	**0.242**	**0.171**	**0.104**
Msa045	1	1	3	5	0.00	0.00	0.27	0.40	0	0	0.000	0.003
Msc029	1	3	3	1	0.00	0.27	0.42	0.00	0	0.705	**0.738**	0
Msa035	1	3	-	5	0.00	0.38	-	0.30	0	0.007	-	**0.650**
Msa061	2	3	2	4	0.03	0.32	0.12	0.67	0.000	**0.988**	**0.531**	0.000
Msa077	1	2	5	8	0.00	0.29	0.27	0.77	0	0.000	**0.998**	0.000
Msa108	3	4	4	4	0.34	0.43	0.39	0.59	0.001	**0.982**	**0.707**	0.021
Msb023	6	5	5	4	0.54	0.42	0.60	0.58	**0.149**	**0.525**	**0.108**	0.000
Msa026	3	1	5	2	0.40	0.00	0.51	0.33	**0.990**	0	**0.240**	0.000
Msa069	3	4	1	3	0.06	0.53	0.00	0.41	**0.997**	0.000	0	**0.649**
Msa032	1	4	-	2	0.00	0.57	-	0.13	0	0.025	-	0.000
Total	30	42	40	58					0.000	0.000	0.028	0.000

Values in bold showed SSRs primer pairs in HWE balance; 0 monomorphic:- No amplification

Table 3: Microsatellite markers, observed (Ho) and expected (HE) heterozygosity and F-statistics (FS values were computed as described by Weir and Cockerham (1984) (W and C)

	Ho				HE				FS (W and C)			
Locus	*X. maculatus*	*X. helleri*	*P. reticulata*	*P. latipinna*	*X. maculatus*	*X. helleri*	*P. reticulata*	*P. latipinna*	*X. maculatus*	*X. helleri*	*P. reticulata*	*P. latipinna*
Msa120	0.00	0.06	0.33	0.39	0.00	0.06	0.26	0.54	0.0000	-0.0303	-0.2571	0.2733
Msb036	0	0.03	0	0.42	0	0.06	0	0.50	0	0.5000	0	0.1764
Msb012	0.00	0	0.40	0.34	0.00	0	0.30	0.24	0.0000	0	-0.3131	-0.3987
Msa018	0.03	0.50	-	0.13	0.03	0.39	-	0.17	0.0000	-0.2897	-	0.2405
Msa090	0	0.38	0.20	0.23	0	0.22	0.13	0.28	0	-0.2500	-0.5789	0.1711
Msa045	0	0	0.03	0.15	0	0	0.03	0.19	0	0	0.0000	0.1832
Msc029	0	0.06	0.06	0	0	0.05	0.06	0	0	-0.1429	-0.1111	0
Msa035	0	0.25	-	0.10	0	0.14	-	0.09	0	-0.7778	-	-0.0968
Msa061	0.00	0.12	0.13	0.84	0.00	0.11	0.11	0.61	0.0000	-0.0452	-0.1765	-0.3807
Msa077	0	0.03	0.26	0.34	0	0.03	0.23	0.34	0	0.0000	-0.1594	0.0109
Msa108	0.16	0.18	0.30	0.13	0.13	0.15	0.22	0.16	-0.2500	-0.1892	-0.3289	0.2045
Msb023	0.22	0.53	0.40	0.63	0.42	0.40	0.31	0.57	0.4750	-0.3281	-0.2800	-0.0909
Msa026	0.06	0	0.80	0.00	0.06	0	0.57	0.00	-0.0526	0	-0.3990	0.0000
Msa069	0.06	0.65	0	0.42	0.06	0.44	0	0.36	-0.0196	-0.4808	0	-0.1684
Msa032	0	0.28	-	0.00	0	0.23	-	0.04	0	-0.2273	-	1.0000
Mean	0.05	0.22	0.26	0.27	0.07	0.16	0.20	0.27	0.03	-0.19	-0.26	0.09

0: Monomorphic; -: No amplification

Table 4: Pairwise Linkage Disequilibrium (LD) between 15 microsatellite primer pairs

Loucs	Msa120	Msb036	Msb012	Msa018	Msa090	Msa045	Msc029	Msa035	Msa061	Msa077	Msa108	Msb023	Msa026	Msa069	Msa032
Msa120															
Msb036	Highly sign														
Msb012	0.010	0.000													
Msa018	0.009	0.181	0.201												
Msa090	0.023	0.002	Not possible	0.070											
Msa045	0.071	0.298	0.001	0.085	0.499										
Msc029	Not possible	Not possible	Not possible	Not possible	Not possible	Not possible									
Msa035	0.075	0.039	0.028	0.033	0.133	0.054	Not possible								
Msa061	Highly sign.	Highly sign	0.001	0.036	0.000	0.018	Not possible	0.000							
Msa077	0.219	0.002	Not possible	0.398	0.059	0.049	Not possible	0.142	0.003						
Msa108	0.010	0.096	0.600	0.290	0.016	0.701	Not possible	Not possible	0.015	0.005					
Msb023	0.000	Highly sign.	0.002	0.349	0.000	0.685	0.198	0.146	Highly sign.	0.000	0.001				
Msa026	0.018	Not possible	0.000	Not possible	0.067	1.000	Not possible	Not possible	0.124	Highly sign.	0.003	0.025			
Msa069	0.000	Highly sign.	0.210	0.003	0.009	0.022	Not possible	0.043	Highly sign	0.046	0.162.	0.000	Not possible		
Msa032	0.173	0.166	Not possible	0.016	0.282	Not possible	Not possible	Not possible	0.167	Not possible	0.504	0.285	Not possible	0.285	s

Light gray: Significant association between markers ($p<0.05$). Black highlighted: No possible association. White boxes: Not significant

Table 5: Overall and per locus F_{ST} and R_{ST} values for four different species of live-bearer fish

Locus	F_{ST}	R_{ST}
Msa120	0.515	0.657
Msb036	0.604	0.824
Msb012	0.559	0.773
Msa018	0.583	0.842
Msa090	0.539	0.822
Msa045	0.613	0.695
Msc029	0.749	0.897
Msa035	0.642	0.301
Msa061	0.563	0.198
Msb077	0.535	0.600
Msa108	0.402	0.046
Msb023	0.354	0.049
Msa026	0.499	0.061
Msa069	0.562	0.696
Msa032	0.699	0.989
Mean	0.559	0.6237

Table 6: Pair-wise comparisons among species

	X. maculatus	*X. helleri*	*P. reticulata*	*P. latipinna*
X. maculatus	-	0.7552	1.2013	1.2735
X. helleri	0.5175	-	0.8954	0.9269
P. reticulata	0.7162	0.3312	-	0.6207
P. latipinna	0.8665	0.8090	0.5683	-

Values below diagonal are estimates of pairwise FST and values above diagonal are estimates of genetic distance fitting in the equation of $Dσ^2 = FST (1-F_{ST})$ (Rousset, 1997)

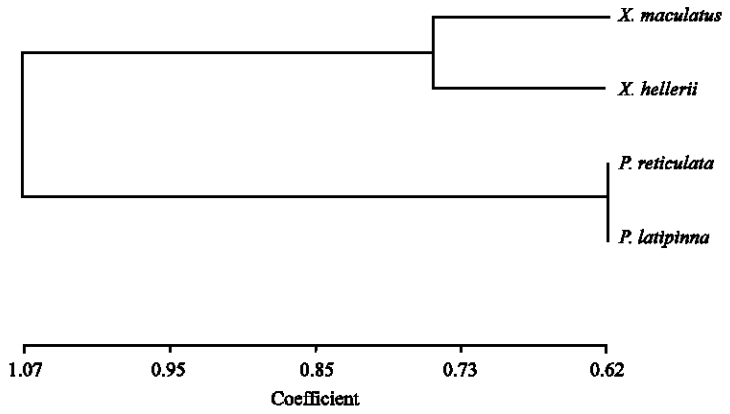

Fig. 1: SAHN-clustering tree plot based on pairwise genetic distance ($Dσ^2$) on four species of live-bearer fishes available in Malaysian market

DISCUSSION

The allelic diversities were low (mean number of alleles ranged from 1 to 8) in the four live bearer fish evaluated. Such observation is expected in cultured fish. Similar observation was found by Norris *et al.* (1999) in farmed Atlantic salmon which showed lower diversity in comparison with those from the wild. Possible explanation for such occurrence would be small population size,

genetic drift, geographical isolation and limited gene flow (Lowe *et al*., 2004; Norris *et al*., 1999). Genetic drift due to geographical isolation or migration will limit the gene flow and subsequently causing low allelic diversity in a newly colonized population. However, such events are more likely to occur in wild population. Another possible reason is artificial selection. As, most of the sample taken in this study was largely cultured, human intervention in selecting fish having favorable traits in breeding will eventually leads to reduction in allele diversity.

Although, there were differences between H_O and H_E in all the four species of live-bearer with an exception on *P. latipinna*, the value of differences were small ranging from 0.02 to 0.06. On average, both *X. helleri* and *P. reticulata* showed H_O higher than H_E, theoretically suggesting heterozygous excess. Possible explanation on the excess of heterozygosity in this study might be due to the type of breeding practices carried out in cultured aquarium fish. Hybridization by crossbreeding to enhance colour and appearance might be causal factor that increased the heterozygosity level on both of the species groups. There is no sign of inbreeding or outbreeding in *P. latipinna* detected as both H_O and H_e values were similar Fifteen years of captive selective breeding either through inbreeding or crossbreeding might not be long enough to contribute significantly to any changes in heterozygosity level (Anderson and Hayes, 2005). This study did not detect any drastic heterozygosity reduction in these four species of live-bearer despite being a cultured fish species. This result was in agreement with the findings by Norris *et al*. (1999) which similarly did not show reduction of heterozygosity in farmed fish. This study also demonstrates that low allelic diversity does not have a direct correlation with the level of heterozygosity. Similar observation was also found by Norris *et al*. (1999) which stated that decrease in allelic diversity is not detectable from levels of heterozygosity.

The average estimator of genetic differentiation across the species group shown by R_{ST} was higher than F_{ST}. Such observation was in agreement with Slatkin (1995), which stated that F_{ST} tends to understimatesthe true level of genetic differentiation. The pair-wise comparison of F_{ST} was high (F_{ST}>0.25) between the four species of live-bearers. Such finding was expected as the four species of live-bearer under study belongs to different taxonomic groups. The highest level of genetic differentiation was found between *P. latipinna* and *X. maculatus* followed by *X. helleri*. Lower level of genetic differentiation was found between *P. latipinna* and *P. reticulata*. This result was expected as closely related taxa generally exhibit lower level of genetic differentiation between them than distantly related taxa (Lowe *et al*., 2004).

The SAHN clustering tree plot showed that both *X. maculatus* and *X. helleri* were grouped in the same cluster while both *P. reticulata* and *P. latipinna* were grouped in another cluster. The clustering pattern of both *Xiphophorus* and *Poecilia* species as revealed by molecular markers seems to be in accordance with their taxonomy. This finding is in agreement with phylogenetic studies by Meyer *et al*. (1994) which showed that *X. helleri* appeared to be the sister group of *X. maculatus* and *P. reticulata* appeared to be an outgroup taxon. However, *P. latipinna* species group was not included in the study by Meyer *et al*. (1994) and made such comparison impossible.

CONCLUSION

The microsattellites (SSRs) markers applied in this study had successfully revealed low allelic diversity in these four cultured species of live bearer fishes. The grouping of both *Xiphophorus* and *Poecilia* species revealed by molecular marker in this study seem to be in accordance with their respective genus that corroborates with their taxonomy. Thus the data gathered from this study provide baseline information in understanding genetic variation of cultured aquarium fish for resource management and conservation.

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