Genetic and Morphological Variation among Hatchery Stocks of Thai Silver Barb *Barbonymus gonionotus* (Bleeker, 1850)

Santi Pannusa[1], Supawadee Poompuang[1,*] and Wongpathom Kamonrat[2]

ABSTRACT

Microsatellite loci and truss morphometrics were used to discriminate eight hatchery stocks of silver barb (*Barbonymus gonionotus*) in Northeast Thailand. The results showed significant genetic ($P < 0.0017$) and morphological differences ($P < 0.0017$) in 18 and 25 out of 28 pair-wise comparisons, respectively. However, cluster analyses revealed a weak association between genetic differentiation and morphometric variation. Principal components analysis identified three truss measurements in the abdominal regions as the most powerful variables to distinguish stocks. Discriminant function analysis, based on 13 truss elements, assigned individuals to four distinct groups, and one group consisted of samples from four stocks. Among distinct stocks that showed variation in body shape, the Buri Ram samples displayed the largest body size as a result of breeding selection. The identity of a stock could be constructed from three measurements for body shape that were significantly correlated. Findings demonstrated the use of the truss morphometric approach in discriminating different hatchery stocks of silver barb within the studied stations.

Keywords: *Barbonymus gonionotus*, discriminant analysis, genetic diversity, microsatellites, truss morphometrics

INTRODUCTION

It is well understood that genetic diversity is necessary for survival of a species as it allows the species to respond to short-term and long-term changes in the environment; short-term environmental changes such as natural disasters and pollution occur quickly and affect the whole population directly whereas long-term environmental changes occur slowly and allow the species an opportunity to adapt (Allendorf and Luikart, 2007). Therefore, in a breeding program, the establishment of a base population with broad genetic diversity is highly recommended to ensure long-term genetic response and minimize the risks of inbreeding and genetic drift (Gjedrem and Baranski, 2009). Moreover, genetically divergent strains should be developed for aquaculture species as protection against uncertainty from changes in economic value to consumers and farmer preferences (Tave, 1993). Given these conditions, there is a need for hatchery managers to monitor genetic changes in the breeding stocks and to assess phenotypic differentiation among divergent strains in order to obtain data for constructing the identity of each strain. Genetic variation and multivariate morphometrics as well as the combination of these two methods are

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[1] Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.

[2] Inland Fisheries Research and Development Bureau, Department of Fisheries, Ministry of Agriculture and Cooperatives, Bangkok 10900, Thailand.

* Corresponding author, e-mail: supawadee.p@ku.ac.th

widely used approaches to discriminate between stocks of aquaculture species, both from wild and hatchery origins (Corti *et al.*, 1988; Hossain *et al.*, 2010; Arechavala-Lopez *et al.*, 2011; Hassanien *et al.*, 2011; Rogdakis *et al.*, 2011). Many studies have demonstrated that both methods are effective tools for stock discrimination, although inconsistency between genetic and morphometric analyses has been reported in some investigations (Eknath *et al.*, 1991; Vasconcellos *et al.*, 2008) due to morphological variation being influenced by genetic as well as environmental factors.

Silver barb (*Barbonymus gonionotus*) is a freshwater species of the Order Cypriniformes, with wide distribution throughout Southeast Asia from the Mekong River basin to the Malay Peninsula and Indonesia (Smith, 1945). Due to the economic importance of this species, the Thai Department of Fisheries (DOF) has maintained large numbers of captive stocks of silver barb in fisheries stations throughout the country (Kamonrat, 1996). Several breeding programs have been established at local fisheries stations to support aquaculture production of this species. In the present study, microsatellites and truss morphometrics were used to assess the body shape and genetic differences among hatchery stocks of silver barb obtained from eight fisheries stations in Northeast Thailand. In addition to growth, body shape is a trait of interest for genetic improvement of silver barb. However, long-term domestication and selective breeding are known to have reduced the diversity of hatchery stocks, leading to genetic and phenotypic changes from their founder populations (Gjedrem and Baranski, 2009). Therefore, the objectives of this study were to assess genetic relationships among eight stock samples and to determine whether morphometrics would be an appropriate tool to distinguish different stocks of silver barb. In addition, discrete morphometric characters were identified in order to construct a stock signature for body shape.

MATERIALS AND METHODS

Fish collection

Samples of silver barb broodstocks (30 males and 30 females) used in this study were obtained from each of eight fisheries stations of the DOF in lower Northeast Thailand in the provinces of Nakhon Ratchsima (NAK), Surin (SUR), Sisaket (SIS), Ubon Ratchthani (UBO), Yasothon (YAS), Mahas Sarakham (MAH), Khon Kaen (KHO) and Buri Ram (BUR). The sex of the fish was determined through manual gamete expression. The ages of the fish were 1–3 yr with the average (± SD) body weight being 568.4 ± 26.2 and 984.5 ± 32.6 g for males and females, respectively. Pectoral fin clips were taken and preserved in 95% ethanol for genetic analyses.

Microsatellite genotyping

Genomic DNA was extracted from the pectoral fin clips, using a standard phenol-chloroform extraction procedure (Taggart *et al.*, 1992). Genotyping was performed at four microsatellite loci, *Bgon08* (Mcconnell *et al.*, 2001) and at *Pgon-69*, *Pgon-75* and *Pgon-79* (Kamonrat *et al.*, 2002). The polymerase chain reaction (PCR) was performed in a 5 µL reaction volume which contained 20 ng of genomic DNA, 1× PCR buffer, 1.5 mM MgCl₂, 100 µM of dNTPs, 0.25 µM of each primer and 0.2 U of *Taq* DNA polymerase (Fermentas; Vilnius, Lithuania). The PCR profile was as follows: initial denaturation at 95°C for 5 min; 40 cycles at 95°C for 30 sec, annealing temperature for 30 sec and 72°C for 1 min; followed by 1 cycle at 72°C for 5 min in a PTC-100 Programmable Thermal Controller (MJ Research; St. Bruno, Quebec, Canada). Following amplification, reaction products were mixed with sequencing dye (0.1% bromphenol blue, 0.1% xylene cyanol, 2% of 0.5 M ethylenediaminetetraacetic acid (EDTA) at pH 8.0 and 98% formamide). The reaction mixtures were heated for 5 min at 95°C and subjected to electrophoresis on

a 4.5% denaturing polyacrylamide gel in 1× Tris-borate-EDTA running buffer at 60 W for 2–3 hr in a sequencing gel apparatus (Bio-Rad; Hercules, CA, USA). Gels were denatured at 100°C for 30 min before electrophoresis. DNA bands in gels were visualized and photographed using Gel Documentation (Advanced American Biotechnology (AAB); Fullerton, CA, USA). Allele sizes were estimated by comparison with a 10-bp DNA ladder (Invitrogen; Carlsbad, CA, USA) using AAB software (Advanced American Biotechnology, Fullerton, CA, USA).

Genetic data analysis

The program MICRO-CHECKER version 2.2.0 (Van Oosterhout *et al.*, 2004) was used to test for the presence of null alleles. Genetic variation within populations based on the mean number of alleles per locus (A), allelic richness (A_r), and observed (H_o) and expected (H_e) heterozygosities were computed using GENEPOP version 4.0 (Raymond and Rousset, 1995). Hardy-Weinberg expectations were tested by the exact *P* values and calculated using a Markov Chain Monte-Carlo randomization method (Guo and Thompson, 1992) using ARLEQUIN version 3.11 (Excoffier *et al.*, 1992). A Bonferroni correction was used to adjust significance levels for multiple tests (Rice, 1989). Genetic differences among stocks were determined as pair-wise F_{ST} values using ARLEQUIN version 3.11. The TFPGA program (Miller, 1997) was used to calculate Nei's genetic distances which were used to construct a unweighted pair group method with arithmetic mean (UPGMA) dendrogram and to test population grouping using bootstrapping analysis.

Morphometric measurements and analysis

Male *B. gonionotus* were lightly anesthetized with clove oil (1 mL/2.5 L of water) for 2–3 min and placed on a light-colored background on their right side and medial fins were pinned in place. Females were excluded from the morphometric analysis due to distortion of body shape caused by enlarged ovaries. A centimeter scale was included in the images to ensure calibration to the nearest 0.01 cm. Images were taken using a D40X digital camera (Nikon; Tokyo, Japan) mounted on a 190XPROB tripod (Manfrotto; Cassola, Italy). The truss network method (Bookstein *et al.*, 1985) was used to characterize whole-body shape. Eleven homologous landmarks were identified and 23 distance measurements were analyzed. Figure 1 displays the location of the landmarks and distances that were plotted on the images using SigmaScan Pro version 4.0 (www.sigmaplot. com).

Principal components analysis (PCA) was used to describe the body shape independently of the fish size and to outline groups of samples using the Paleontological Statistics software (PAST) (Hammer *et al.*, 2015) by comparing the shape differences of all eight stocks and between pairs of stock samples. To eliminate the effect of fish length on the first principal component (Bookstein *et al.*, 1985), all truss morphometric data were standardized to fish fork length (FL) using the method described by Reist (1985). Cluster analysis based on Euclidean distances was used to determine grouping of stocks and to construct a UPGMA dendrogram using the computer program PAST. Multivariate analysis of variance (MANOVA) was used to examine differences between pairs of stock samples using PAST. Discriminant function analysis was used to estimate the mathematical function for the classification of individuals into groups using IBM SPSS Statistics 22 (Chicago, IL, USA). This analysis separates individuals based on a priori recognition of groups. In addition, the scores of individual fish and the corresponding Euclidean distances from the group centroids were calculated for validation of the samples (Von Cramon-Taubadel *et al.*, 2005; Hossain *et al.*, 2010; Khan *et al.*, 2012). ANOVA was used to test for differences in truss measurements between stocks using IBM SPSS Statistics 22.
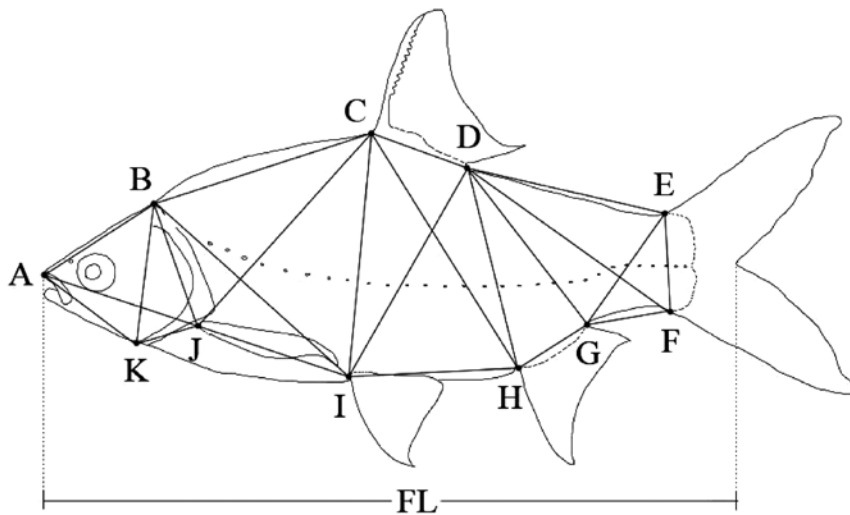
Figure 1 Locations and distances between landmarks for 23 truss measurement variables (TE). TE1 (A–B: anterior tip of snout at upper jaw to posterior most aspect of neurocranium); TE2 (B–C: posterior most aspect of neurocranium to origin of dorsal fin); TE3 (C–D: origin of dorsal fin to insertion of dorsal fin); TE4 (D–E: insertion of dorsal fin to anterior attachment of dorsal membrane from caudal fin); TE5 (E–F: anterior attachment of dorsal membrane from caudal fin to anterior attachment of ventral membrane from caudal fin); TE6 (F–G: anterior attachment of ventral membrane from caudal fin to insertion of anal fin); TE7 (G–H: insertion of anal fin to origin of anal fin); TE8 (H–I: origin of anal fin to insertion of pelvic fin); TE9 (I–J: insertion of pelvic fin to origin of pectoral fin); TE10 (J–K: origin of pectoral fin to posterior most point of maxillary); TE11 (K–A: posterior most point of maxillary to anterior tip of snout at upper jaw); TE12 (A–J: anterior tip of snout at upper jaw to origin of pectoral fin); TE13 (J–B: origin of pectoral fin to posterior most aspect of neurocranium); TE14 (B–K: posterior most aspect of neurocranium to posterior most point of maxillary); TE15 (B–I: posterior most aspect of neurocranium to insertion of pelvic fin); TE16 (I–C: insertion of pelvic fin to origin of dorsal fin); TE17 (C–J: origin of dorsal fin to origin of pectoral fin); TE18 (C–H: origin of dorsal fin to origin of anal fin); TE19 (H–D: origin of anal fin to insertion of dorsal fin); TE20 (D–I: insertion of dorsal fin to insertion of pelvic fin); TE21 (D–G: insertion of dorsal fin to insertion of anal fin); TE22 (G–E: insertion of anal fin to anterior attachment of dorsal membrane from caudal fin); and TE23 (F–D: anterior attachment of ventral membrane from caudal fin to insertion of dorsal fin). FL = standard fork length.

RESULTS

Genetic analysis revealed that all eight stocks of silver barb exhibited moderate to high genetic variation, with the total number of alleles across stock samples ranging from four to 11 alleles per locus (Table 1). Allele richness varied from 4.6 (BUR) to 8.0 (SIS). Mean H_o across loci ranged from 0.54 ± 0.27 (KHO) to 0.76 ± 0.19 (UBO) and mean H_e ranged from 0.61 ± 0.19 (KHO) to 0.77 ± 0.08 (NAK). Significant departures from Hardy-Weinberg expectation were observed at one locus in five samples and at two loci in three samples. There was no significant linkage disequilibrium

for any pairs of loci ($P > 0.008$ after Bonferroni correction). The global F_{ST} value of 0.0326 (95% confidence interval: 0.0253–0.0418) indicated significant genetic differentiation among stocks, with 18 of 28 pair-wise stock sample comparisons exhibiting significant F_{ST} values ranging from 0.0256 to 0.0917 ($P < 0.0017$) after Bonferroni correction for multiple testing (Table 2). Genetic dissimilarity was highest between NAK and KHO stocks ($F_{ST} = 0.0917$). The UPGMA dendrogram constructed from the D_A matrix revealed the genetic relationships of three groups (Figure 2a). Group 1 consisted of three stocks—BUR, SUR and KHO. Four stocks—UBO, MAH, SIS and YAS—were clustered in Group 2 while the NAK

formed a unique branch clearly separated from the rest of the stocks in Group 3.

Analysis of morphometric data revealed that PC1 and PC2 accounted for 60.3% and 10.4% of the total variation in fish body shape, respectively. Table 3 shows the component loading values of each variable on principal component 1 (PC1) and PC2. The variation in measurements of three truss measurements, including TE16 (distance from dorsal fin front to abdominal fin front), TE19 (depth between end of dorsal fin and origin of anal fin) and TE20 (depth between end of dorsal fin and origin of abdominal fin), contributed the maximum loadings of PC1. The measurements of TE 9 (distance from insertion of

Table 1 Stocks of samples (number of individuals sampled ± SD) and genetic variation parameters of silver barb stocks determined from four microsatellite loci.

Stock (N)	Number of . alleles	A_R	H_O	H_E	F_{IS}
NAK (30)	8.00 ± 2.58	7.76 ± 2.45	0.70 ± 0.12	0.77 ± 0.08	0.09
BUR (30)	4.75 ± 0.96	4.65 ± 0.87	0.64 ± 0.24	0.65 ± 0.07	0.03
SUR (30)	6.50 ± 1.29	6.29 ± 1.09	0.69 ± 0.26	0.75 ± 0.03	0.08
SIS (30)	8.25 ± 2.36	8.03 ± 2.19	0.66 ± 0.24	0.74 ± 0.06	0.12
UBO (30)	7.25 ± 1.26	6.91 ± 1.10	0.76 ± 0.19	0.73 ± 0.07	-0.03
YAS (29)	7.00 ± 2.16	6.74 ± 1.84	0.67 ± 0.12	0.71 ± 0.11	0.05
MAH (30)	7.00 ± 1.15	6.79 ± 1.03	0.60 ± 0.19	0.69 ± 0.09	0.14
KHO (30)	6.00 ± 1.15	5.81 ± 1.20	0.54 ± 0.27	0.61 ± 0.19	0.17

A_r = allelic richness; H_o = observed heterozygosity; H_e = expected heterozygosity; F_{IS} = fixation index.

NAK = Nakhon Ratchsima; BUR = Buri Ram; SUR Surin; SIS = Sisaket; UBO = Ubon Ratchthani; YAS = Yasothon;

MAH = Mahas Sarakham; KHO = Khon Kaen.

Table 2 Estimation of pairwise population differentiation. Overall genetic differentiation, values are above diagonal; P values for MANOVA test are below diagonal.
        *Significant at $P < 0.000357$ after Bonferroni correction.

	NAK	BUR	SUR	SIS	UBO	YAS	MAH	KHO
NAK		0.085*	0.021*	0.029*	0.014	0.032*	0.043*	0.092*
BUR	3.24×10^{-12}*		0.016*	0.059*	0.055*	0.051*	0.069*	0.027*
SUR	1.15×10^{-9}*	8.24×10^{-8}*		0.014	0.007	0.012	0.025*	0.037*
SIS	3.35×10^{-7}*	7.54×10^{-5}*	1.58×10^{-5}*		0.003	0.007	0.002	0.053*
UBO	3.26×10^{-9}*	7.83×10^{-9}*	1.31×10^{-7}*	3.76×10^{-5}*		0.005	0.005	0.049*
YAS	9.00×10^{-8}*	6.86×10^{-8}*	3.16×10^{-8}*	0.00085	7.86×10^{-5}*		0.015	0.023
MAH	4.90×10^{-8}*	7.00×10^{-9}*	8.44×10^{-8}*	0.00037	9.25×10^{-5}*	0.01570		0.061*
KHO	7.06×10^{-9}*	6.47×10^{-9}*	1.68×10^{-5}*	5.37×10^{-5}*	1.51×10^{-6}*	4.26×10^{-6}*	7.67×10^{-6}	

NAK = Nakhon Ratchsima; BUR = Buri Ram; SUR Surin; SIS = Sisaket; UBO = Ubon Ratchthani; YAS = Yasothon;

MAH = Mahas Sarakham; KHO = Khon Kaen.

Table 3 Component loadings for the principal components (PC1 and PC2) of truss morphometric characters (TE1–TE23; see Figure 4 for details) of Thai silver barb. Bold figures indicate maximum loadings of three characters for PC1 and PC2.

Character	PC1	PC 2
FL	0	0
TE1	0	0.31
TE2	0.16	-0.11
TE3	0.02	-0.1
TE4	-0.05	-0.08
TE5	0.06	0.01
TE6	-0.15	0.09
TE7	0.04	-0.01
TE8	0.13	-0.07
TE9	0.12	**0.41**
TE10	-0.01	**0.53**
TE11	-0.02	**0.37**
TE12	0.03	0.2
TE13	0.07	0.34
TE14	0.05	0.05
TE15	0.21	0.04
TE16	**0.47**	0.2
TE17	0.3	-0.04
TE18	0.38	-0.15
TE19	**0.41**	-0.05
TE20	**0.43**	0.18
TE21	0.23	-0.09
TE22	0.02	0.07
TE23	0.02	-0.06

FL = standard fork length.

pelvic fin to origin of pectoral fin), TE10 (distance from origin of pectoral fin to posterior most point of maxillary) and TE11 (distance from posterior most point of maxillary tip of snout at upper jaw) had the largest loadings for PC2. The PCA identified clusters of all eight stock samples and showed some that overlapped (Figure 3). However, the cluster analysis separated individuals into eight groups corresponding to the stock samples as shown in the dendrogram in Figure 2b. Three stocks—KHO, NAK and SUR—were clustered in the same group. The second group consisted of five stocks—YAS, SIS, UBO, MAH and BUR. To further differentiate individuals between pairs of stocks, PCA analyses were performed for 28 combinations followed by a MANOVA test. The results showed that body shape differences were significant ($P < 0.00038$) between all stocks except for three pairs of stocks, namely YAS-SIS, YAS-MAH and MAH-SIS (Table 2).

Discriminant functions (DFs) were constructed based on 13 truss elements (TE1, TE2, TE3, TE8, TE9, TE10, TE13, TE14, TE15, TE16, TE17, TE19 and TE20). The results revealed that the first and the second DF functions accounted for 37.5% and 23.5 % of the total variation, respectively. The first DF function distinguished the NAK, KHO, SUR and BUR samples from the
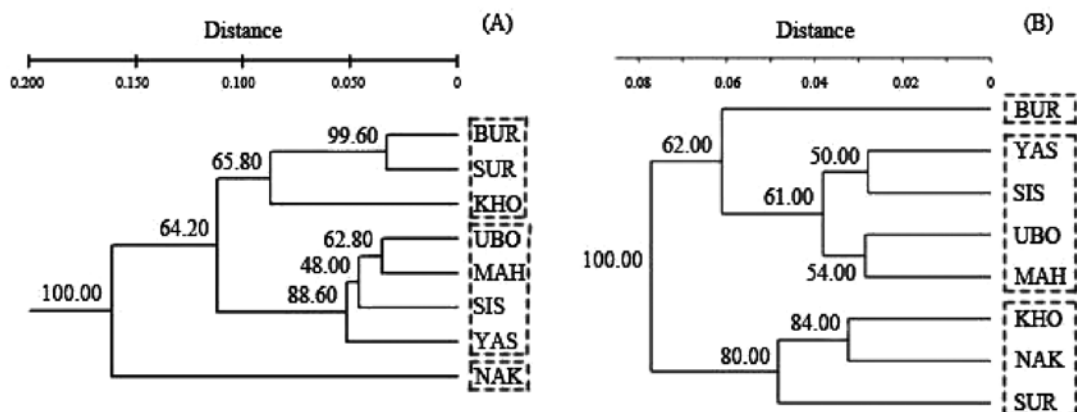

Figure 2 Unweighted pair group method with arithmetic mean dendrograms based on (A) Nei's genetic distance; (B) and Euclidean distance. (NAK = Nakhon Ratchsima; BUR = Buri Ram; SUR Surin; SIS = Sisaket; UBO = Ubon Ratchthani; YAS = Yasothon; MAH = Mahas Sarakham; KHO = Khon Kaen).

group of four stocks (SIS, UBO, YAS and MAH) that were similar to each other. Discrimination plots based on two mathematical functions showed a separation of group centroids from four stocks (NAK, KHO, SUR and BUR) in discriminant space, whereas those from the remaining four stocks overlapped with each other (Figure 4). According to the standardized canonical discriminant function coefficients, the most discriminating variable for the first DF function was TE 16. Furthermore, using the principal components of body shape, the discriminant analysis showed that 78% of fish were correctly assigned to their origin (Table 3). Within the group of four distinct stocks, the BUR samples were differentiated by larger truss elements. Table 4 shows the ANOVA and differences in truss measurements between the four stocks that can be used as a unique signature for each stock.

DISCUSSION

Morphometric and genetic analyses are commonly used methods to characterize wild and cultured stocks of aquaculture species. The variations in morphometric characters within- and between hatchery stocks are known to be influenced by genetics (stock origin) as well as factors related to the rearing conditions. A morphometric approach has been suggested as an alternative to distinguish stocks when genetic and morphometric data are highly correlated (Corti *et al.*, 1988). Although association between genetic divergence at neutral loci and phenotypic variation remains controversial (Lewontin, 1984; Allendorf and Phelps, 1998), significant morphology differences were reported between wild and farmed strains of Mediterranean gilthead seabream and European seabass (Arechavala-Lopez *et al.*, 2011) and between wild and hatchery-reared Atlantic salmon (Von Cramon-Taubadel *et al.*, 2005). Relatively few differences in morphology were observed among eight founder stocks of Nile tilapia used for development of the GIFT (Genetically Improved Farmed Tilapia) strain (Eknath *et al.*, 1991). In common carp, morphological divergence between hatchery stocks was apparently due to
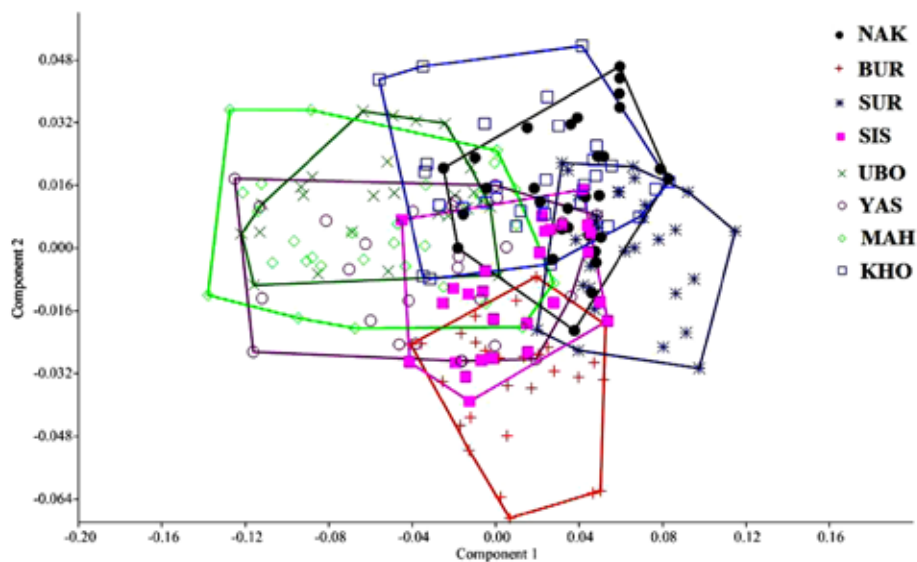

Figure 3 Results of the principal components analysis on truss variables for body shape differences among stocks. Principal component 1 and principal component 2 accounted for 60.3% and 10.4% of the total variation. Each stock is outlined with a polygon. (NAK = Nakhon Ratchsima; BUR = Buri Ram; SUR Surin; SIS = Sisaket; UBO = Ubon Ratchthani; YAS = Yasothon; MAH = Mahas Sarakham; KHO = Khon Kaen).
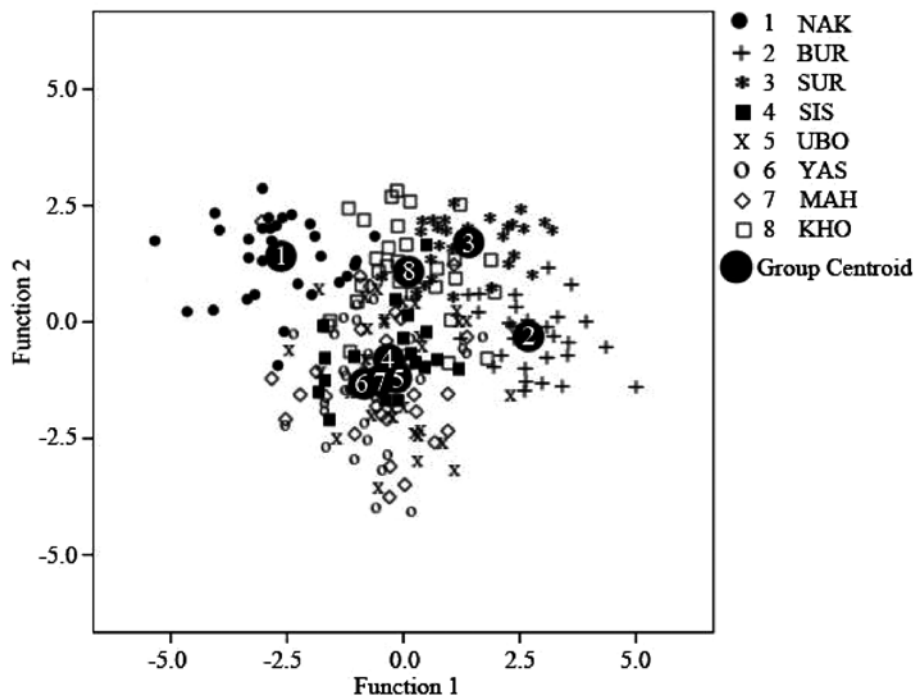
Figure 4 Scatter plot of discriminant functions 1 and 2 for body shape variation with group centroids of eight hatchery populations of silver barb. (NAK = Nakhon Ratchsima; BUR = Buri Ram; SUR Surin; SIS = Sisaket; UBO = Ubon Ratchthani; YAS = Yasothon; MAH = Mahas Sarakham; KHO = Khon Kaen).

Table 4 Classification results for the discriminant function analyses of silver barb broodstock (77.8% of all samples were correctly classified). Values in rows indicate the number of each sample group classified as a member of each predicted group. Percentage values of total sample size are given in parentheses.

Sample group	Predicted group								Total (N)
	NAK	BUR	SUR	SIS	UBO	YAS	MAH	KHO	
NAK	28 (93.3)	0	1(3.3)					1(3.3)	30
BUR	0	26 (86.7)	1(3.3)	2(6.7)	1(3.3)				30
SUR	1(3.33)	0	29(96.7)						30
SIS	0	1(3.3)	2 (6.7)	19 (63.3)	0	5	0	3	30
UBO	0	0	0	0	24 (80.0)	3 (10.0)	1 (3.3)	2 (6.7)	30
YAS	0	1 (3.5)	1(3.5)	2(6.9)	3 (10.3)	17 (58.6)	4 (13.8)	1(3.5)	29
MAH	0	0	2 (6.7)	3 (10.0)	0	5 (16.7)	18 (60.0)	2 (6.7)	30
KHO	0	0	2 (6.7)	1 (3.3)	1(3.33)	0	1 (3.3)	25 (83.3)	30

NAK = Nakhon Ratchsima; BUR = Buri Ram; SUR Surin; SIS = Sisaket; UBO = Ubon Ratchthani; YAS = Yasothon;

MAH = Mahas Sarakham; KHO = Khon Kaen.

Table 5 ANOVA test for morphological differences among four silver barb stocks based on measurements of three truss elements (± SD).

Variables	Sample Group				F	P Value
	NAK	BUR	SUR	KHO		
TE 16	6.54 ± 0.31	8.27 ± 0.71	6.31 ± 0.44	7.08 ± 0.49	90.16	0.00*
TE 19	5.30 ± 0.24	7.14 ± 0.61	5.39 ± 0.38	5.92 ± 0.42	114.69	0.00*
TE 20	6.43 ± 0.29	8.14 ± 0.67	6.11 ± 0.41	7.01 ± 0.48	102.98	0.00*

*$P < 0.001$

NAK = Nakhon Ratchsima; BUR = Buri Ram; SUR Surin; SIS = Sisaket; UBO = Ubon Ratchthani; YAS = Yasothon; MAH = Mahas Sarakham; KHO = Khon Kaen.

genetic factors as confirmed by the significant relationship between genetic and morphometric distances (Corti *et al.*, 1988). The results from the present study demonstrated that the morphological variation detected between male broodstocks of silver barb were supported to a certain degree by the genetic analysis. For instance, the cluster analyses revealed identical groupings of the four stocks—YAS, SIS, UBO and MAH—by morphometric measurements and genetic data, whereas the groupings of the remaining stocks were different. Stock transfers between fisheries stations in the northeast region have occurred for more than 20 years (Kamonrat, 1996). The founder stocks of the YAS, SIS, UBO and MAH stations consisted of brood fish from the Buri Ram (BUR) station and those originating from the Mekong River (Rungtongbaisuree *et al.*, 2012). Therefore, it was likely that stock movement among stations has resulted in non-significant pairwise F_{ST} values between these stocks. Consequently, the levels of morphology differentiation found within this group were more subtle than those observed from the rest of the stock samples.

On the contrary, cluster analysis also indicated the discrepancy between morphometric and genetic patterns, that is, the groupings of BUR, SUR, KHO and NAK stations. Among these four stocks, there was no correlation between the Euclidean and Nei's genetic distances, suggesting that morphometric and genetic differentiations were not associated. The stocks, BUR and SUR, which shared the same origin of the Mekong

River, were genetically similar because the SUR station obtained its founder stock from the BUR station. The differences in their morphology were apparently due to the selection programs of the BUR stock which underwent selective breeding to improve growth over four generations (6–8 yr) (Rungtongbaisuree *et al.*, 2012). In contrast, there are no records of selection programs in other stocks of silver barb. Despite having the highest genetic differentiation in this study (pairwise $F_{ST} = 0.0917$), the NAK and KHO stocks were clustered in the same group as the SUR samples. The current KHO stock originated from the Mekong River and has been propagated for less than 5 yr (ICLARM, 2000). The NAK stock which was genetically distinct from the rest of the stock samples was obtained from a private hatchery outside the studied stations, with no record of selective breeding. For stocks sharing the same original sources, it is likely that environmental factors related to rearing conditions such as the stocking density and quality of food, may also have contributed to body shape distinction. These factors have been suggested to have significant impact on body shape differentiation between wild and hatchery-reared populations of Atlantic salmon at various growth stages (Von Cramon-Taubadel *et al.*, 2005) and between cultured and wild populations of African catfish (*Clarias gariepinus*) (Solomon *et al.*, 2015). Moreover, to some extent, differences in individuals' ages may also contribute to body shape differences among stocks of silver barb because the age of brood fish

used in this study ranged from one to three years old.

There were morphological differences that allowed for stock identification in at least four stocks—NAK, BUR, SUR and KHO. The results from this study provide sufficient evidence to show that these four stocks can be distinguished using the truss morphometric approach. First, the MANOVA test for pairwise morphometry differentiation suggested significant differences in body shape ($P < 0.05$) between 25 of the 28 sample pairs. Because the use of an inadequate sample size in terms of the number of fish studied (N) relative to the number of truss elements (P) may fail to capture morphological variation, Kocovsky *et al*. (2009) suggested an N to P ratio in the range 5–8 for the analysis of fish shape. In the present study, an N:P ratio of 10 (239/23) was sufficient to achieve reliable outcomes from PCA. Second, the discriminant function analysis separated individuals into four distinct groups and one group consisted of four stocks with similar morphology. This was further confirmed by significant differences among the group centroids ($P < 0.001$). Third, the discriminant function analysis assigned the unknown individuals to four distinct stocks with high accuracy. For instance, NAK which was genetically distinct from the rest of the stocks was assigned to the origin with 93% accuracy, followed by SUR, BUR and KHO. As expected, the samples from four stocks (MAS, SIS, UBO and YAS) which displayed similar body shapes were assigned to their stocks of origin with lower levels of accuracy (58–80%).

The amount of morphological and genetic distinction detected among silver barb broodstock allows hatchery managers to identify stock by origin based on their morphometric characters for four stocks, NAK, KHO, SUR and BUR (Table 4). More importantly, the results suggested that morphometric analysis provides a useful tool for selecting male brood fish to improve traits that are highly correlated with body shape measurements.

For instance, the truss measurements, TE16, TE19 and TE20, which represent fish body depth, showed highly significant correlations (0.89, 0.98 and 0.87, respectively, between TE16 and TE19; TE16 and TE20; and TE19 and TE20). These measurements were powerful variables in discriminating stocks and perhaps useful parameters for the selection and prediction of important production traits such as growth, carcass percentage and fillet yield. Body shape measurements have been used for the prediction of fillet weight and fillet yield in common carp, *Cyprinus carpio* (Cibert *et al.*, 1999), tilapia, *Oreochromis niloticus* (Rutten *et al.*, 2004), river catfish, *Pangasius hypophthalmus* (Tseng *et al.*, 2009) and rainbow trout, *Oncorhynchus mykiss* (Haffray *et al.*, 2012).

In conclusion, the present study suggested that differences in morphology of silver barb could be attributed to genetic differentiation between stocks. More importantly, this study demonstrated that a truss morphometric approach can be used as an alternative to microsatellite analysis in discriminating different hatchery stocks of silver barb from the northeast region. Truss measurements provide a simple tool and may be useful for constructing the stock identity and predicting production traits in selective breeding programs. However, the truss morphometric approach was less effective when the silver barb broodstock showed high levels of genetic similarity.

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