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# Morphometric Variations and Fishery Unit Assessment of *Cyclocheilichthys apogon* (Actinopterigii: Cyprinidae) from Three-Different Rivers in North-Eastern Thailand

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## ABSTRACT

Beardless barb, *Cyclocheilichthys apogon* (Valenciennes, 1842) is a freshwater fish of importance as source of low-cost protein in Lower Mekong Region. The present study applied multivariate morphometric technique to identify fishery management units of *C. apogon* from six populations of three different river drainages: Pong, Chi, and Mun Rivers. Thirty-two truss measures and standard length were obtained using digital calliper from 291 fish individuals, and raw measured data were then subjected to allometric equation to remove size-dependent variation prior further statistical analyses. Multivariate analysis of variance (MANOVA) indicated highly significant differences in morphometric characters between populations ($p < 0.01$). The first three principal axes of principal component analysis (PCA) explained 49.29% of total variance. The PCA also revealed that morphological variations related to the characters of head depth, body length, body depth, caudal peduncle length and depth. In discriminant function analysis (DFA), the first two discriminant functions accounted for 72.00% of total variation, and discriminated fish samples into three major groups following to their collecting drainages. Furthermore, 96.29 and 90.56% of fish samples were correctly classified into their respect populations with original and cross-validated tests, respectively. The reliable morphometric variations in the present study suggest that management unit of *C. apogon* should define relied on the isolation of river drainage. Moreover, the study also indicates the involvement of environmental conditions in morphological adaptation, providing useful information for the sustainable conservation of this fish.

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**AK collected samples, performed laboratory analysis, and wrote the article. PJ supervised the work, helped in analysis, and wrote and revised the article.**

**Key words**
**Morphological variation, Morphometrics, Ecomorphology, Cyprinids, Fishery management unit.**

## INTRODUCTION

Beardless barb, *Cyclocheilichthys apogon* (Valenciennes, 1842) is an important food fish particular in lower Mekong region. By having beautiful body colour and patterns, this fish is popular in aquaculture as the ornamental fish (Rainboth, 1996; Vidthayanon, 2012). Thedistribution of this fish species is throughout southeast Asia (Kottelat, 2001; Vidthayanon, 2012). It is usually found in a various habitats from small pond to large lake (Rainboth, 1996) as well as small- throughout large-sized rivers (Kottelat, 2001). The wide range of distribution of this fish probably indicate the formation of stock units based on different ecological conditions of each separated habitats (Akbarzadeh *et al*., 2009).

Understanding of population structure is an important consideration in developing plans for effective fishery management programmes (Cronin-Fine *et al*., 2013; Hoggarth, 2006). Each population stocks usually have specific biological attributes that must be taken into account in fishery management (Secor, 2014). The appropriate management plans for each fishery stock will yield high production (Begg *et al*., 1999) as well as protect population diversity for further fishery use (Turan, 2004). Lacking of knowledge on population biology can also be problematic issues such as the loss of genetic diversity, the decrease of population, and overfishing (Begg *et al*., 1999; Smith *et al*., 1991).

Many techniques are applied for understanding population structure in many fish species in order to plan for further beneficial use (Cardin *et al*., 2005; Hoggarth, 2006). Morphometric analysis especially the truss network technique is frequently used in identification of stock and population structure in various animals including fish (Cardin *et al*., 2005; Pazhayamadom *et al*., 2015). The basis of truss network method involved with the measurements of distances between anatomical landmarks forming and reticular network covering the entire fish body (Strauss and Bookstein, 1982; Turan, 1999). The effective in capturing morphological variations regarding shape

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variability rather than traditional measurement (Cavalcanti *et al*., 1999) has made the truss network method to become in use more frequently in describing and identifying morphological variability of intraspecific fish groups (Mir *et al*., 2013; Pazhayamadom *et al*., 2015).

The objectives of this study were to examine morphological variations of *C. apogon* habiting in different geographical locations in north-eastern Thailand. A truss network morphometric method was selected and applied on the fish external morphology to provide meaningful information in an identification of fishery units for further use in conservation and management purposes.

## MATERIALS AND METHODS

*Sample collection*

Samples of *C. apogon* were collected in total of 291 individual fish from six populations of three-different rivers in north-eastern Thailand (Table I, Fig. 1). Two populations of 19 and 37 fish from Ubolratana dam (URD1 and URD2) were in reservoir locating along the river Pong. Three populations of 37, 77 and 31 fish from Kaeng Lawa (KLW1 and KLW2) and Kaeng Nam Ton (KNT), and a population of 28 fish from Huai Chorakhe Mak (HCM) were collected from slow-moving water of the river Chi and Mun, respectively.

The fish samples were identified for the right type of fish species based on the identification key of Rainboth (1996) and Kottelat (2001). The identified fish were labelled with a specific code for further traceability and then kept in -20 °C prior for further analysis through truss network method.

*Morphometric measurement*

The identified fish samples were soaked in running-tap water to make their body soften before placing the right sides posture on a polystyrene board. The landmarks were defined on the basis of homologous points of the external morphology among the specimens with slightly modification from Armbruster (2012). The morphometric valuables were measured on the fish left side to the nearest 0.01 mm with digital callipers based on truss-network system (Strauss and Bookstein, 1982). Thirty-two morphometric variables and standard length (SL) were obtained from 14 anatomical landmarks (Fig. 2). The gender of specimen was identified by dissecting fish body to examine the gonads inside. The specimens were fix in 10% neutral buffered formalin for at least 7 days and changed to preserve in 70% ethanol for voucher-specimen collection.

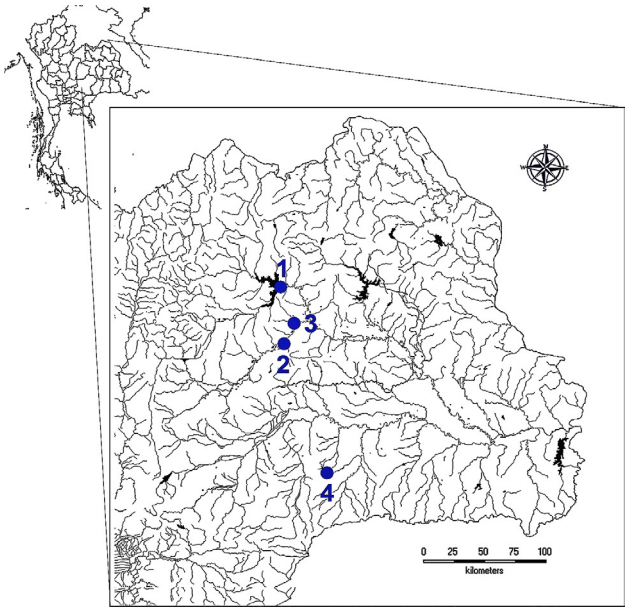


Fig. 1. Locations of sampling sites: 1-Ubolratana dam (URD), 2-Kaeng Lawa (KLW), 3-Kaeng Nam Ton (KNT) and 4-Huai Chorakhe Mak (HCM).

**Table I.- Sampling site localities, collection date, population code, and sample sizes of specimens used for this study.**

| River basin | Sampling sites | Geographical coordinate | Collection date | Population | Sample size | | |
|---|---|---|---|---|---|---|---|
| | | | | | F | M | total |
| Pong | Ubolrattana Dam, Ubolratana District, Khon Kaen Province | N 16 43.060 E 102 37.187 | November, 2012 | URD1 | 7 | 12 | 19 |
| | | | April, 2013 | URD2 | 22 | 15 | 37 |
| Chi | Kaeng Lawa, Ban Phai District, Khon Kaen Province | N 16 09.644 E 102 39.860 | April, 2013 | KLW1 | 55 | 15 | 37 |
| | | | November, 2013 | KLW2 | 45 | 29 | 77 |
| | Kaeng Nam Ton, Mueang District, Khon Kaen Province | N 16 23.746 E 102 45.903 | November, 2013 | KNT | 19 | 12 | 31 |
| Mun | Huai Chorakhe Mak, Mueang District, Burirum Province | N 14 54.293 E 103 01.270 | April, 2013 | HCM | 16 | 12 | 28 |
| | | | | | 164 | 127 | 291 |

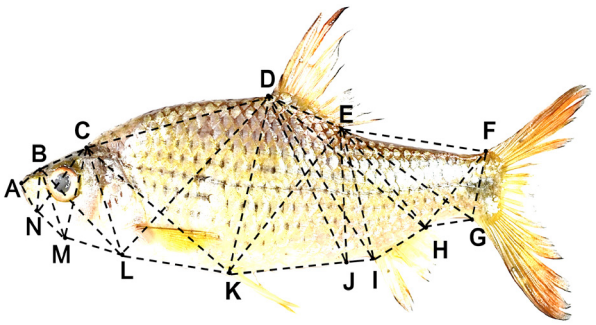


Fig. 2. Schematic image of beardless barb *Cyclocheilichthys apogon* showing 32 truss-network measurements constructed from 14 anatomical landmarks.

*Statistical analysis*

All measured variables was subjected to an allometric transformation equation (Elliott *et al*., 1995; Reist, 1985) in order to get rid of size-dependent variation from shape information:

$$M_{adj} = M\,(L_s/L_0)^b$$

Where, $M_{adj}$ is the size-adjusted measurement, $M$ is the original measurement, $L_0$ is the standard length of the fish specimen, $L_s$ is the overall mean of standard length for all fish from all samples in each analysis, and $b$ is an adjusted coefficient estimated from the observed data as the slope of *log M* against *log* $L_0$ using all fish in each group. The transformation efficiency was confirmed by testing the correlation significances between the adjusted variables and standard length (Turan, 1999).

Multivariate analysis of variance (MANOVA) was performed in order to evaluate the statistically significant difference between sex and among populations. In addition, a univariate analysis of variance (ANOVA) was performed to examine the statistical differences of each morphometric character for sexual and population effects, respectively. The significant characters ($p$<0.05) were then subjected to subsequently statistical analyses.

Principal component analysis (PCA) was then implied in order to elucidate patterns of morphological variations between sexes and among populations. The PCA can use for reducing redundancy among the variables and in extracting sets of independent variables that meaningful contributed with morphological differentiation. The univariate *t*-test and ANOVA were applied to the loading scores of PCA in order to determine significant differences in patterns of morphological variations between sexes and among populations, respectively.

Linear discriminant function analysis (DFA) was executed to predict and classify each specimen to their respective populations based on their morphometric features. Furthermore, the percentages of correct classification were calculated and a cross-validated test was performed to estimate the expected actual error rates of the classification.

All statistical analyses were performed using computer programme R version 3.2.1 (R Core Team, 2016).

## RESULTS

According to the allometric transformation, the correlation analysis revealed no significant correlation

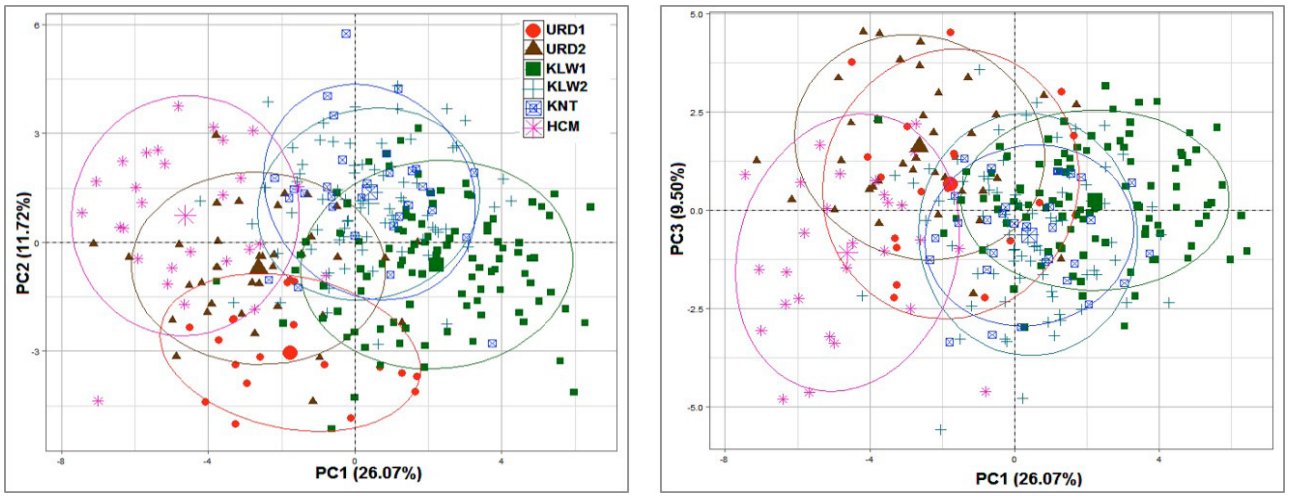


Fig. 3. Bivariate scatterplots of *Cyclocheilichthys apogon* individuals along PC1-PC2 and PC1-PC3 axes of principal component analysis (PCA) for testing morphological variations among populations.

between transformed truss measurements and standard length (SL) ($r$ <0.03, $p$>0.05; data not shown). There was an evidence which indicated that size-dependent effects had been efficiently removed from shape information.

Multivariate analysis of variance (MANOVA) on transformed truss variables (Table II) presented significant differences between sexes ($p$<0.01) and populations ($p$<0.01), whereas interaction effect of sex and populations was not significantly different ($p$>0.05).

*Patterns of morphological variability among populations*

Univariate ANOVA demonstrated that all transformed variables were significantly different between populations (Table III), and the PCA also revealed the first three principal components (PC1 – PC3) accounted for 47.29% of total variation (Table IV). The PC1 accounted for 26.07% of total variation, whereas the PC2 and PC3 explained 11.72 and 9.50% of total variation, respectively.

**Table II.- Results of multivariate analysis of variance (MANOVA) on transformed truss-network variables for testing effects of sex and population on morphometric variability.**

| | d.f. 1 | d.f. 2 | Wilk's | F-value |
|---|---|---|---|---|
| Sex | 1 | 32 | 0.68197 | 3.6141 ** |
| Populations | 5 | 160 | 0.00634 | 13.7077 ** |
| Sex × Population | 5 | 160 | 0.52947 | 1.0561 |

**, highly significantly different ($p$<0.01).

**Table III.- Descriptive statistic ($\bar{X}$ ± S.D.) of each truss-network measurement of female and male *Cyclocheilichthys apogon*, and *F*-statistics of ANOVA for testing morphological variations among populations.**

| Var. | Descriptive statistic ($\bar{X}$ ± S.D.) | | | | | | ANOVA |
|---|---|---|---|---|---|---|---|
| | URD1 (n=19) | URD2 (n=37) | KLW1 (n=99) | KLW2 (n=77) | KNT (n=31) | HCM (n=28) | (F-value) |
| AB | 6.08 ± 0.67 | 5.22 ± 0.49 | 6.08 ± 0.67 | 5.24 ± 0.61 | 4.59 ± 0.59 | 4.62 ± 0.43 | 23.496** |
| AN | 6.03 ± 0.70 | 6.04 ± 0.65 | 6.03 ± 0.70 | 5.38 ± 0.62 | 5.34 ± 1.16 | 5.14 ± 0.47 | 48.049** |
| BC | 11.24 ± 1.33 | 11.17 ± 0.73 | 11.24 ± 1.33 | 11.21 ± 1.11 | 9.69 ± 1.16 | 10.12 ± 0.78 | 6.9692** |
| BL | 20.86 ± 2.10 | 18.52 ± 1.48 | 20.86 ± 2.10 | 20.31 ± 2.00 | 18.10 ± 1.94 | 17.60 ± 1.80 | 10.17** |
| BM | 13.09 ± 1.32 | 11.84 ± 0.85 | 13.09 ± 1.32 | 12.27 ± 1.11 | 11.25 ± 1.57 | 11.00 ± 0.92 | 16.823** |
| BN | 8.86 ± 0.92 | 8.22 ± 0.64 | 8.86 ± 0.92 | 7.99 ± 0.84 | 7.17 ± 1.00 | 7.10 ± 0.71 | 29.434** |
| CD | 42.87 ± 4.54 | 39.31 ± 2.54 | 42.87 ± 4.54 | 40.48 ± 3.74 | 35.43 ± 4.41 | 34.71 ± 3.10 | 9.8771** |
| CK | 42.01 ± 4.65 | 38.03 ± 2.69 | 42.01 ± 4.65 | 39.56 ± 3.72 | 33.90 ± 4.22 | 33.29 ± 3.01 | 14.255** |
| CL | 20.69 ± 2.03 | 19.08 ± 1.34 | 20.69 ± 2.03 | 19.94 ± 1.71 | 17.23 ± 2.07 | 17.53 ± 1.46 | 13.858** |
| CM | 18.42 ± 1.84 | 17.34 ± 1.12 | 18.42 ± 1.84 | 17.41 ± 1.45 | 14.99 ± 1.91 | 15.89 ± 1.17 | 19.11** |
| CN | 17.41 ± 1.71 | 16.52 ± 0.99 | 17.41 ± 1.71 | 16.50 ± 1.34 | 14.19 ± 1.62 | 14.88 ± 1.05 | 12.462** |
| DE | 17.33 ± 1.67 | 16.37 ± 0.76 | 17.33 ± 1.67 | 16.65 ± 1.58 | 15.01 ± 1.61 | 14.85 ± 0.90 | 2.9346* |
| DH | 45.47 ± 4.46 | 42.68 ± 2.18 | 45.47 ± 4.46 | 43.93 ± 3.74 | 38.28 ± 4.83 | 38.81 ± 2.57 | 25.934** |
| DI | 41.26 ± 4.27 | 39.47 ± 2.28 | 41.26 ± 4.27 | 39.52 ± 3.67 | 34.42 ± 4.42 | 34.85 ± 2.60 | 62.991** |
| DJ | 39.64 ± 4.16 | 37.73 ± 2.47 | 39.64 ± 4.16 | 38.04 ± 3.53 | 33.27 ± 4.57 | 33.48 ± 2.73 | 50.486** |
| DK | 38.21 ± 4.43 | 35.36 ± 2.49 | 38.21 ± 4.43 | 36.30 ± 3.62 | 31.63 ± 4.49 | 31.62 ± 3.04 | 25.636** |
| DL | 49.84 ± 5.01 | 46.51 ± 3.17 | 49.84 ± 5.01 | 46.78 ± 4.21 | 40.89 ± 5.32 | 40.95 ± 3.12 | 29.192** |
| EF | 33.08 ± 3.70 | 30.55 ± 2.39 | 33.08 ± 3.70 | 31.21 ± 2.74 | 27.98 ± 3.89 | 28.01 ± 2.95 | 14.671** |
| EG | 37.82 ± 4.22 | 35.09 ± 2.14 | 37.82 ± 4.22 | 36.12 ± 2.95 | 31.80 ± 4.51 | 31.64 ± 2.76 | 16.343** |
| EH | 29.25 ± 3.25 | 27.40 ± 1.65 | 29.25 ± 3.25 | 28.65 ± 2.37 | 24.30 ± 3.57 | 24.95 ± 2.15 | 30.448** |
| EI | 28.19 ± 3.20 | 27.08 ± 1.68 | 28.19 ± 3.20 | 27.50 ± 2.50 | 23.30 ± 3.34 | 23.79 ± 2.11 | 55.531** |
| EJ | 28.59 ± 3.20 | 27.20 ± 1.81 | 28.59 ± 3.20 | 27.92 ± 2.54 | 23.84 ± 3.61 | 24.21 ± 2.26 | 50.031** |
| EK | 38.83 ± 4.49 | 35.84 ± 2.36 | 38.83 ± 4.49 | 37.60 ± 3.57 | 32.91 ± 4.84 | 32.77 ± 2.86 | 40.696** |
| FG | 13.46 ± 1.58 | 12.95 ± 0.81 | 13.46 ± 1.58 | 13.14 ± 1.22 | 11.22 ± 1.48 | 11.23 ± 0.92 | 44.872** |
| FH | 19.86 ± 2.25 | 18.36 ± 1.67 | 19.86 ± 2.25 | 19.14 ± 1.76 | 16.69 ± 2.14 | 16.29 ± 1.58 | 10.045 ** |
| GH | 12.65 ± 1.72 | 11.22 ± 1.21 | 12.65 ± 1.72 | 11.73 ± 1.25 | 10.79 ± 1.62 | 9.84 ± 1.13 | 2.3604 * |
| HI | 12.87 ± 1.19 | 11.70 ± 0.96 | 12.87 ± 1.19 | 12.50 ± 1.24 | 10.94 ± 1.41 | 11.13 ± 0.66 | 2.7719 * |
| IJ | 5.54 ± 0.84 | 5.26 ± 0.69 | 5.54 ± 0.84 | 5.08 ± 0.80 | 4.42 ± 0.88 | 4.77 ± 0.49 | 21.675 ** |
| JK | 26.17 ± 3.43 | 24.39 ± 2.63 | 26.17 ± 3.43 | 25.29 ± 2.74 | 22.96 ± 4.02 | 22.61 ± 2.07 | 35.051 ** |
| KL | 30.58 ± 3.45 | 28.16 ± 2.55 | 30.58 ± 3.45 | 27.98 ± 2.95 | 23.92 ± 3.24 | 23.57 ± 2.08 | 16.076 ** |
| LM | 10.52 ± 1.36 | 8.82 ± 1.24 | 10.52 ± 1.36 | 10.31 ± 1.46 | 8.74 ± 1.05 | 8.89 ± 1.55 | 9.3031 ** |
| MN | 5.85 ± 0.74 | 4.79 ± 0.70 | 5.85 ± 0.74 | 5.72 ± 0.68 | 5.11 ± 0.81 | 5.08 ± 0.48 | 25.95 ** |

*, significant difference ($p$<0.05); **, highly significant difference ($p$<0.01).
AB, snout length; AN, mouth length; BC, forehead length; BL, BM, BN, CL, CM, CN, head depth; CD, pre-dorsal length; CK, diagonal body depth; DE, dorsal fin-base length; DH, DI, DJ, DK, DL, diagonal depth of foretrunk; EF, post-dorsal length; EG, EH, diagonal length of caudal peduncle; EI, EJ, EK, diagonal depth of post-trunk; FG, precaudal depth; FH, diagonal length of caudal peduncle; GH, caudal peduncle length; HI, anal fin-base length; IJ, JK, abdomen length; KL, pectoral length; LM, MN, lower head length.

The bivariate plots of PCA also showed some separations of morphological variations among populations (Fig. 3). Populations URD1, URD2 and HCM distributed on the negative side of PC1 and tended to separate from population KLW1, KLW2 and KNT which distributed on the positive PC1 axis. Populations URD1 and URD2 were also separated from population HCM by PC2.

In addition, the ANOVA on loadings scores of the first three PCs indicated significant differences in patterns of morphological variations among populations (Fig. 4). The pairwise-multiple comparisons showed that PC1 (Fig. 4a) grouped the samples into four groups including URD1 and URD2; KLW1; KLW2 and KNT; and HCM. These variations associated with the variations in head depth (CM), forepart body length (DL), hind-part body length (DH, DI, DJ, EJ, EK), body depth (DK, EH), caudal peduncle length (EG, EH) and caudal peduncle depth (FG).

The second index (Fig. 4b), PC2 clustered samples into three groups including URD1 and KLW1, URD2, and KLW2, KNT and HCM. Such groupings have morphological differentiations in head characters (AN, BN, CM, CN), body depth (EH) and caudal peduncle length (EG).

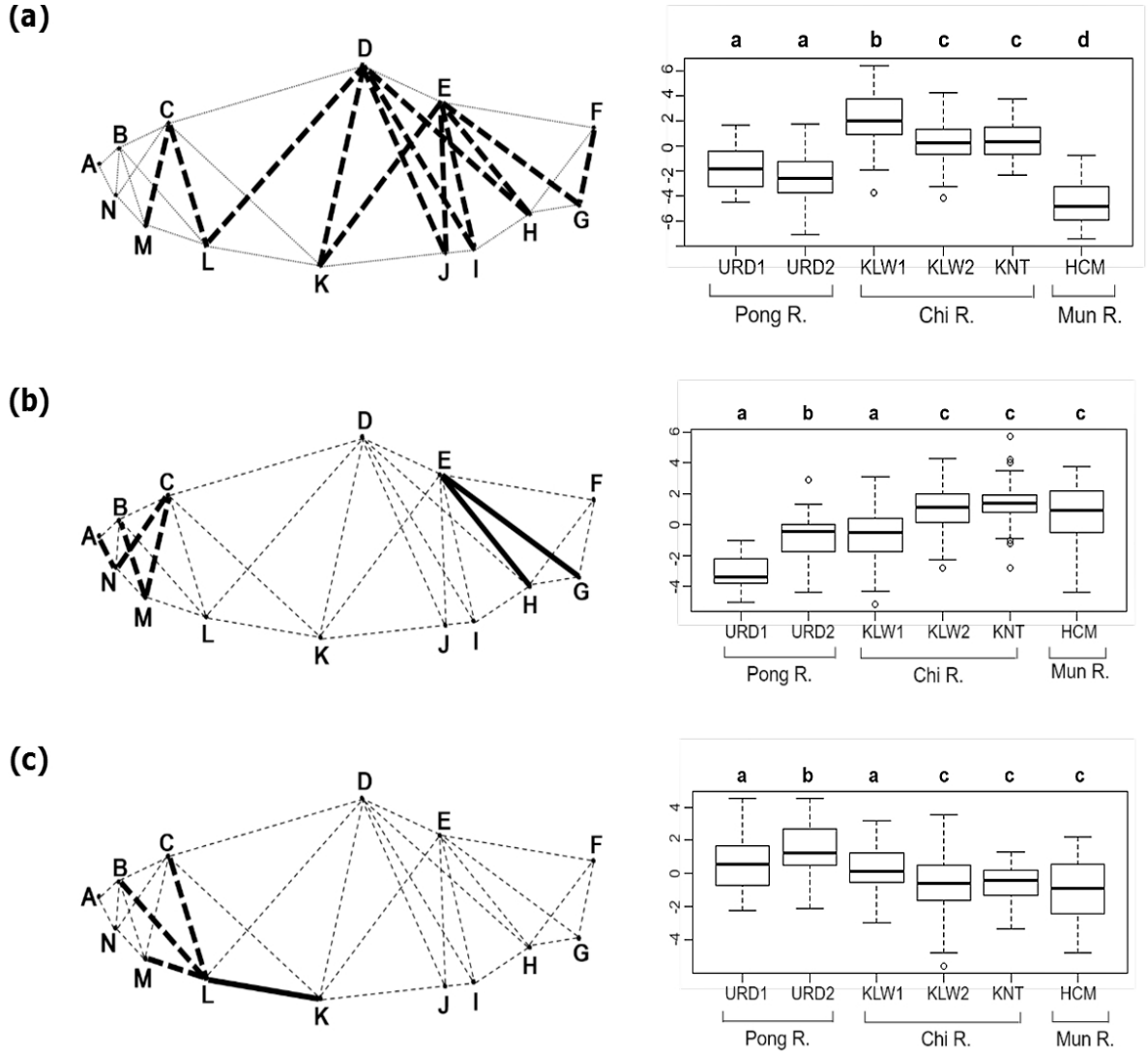


Fig. 4. Patterns of morphological variation derived from the first three principal components (PC1-PC3) of principal component analysis (PCA) and multiple-pairwise comparisons for testing morphological variabilities among populations of *Cyclocheilichthys apogon*.
For abbreviations, see Table III

The PC3 yielded population separations similar to PC2 (Fig. 4c) in correlation with the variations in head features (BC, CL, LM), thoracic length (KL) and forepart body length (DL).

**Table IV.- Results of principal component analysis (PCA) and analysis of variance (ANOVA) of factors scores for investigating morphological variations among populations.**

| Variables | Factor loadings | | |
|---|---|---|---|
| | PC1 | PC2 | PC3 |
| AB | 0.230 | -0.377 | 0.022 |
| AN | -0.244 | -0.598 | 0.028 |
| BC | -0.325 | -0.268 | 0.086 |
| BL | -0.172 | -0.223 | -0.896 |
| BM | -0.311 | -0.428 | -0.173 |
| BN | -0.172 | -0.746 | 0.051 |
| CD | -0.400 | -0.005 | -0.062 |
| CK | -0.412 | -0.379 | 0.060 |
| CL | -0.516 | -0.411 | -0.612 |
| CM | -0.479 | -0.570 | 0.072 |
| CN | -0.310 | -0.615 | 0.160 |
| DE | -0.092 | -0.209 | -0.021 |
| DH | -0.768 | 0.239 | -0.019 |
| DI | -0.875 | -0.049 | 0.105 |
| DJ | -0.892 | 0.095 | 0.004 |
| DK | -0.797 | -0.086 | -0.121 |
| DL | -0.615 | -0.212 | 0.414 |
| EF | -0.435 | 0.422 | 0.135 |
| EG | -0.583 | 0.477 | 0.101 |
| EH | -0.718 | 0.467 | -0.010 |
| EI | -0.898 | 0.209 | 0.082 |
| EJ | -0.871 | 0.241 | 0.010 |
| EK | -0.713 | 0.087 | -0.208 |
| FG | -0.662 | 0.007 | 0.108 |
| FH | -0.295 | 0.085 | 0.163 |
| GH | 0.088 | 0.088 | 0.118 |
| HI | 0.157 | -0.007 | -0.109 |
| IJ | -0.123 | -0.360 | 0.179 |
| JK | -0.411 | 0.220 | -0.189 |
| KL | -0.025 | -0.308 | 0.796 |
| LM | 0.011 | -0.118 | -0.844 |
| MN | 0.116 | 0.417 | -0.168 |
| EigenValue | 8.3421 | 3.7506 | 3.3090 |
| % variation | 26.07% | 11.72% | 9.50% |
| **F-value** | **81.03**** | **32.00**** | **15.09**** |

**, highly significantly different ($p$<0.01).
For abbreviations, see Table III

*Discrimination of population using morphometric characteristics*

The discriminant function analysis (DFA) revealed five discriminant functions which could be used as morphological descriptors for classifying the samples into their own groups (Table V). The first two discriminant functions which were meaningful for DFA (Eigenvalue > 1) accounted for 72% of total variation among population. The first discriminant function (DF1) accounted for 49.33% of total variation. The measurements from head (AN, BN, LM) and hind-part body length (EL, HI) highly

**Table V.- Structure matrix of discriminant functions obtained from discriminant function analysis (DFA) on truss-network variables.**

| Variables | Discriminant function (DF) | | | | |
|---|---|---|---|---|---|
| | DF1 | DF2 | DF3 | DF4 | DF5 |
| AB | -0.092 | -0.166 | 0.292 | -0.345 | -0.356 |
| AN | 0.616 | -0.129 | -0.491 | -0.149 | -0.025 |
| BC | 0.156 | 0.036 | 0.164 | -0.025 | 0.329 |
| BL | 0.111 | 0.251 | -0.117 | 0.407 | -0.288 |
| BM | 0.331 | 0.206 | -0.387 | 0.056 | -0.309 |
| BN | 0.561 | -0.255 | -0.107 | -0.119 | -0.265 |
| CK | 0.344 | 0.219 | 0.077 | -0.030 | -0.101 |
| CL | 0.429 | 0.091 | 0.276 | 0.095 | -0.159 |
| CM | 0.304 | 0.300 | 0.228 | 0.182 | -0.097 |
| CN | 0.375 | 0.198 | 0.365 | -0.179 | -0.059 |
| DE | 0.317 | -0.095 | 0.351 | -0.160 | 0.037 |
| DJ | 0.133 | -0.089 | -0.080 | -0.153 | 0.185 |
| DK | 0.233 | 0.441 | 0.192 | -0.064 | 0.296 |
| DL | 0.517 | 0.375 | 0.197 | -0.264 | 0.328 |
| EF | 0.220 | 0.584 | 0.195 | -0.339 | 0.374 |
| EG | 0.164 | 0.581 | 0.149 | -0.163 | 0.090 |
| EH | 0.424 | 0.224 | 0.198 | -0.393 | 0.096 |
| EI | 0.146 | 0.490 | -0.002 | -0.112 | -0.050 |
| EJ | 0.148 | 0.461 | -0.004 | -0.038 | 0.161 |
| EK | 0.071 | 0.526 | 0.277 | 0.107 | 0.249 |
| FG | 0.345 | 0.471 | 0.297 | -0.044 | 0.393 |
| FH | 0.156 | 0.610 | 0.258 | -0.051 | 0.363 |
| GH | 0.072 | 0.681 | -0.005 | 0.051 | 0.185 |
| HI | 0.508 | 0.176 | 0.119 | 0.082 | 0.419 |
| IJ | 0.337 | 0.154 | -0.015 | 0.194 | 0.047 |
| JK | 0.087 | -0.057 | -0.258 | 0.063 | -0.055 |
| KL | -0.118 | -0.172 | 0.011 | 0.099 | -0.052 |
| LM | 0.592 | -0.124 | 0.011 | 0.136 | -0.303 |
| MN | -0.020 | 0.639 | -0.216 | -0.126 | 0.234 |
| Eigenvalue | 3.8967 | 1.8510 | 0.9886 | 0.6947 | 0.4681 |
| % variation | 49.33% | 23.43% | 12.52% | 8.79% | 5.93% |

For abbreviations, see Table III

**Table VI.- Percentage of specimens classified in each group from original and cross-validation tests of discriminant function analysis (DFA) on truss-network data.**

| Predicted populations | Original populations | | | | | | Global accuracy |
|---|---|---|---|---|---|---|---|
| | URD1 | URD2 | KLW1 | KLW2 | KNT | HCM | |
| Original test | | | | | | | |
| URD1 | **100** | 0 | 0 | 0 | 0 | 0 | |
| URD2 | 0 | **97.30** | 0 | 2.70 | 0 | 0 | |
| KLW1 | 0 | 0 | **95.96** | 4.04 | 0 | 0 | **96.29** |
| KLW2 | 0 | 1.30 | 6.49 | **90.91** | 1.30 | 0 | |
| KNT | 0 | 0 | 6.45 | 0 | **93.55** | 0 | |
| HCM | 0 | 0 | 0 | 0 | 0 | **100** | |
| **Cross-validation test** | | | | | | | |
| URD1 | **100.00** | 0 | 0 | 0 | 0 | 0 | |
| URD2 | 2.70 | **91.90** | 0 | 2.70 | 0 | 2.70 | |
| KLW1 | 0 | 2.02 | **88.89** | 6.06 | 2.02 | 1.01 | **90.59** |
| KLW2 | 0 | 1.30 | 7.79 | **89.61** | 1.30 | 0 | |
| KNT | 0 | 0 | 9.68 | 6.45 | **83.87** | 0 | |
| HCM | 0 | 3.57 | 0 | 3.57 | 3.57 | **89.29** | |

For abbreviations, see Table III

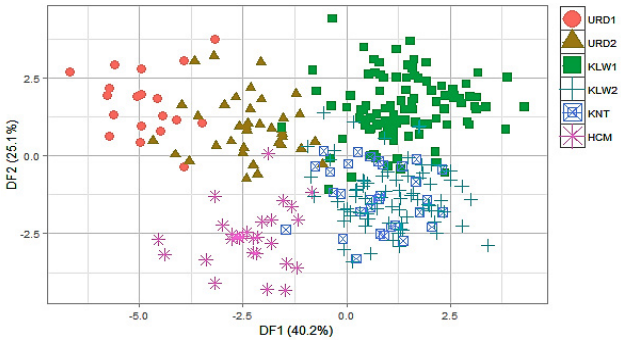


Fig. 5. Discrimination plot of *Cyclocheilichthys apogon* individuals along DF1-DF2 axes of discriminant function analysis (DFA) on truss-network variables showing three separated groupings of the samples according to their river drainages.

contributed to DF1. The second discriminant function (DF2) explained 23.43% of total variation, and this function highly correlated with the measurements from head (MN), body depth (EJ), hind-part body length (EI, EK), and caudal peduncle traits (EF, EG, GH, FG, GH). It indicated that all of those variables were important in discrimination of morphological variation in *C. apogon* populations.

The discriminant plot of fish individuals on DF1-DF2 axes (Fig. 5) distinguished samples into three groups regarding river drainage systems of the collecting sites including URD (Pong River basin), KLW and KNT (Chi River basin), and HCM group (Mun River basin).

The global accuracy of classification was 96.29% for original classification and 90.56% for cross-validated test (Table VI). The correct classification rate was highest in population KLW1 (100% for both original and cross-validated tests). For the original test, the corrected classification rates ranged from 90.91 – 100%, and the highest misclassification was the misclassification of population KLW2 into KLW1 (6.49%). For the cross-validated test, the corrected classification ranged from 83.87% - 100%, and the highest misclassification is the classification of KNT into KLW1 (9.68%). These misclassifications may indicate the morphological similarities of the *C. apogon* collected from same river drainage.

## DISCUSSION

The morphological variation of *C. apogon* in the present study occurred in both between different sexes and among population. These findings are consistent to many

previous studies in fish such as the rohu labeo *Labeo rohita* from Ganga basin in India (Mir *et al*., 2013), silver perch *Leiopotheron plumbeus* from three lakes in the Philippines (Quilang *et al*., 2007), Günther's Mouthbrooder *Chromidotilapia guntheri* from three coastal rivers of Africa (Boussou *et al*., 2010), the three populations of orange-fin labeo *Labeo cabasu* from two isolate rivers in Bangladesh (Hossain *et al*., 2010) and spotted snakehead *Channa punctatus* from three Indian rivers (Khan *et al*., 2013).

The results of the present work were also indicative of geographical isolation according river drainage systems of the collecting sites: Pong River (URD populations), Chi River (KLW and KNT populations), and Mun River (HCM population). Most of the fish samples (96.29 and 90.56% with original and cross-validation tests, respectively) were correctly classified into their respect locations by DFA (Table VI) and the discrimination plots of each individual along discriminant axes showed quite separation regarding river isolation (Fig. 5).

Interestingly, morphological similarity was observed in KLW2 and KNT which are geographically isolated populations. This observation may be due to the morphological plasticity to the similar ecological impacts (Mir *et al*., 2013), or due to local migration of the fish between connected locations (Hossain *et al*., 2010; Khan *et al*., 2013). On the other hand, this finding suggest that an insufficient degree of geographical isolation might not be involved the formation of different stock especially if the ecological conditions of the habitats are quite similar.

Regarding the large degree of morphological variation between populations obtaining from the same locality at different times (URD1-URD2 and KLW1-KLW2), significant morphometric differences between those two pairs of populations were observed and resulted in a high correct reclassification rate of each populations (Table VI, Fig. 5). These findings provide further evidence for the complexity of the stock structure within that locations (Zhang *et al*., 2016). The separation of URD1 and URD2 samples may possibly due to isolation of portion of populations within large local habitat area (Mir *et al*., 2013; Turan *et al*., 2004) that may be sufficient to enforce populations to adapt and involve as independent biological entities with specific phenotypes in different ecological conditions (Turan *et al*., 2004). In addition, the differences may also be attributed to spatial variation in environment factors varying in different season during the year. Hydrological regime is considered the key factor driving ecological functioning in river floodplain system (Bunn and Arthington, 2002; Thomaz *et al*., 2007). The water level and water current affecting by differential flooding cycles are causally related to ecosystem attributes in the habitat especially food availability (Cochran-Biederman and Winemiller, 2010; Thomaz *et al*., 2007) which will be affected biological parameters of populations, leading to the differentiation in morphology among that populations.

The plots of the first three principal component axes of PCA (Fig. 4) also confirmed a high degree of morphological variations. The subsequently observed differences in morphology were significantly in overall body shape from head to tail. The variation of size and shape was usually occurred in fish more than other vertebrates and were considered as the involvement of environmental influences (Cadrin, 2000; Wimberger, 1992). Such variations in body depth and caudal peduncle characteristics could possibly be related to environmental conditions in relation to water depth and current flow (Pazhayamadom *et al*., 2015). The adaptation in body depth and caudal peduncle traits may be associated with swimming performance (Boily and Magnan, 2002; Peres-Neto and Magnan, 2004; Webb, 1984), which could also be related to foraging efficiency (Boily and Magnan, 2002; Swain *et al*., 2005) and predator evasiveness (Chipps *et al*., 2004; Swain *et al*., 2005). Adaptation with deep robust body is required for attain faster burst velocity with transient propulsion in the less turbulent water, while the shallow body depth is optimal for periodic propulsion against fast-following water currents (Blake *et al*., 2005; Webb, 1984). The variability in the head parts which reflected for a differential habitat use (Boily and Magnan, 2002; Robinson and Wilson, 1994; Wainwright, 1996; Webster *et al*., 2011), especially regarding the feeding regimes with variable diets (Berchtold *et al*., 2015; Hyndes *et al*., 1997; Wainwright and Richard, 1995). In addition, variation in the head morphology will also attributed to water parameters and current velocity (Langerhans *et al*., 2007). It is well known that the phenotypic plasticity allows fish to adaptively react to environmental changes for fitness by modifications in their physiology and behaviour, which lead to changes in morphology, reproduction and survival (Turan *et al*., 2004). Variations of environmental factors such as water current, flooding patterns, water turbidity, and food availability could also be involved as particular factors in morphological variations during the early development stages when the individual's trait is more susceptible to environment influences (Wimberger, 1992).

Apparently, there is possibility that morphological variabilities among geographically isolated populations observed in the recent work may be correlated to genetic differentiation. Since the fragmentation of habitat localities that prevent the genetic exchange among populations designates the enrichment of the established genetic differences resulting a heighten degree of inter-

population differences (Cadrin, 2000; Poulet *et al*., 2004). The relationship of morphological variation and genetic difference was explained in several fish including the pikeperch *Sander lucioperca* from a fragmented delta (Poulet *et al*., 2004) and Swedish postglacial stickleback *Pungitius pungitius* from coastal and inland lakes (Mobley *et al*., 2011). The correlation between genetic and morphological variations also supported the existence of distinct population groups of *Moenkhausia oligolepis* from different tributaries (Domingos *et al*., 2014).

However, morphological variations observed in isolated populations in the recent study may not be involved with genetic differentiation as in *Coilia ectenes* populations from three-isolated lakes that found no obviously genetic variation in relation to geographical differentiation (Xie, 2012). In contrary with the study of Eurasian perch, *Perca fuuviatilis* that showed the difference of morphology between literal and pelagic populations more closely related to the environmental adaptation than genetic variation (Svanbäck and Eklöv, 2006).

The present study suggests that the extent of morphological divergence probably related to ecological differences which also related to the distances of geographical isolation. The variation of *C. apogon* will be resulted from phenotypic plasticity that allows the fish to consequently suitable to the environmental conditions of the habitats (Wimberger, 1992). A sufficient degree of isolation, which can arise because of geographical distant or flood cycle of the river, will result in notable morphological differentiation as well as genetic variability between stock of *C. apogon* (Cronin-Fine *et al*., 2013; Turan, 2004). An effective planning for fishery management should establish separately based on isolate stock function as basic unit (Begg *et al*., 1999; Cardin *et al*., 2005; Hoggarth, 2006). A failure to account for fishery stock can lead to erosion of biological attributes of population, which would be subsequently accelerated a loss of genetic diversity and a potential decrease of fishery productivity of the species resource (Begg *et al*., 1999; Sterner, 2007; Zhang *et al*., 2016).

## CONCLUSION

The output of the study will provide useful baseline information of *C. apogon* for appropriate management and conservation of the species. The results indicated that fisheries management of *C. apogon* should be considered strategic planning independently along each of the river drainages. However, further study in genetic information is necessary to investigate correlation between morphological variation and genetic attributes of this species, resulting to sufficient information for sustainable utilisation of fishery resources.

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*Statement of conflict of interest*

Authors have declared no conflict of interest.

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