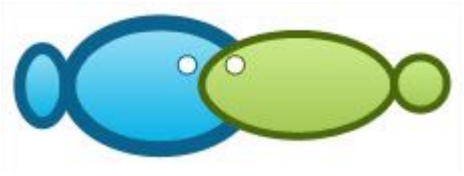

Evaluation of body shape variation of indigenous and exotic *Anabas testudineus* (Bloch, 1792) in Bangladesh by geometric morphometric analyses

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Abstract. Along with the native origin of *Anabas testudineus* in Bangladesh, this species originated from Thailand and Vietnam is also available for aquaculture in Bangladesh. The body shape variations of this species originated from three different regions have not been evaluated yet. In this study, geometric morphometry was used to identify the variations in body shape by delineating landmarks and analyzing the shape by interconnecting landmark distances. The landmark-based geometric morphometrics including 14 landmarks from each sample were analyzed by TPS software series and Past software version 3.0. The result showed 94.21% variance among Bangladeshi, Thai and Vietnamese *A. testudineus* in case of principal component 1 and highest variation was found in principal component 1. Subsequently, the result also showed 3.09% variance in case of principal component 2 and 1.93% variance in case of principal component 3. Females showed more variance compared to males (F, 94.24% vs M, 84.52%). In dendrogram, *A. testudineus* originated from Bangladesh formed a separate cluster whereas Thai and Vietnamese origin formed a sister cluster. This study confirms a considerable variation in shape exists in *A. testudineus* originated from three countries which would be useful to maintain purity of native stock in induced breeding programs.
Key Words: *Anabas testudineus*, landmark, shape variation, aquaculture.

Introduction. *Anabas testudineus* is a popular food fish in Bangladesh found in other South Asian countries such as India, China, Myanmar, Thailand, Vietnam, Pakistan, Sri-Lanka, Philippines and Malaysia (Hossain et al 2012). In Bangladesh, this species is locally known as koi, and it was abundantly available in the freshwater systems such as ponds, lakes, *beels*, small rivers, floodplains (Hussain 2010). Due to overexploitation, destruction of habitats, use of pesticides and degradation of breeding ground the availability of *A. testudineus* has declined (Hussain 2010). In the 1980s, this species contributed 2.8% of total inland fish production which decreased to 0.85% in 1999 (DoF 2000). Artificial breeding technique has been developed recently by Bangladesh Fisheries Research Institute but commercial aquaculture of *A. testudineus* has not flourished due to the less availability of hatchery produced seed and slower growth rate and lower feed conversion ratio (Roy et al 2013).

As the availability of *A. testudineus* decreased, the consumer demand and market price has increased manifold in the past two decades. To fulfill this demand, *A. testudineus* was introduced from Thailand in 2002 by Bangladesh Fisheries Research Institute and from Vietnam in 2010 by Sharnalata Agro Fisheries Limited (Hoque et al 2017). *A. testudineus* originated from Thailand and Vietnam has exhibited better growth rate compared to native species (Ahmed et al 2014; Uddin et al 2017). Currently *A. testudineus* originated from Thailand and Vietnam is extensively cultured in Bangladesh. Being a single species originated from three different countries, *A. testudineus* has morphological similarity. The market price of native *A. testudineus* is two to three folds

higher than the introduced strains. In addition, maintaining purity of a native stock is very important during controlled breeding programs. As a result, a clear identification of *A. testudineus* from Thailand, Vietnam and Bangladesh origin is very important.

Two important methods (a) variations in body shape by geometric morphometry and (b) molecular markers such as COI, Cytb, RAPD, RFLP, AFLP and microsatellite are commonly used to identify a species of different origin (Friedheim 2016). Even though molecular markers are reliable but costly, require sophisticated laboratory set up and skilled personnel (Changizi et al 2013). In comparison, geometric morphometry is a reliable method which is less sophisticated, cost-effective and suitable for field-based studies (Cavalcanti et al 1999). Landmarks refer to randomly selected points on a fish's body which are used to define the shape of individuals, and then variations among the shapes are delineated as separate stock or origin. The landmark based geometric morphometry has been used to know the variations among populations and stocks of different fish species including *Oncorhynchus kisutch* (Swain et al 1991), selected Serranid populations (Cavalcanti et al 1999), *Chalcalburnus chalcoides* (Bagherian & Rahmani 2007), *Trachurus mediterraneus* (Turan 2004). This study aims to know the variations in body shape of *A. testudineus* originated from Bangladesh, Thailand and Vietnam.

Material and Method

Study period and sample collection. The research works were conducted from July 2016 to June 2017 which included sample collection of three population, measurement and recording of data. Bangladeshi *A. testudineus* were collected from a local fisher in Dinajpur (25.70 N 88.68 E). Thai *A. testudineus* were collected from Saidpur substation of Bangladesh Fisheries Research Institute (25.47 N 88.56 E), and Vietnamese *A. testudineus* were collected from Sharnalata Agro Fisheries Limited, Mymensing (24.36 N 90.20 E). The sample size, sex ratio, standard lengths are presented in Table 1.

Table 1

Sample size, sex ratio, standard length of *A. testudienus* originated from Bangladesh,Thailand and Vietnam

Sample origin	*Sample size*	*Sex ratio (F vs M)*	*Standard length (female)*	*Standard length (male)*
Bangladesh	24	1.7:1	9.3-10.3	7.2-9.1
Thailand	25	1.3:1	15.6-20	9.9-14
Vietnam	24	2:1	11.7-14.6	8-13.1

Geometric morphometric data collection. Live fishes were anesthetized using clove oil. Then the left side of each sample was photographed using Cannon EOS 700D (DSLR-lens: 18-55 mm STM) camera. A total of 14 landmark points was selected, and then the distances among the landmarks were calculated using tpsDig2 software version 2.29 (Figure 1) that provides a truss network among landmark points. Afterwards, PAST (Paleontological Statistics Software Package) was used to delineate the variations among landmark points among different individuals.

Statistical analysis. Size variation among the individuals was eliminated using an allometric formula given by Elliott et al (1995): $M_{adj} = M(L_s/L_o)$, where M is the original measurement, M_{adj} is the size-adjusted measurement, L_o is the standard length of fish, L_s is the mean of standard lengths. To meet the assumptions of normality, Shapiro-Wilk test was performed. Then one-way ANOVA of landmark distances was conducted to determine the differences among Bangladeshi, Thai and Vietnamese *A. testudineus*. PCA helps to seek the bulkiness of the disparity and done to know the variation. A dendrogram was also constructed to visualize the phylogenetic relationship. All the data were analyzed using PAST (version 3.0).

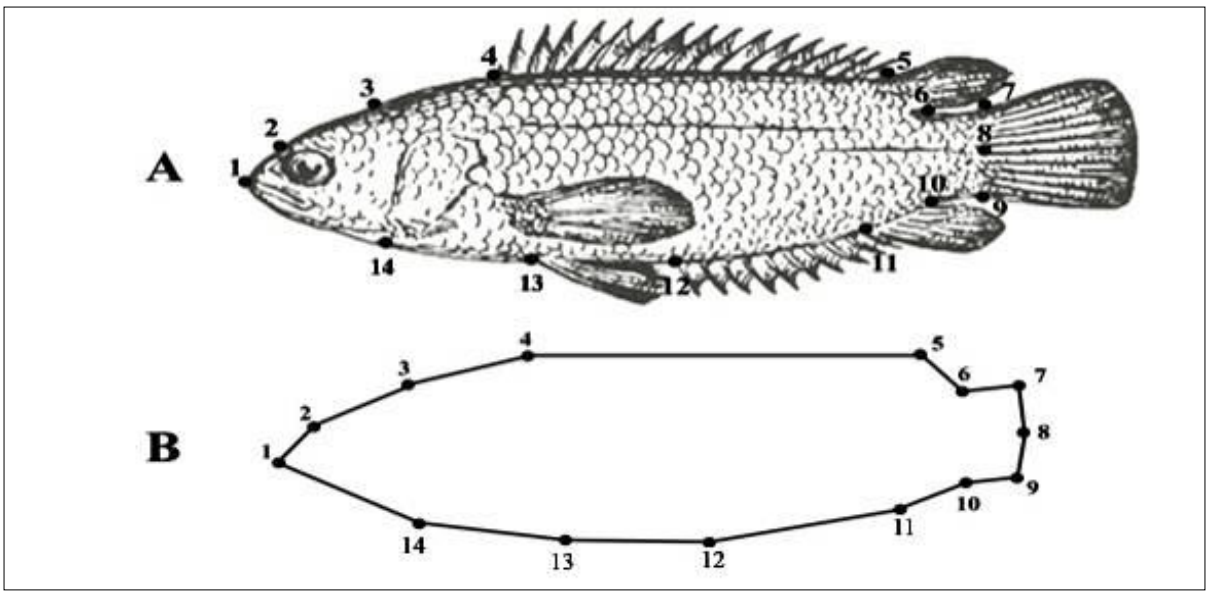

Figure 1. A. Position of 14 landmark points in fish, 1) anterior tip of upper jaw, 2) midpoint indication of eye, 3) forehead (ending of frontal bone), 4) origin of dorsal fin, 5) origin of dorsal soft rays, 6) end of dorsal fin, 7) origin of caudal fin (dorsal origin), 8) midpoint of caudal fin, 9) origin of caudal fin (ventral origin), 10) end of anal fin, 11) origin of anal soft rays, 12) origin of anal fin, 13) origin of pectoral fin, 14) down point indication of operculum. B. Showing truss network among landmark point.

Results. The principal component (PC) analysis revealed considerable variation among Bangladeshi, Thai and Vietnamese *A. testudineus*. The overall variation was estimated for a total of 28 principal components, in which PC 1 represented of 94.21% variation and 163.49 Eigenvalue (Table 2).

Table 2

Eigenvalues and percent variance for Bangladeshi, Thai and Vietnamese *A. testudineus*

PC No.	*Eigenvalue*	*% variance*	*PC No.*	*Eigenvalue*	*% variance*
1	163.49	94.21	15	0.01	0.01
2	5.36	3.09	16	0.01	0.01
3	3.36	1.93	17	0.01	0.01
4	0.59	0.34	18	0.01	0.01
5	0.24	0.14	19	0.01	0.00
6	0.12	0.07	20	0.01	0.00
7	0.10	0.06	21	0.01	0.00
8	0.07	0.04	22	0.00	0.00
9	0.05	0.03	23	0.00	0.00
10	0.03	0.02	24	0.00	0.00
11	0.02	0.01	25	0.00	0.00
12	0.02	0.01	26	0.00	0.00
13	0.01	0.01	27	0.00	0.00
14	0.01	0.01	28	0.00	0.00

In the scatter plot, *A. testudineus* of Bangladeshi origin plotted separately from Thai and Vietnamese origin, but Thai and Vietnamese *A. testudineus* overlapped (Figure 2).

Significant differences were found among Bangladeshi, Thai and Vietnamese *A. testudineus* by one-way ANOVA ($p < 0.05$, $F = 189.5$). Significant differences were also found in *A. testudineus* among the males ($p < 0.05$, $F = 109.2$) and among the females ($p < 0.05$, $F = 159.5$) originated from different countries. When males and females were analyzed separately, 28 principal components were identified in females where PC1 represented 94.24 % variation and 196.50 eigenvalue (Table 3).

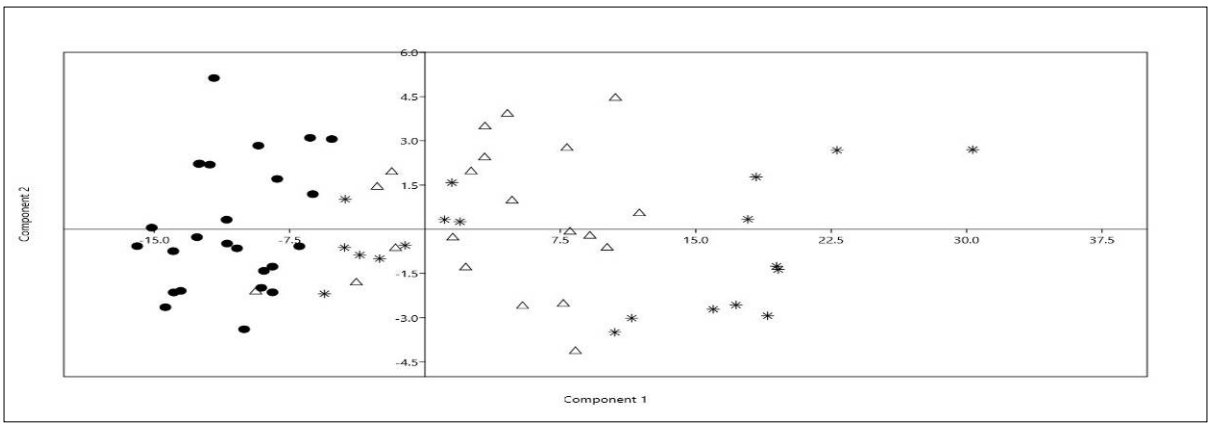

Figure 2. The scatter plot of Bangladeshi, Thai and Vietnamese *A. testudienus*; symbols refer to individuals in Bangladeshi-"Dot", Thai-"Star"; Vietnamese -"Triangle".

Table 3

Eigenvalues and percent variance for Bangladeshi, Thai and Vietnamese female *A. testudineus*

PC No.	*Eigenvalue*	*% variance*	*PC No.*	*Eigenvalue*	*% variance*
1	196.50	94.24	15	0.01	0.01
2	5.53	2.65	16	0.01	0.01
3	3.42	1.64	17	0.01	0.00
4	1.81	0.87	18	0.01	0.00
5	0.36	0.17	19	0.01	0.00
6	0.31	0.15	20	0.00	0.00
7	0.16	0.07	21	0.00	0.00
8	0.14	0.06	22	0.001	0.00
9	0.085	0.04	23	0.00	0.00
10	0.05	0.03	24	0.00	0.00
11	0.04	0.02	25	0.00	0.00
12	0.03	0.01	26	0.00	0.00
13	0.02	0.01	27	0.00	0.00
14	0.01	0.01	28	0.00	0.00

Among males, 25 principal components were identified where PC 1 represented 84.52% variation and 52.64 eigenvalue (Table 4).

Table 4

Eigenvalues and percent variance for Bangladeshi, Thai and Vietnamese male *A. testudineus*

PC No.	*Eigenvalue*	*% variance*	*PC No.*	*Eigenvalue*	*% variance*
1	52.64	84.52	14	0.01	0.01
2	6.92	11.12	15	0.01	0.01
3	1.64	2.63	16	0.00	0.01
4	0.51	0.81	17	0.00	0.01
5	0.23	0.37	18	0.00	0.00
6	0.11	0.17	19	0.00	0.00
7	0.07	0.12	20	0.00	0.00
8	0.04	0.07	21	0.00	0.00
9	0.03	0.04	22	0.00	0.00
10	0.02	0.04	23	0.00	0.00
11	0.01	0.03	24	0.00	0.00
12	0.01	0.02	25	0.00	0.00
13	0.01	0.02			

In the scatter plot, both female and male *A. testudineus* of Bangladeshi origin plotted separately from Thai and Vietnamese origin (Figures 3 and 4).

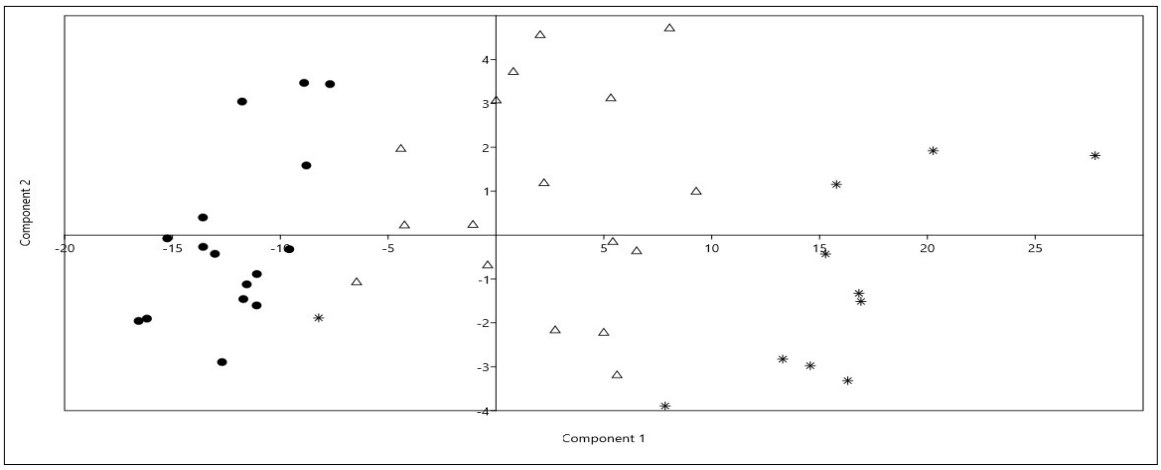

Figure 3. The scatter plot of Bangladeshi, Thai and Vietnamese female *A. testudienus*; symbols refer to individuals in Bangladeshi female-"Dot", Thai female -"Star"; Vietnamese female - "Triangle".

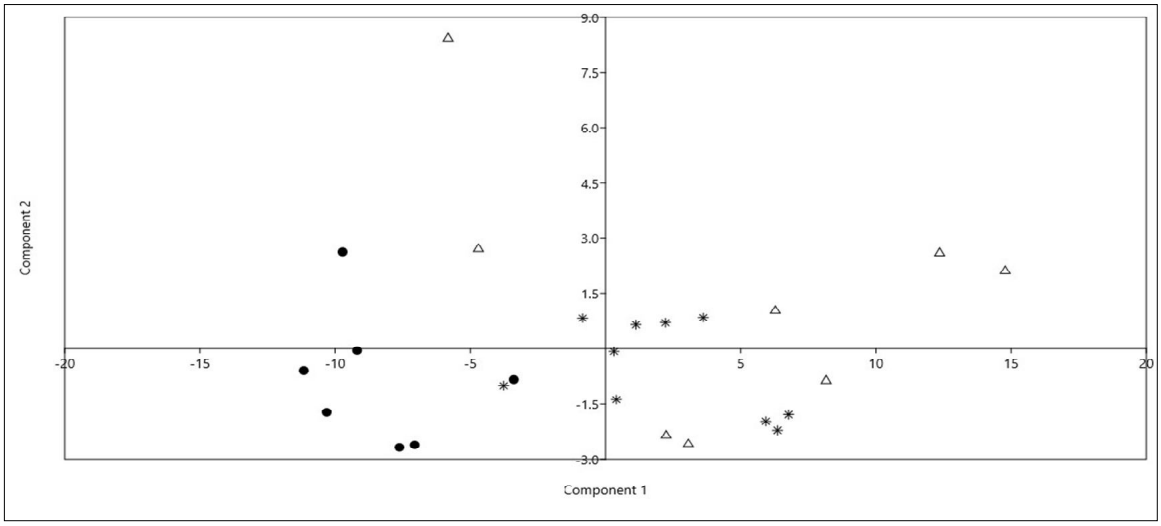

Figure 4. The scatter plot of Bangladeshi, Thai and Vietnamese female *A. testudienus*; symbols refer to individuals in Bangladeshi male-"Dot"; Thai male-"Star"; Vietnamese male-"Triangle".

The UPGMA dendrogram revealed that *A. testudineus* of Bangladeshi origin formed a separate cluster from Thai and Vietnamese *A. testudineus* (Figure 5).

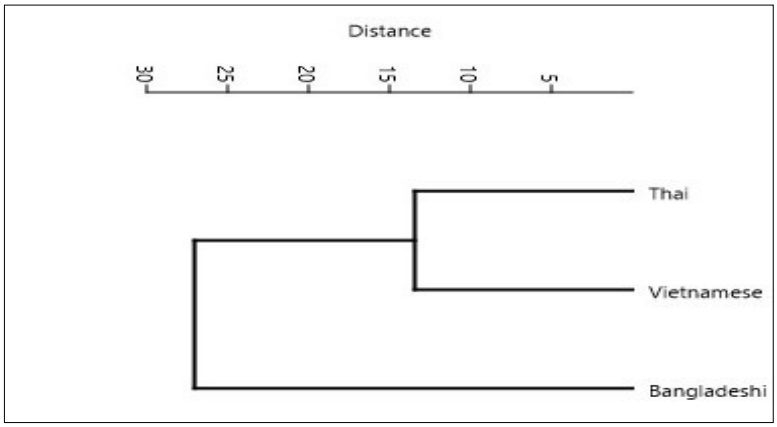

Figure 5. The dendogram showing the separate cluster formation of the three countries originated *A. testudineus*, where the Bangladeshi *A. testudineus* separated from the Thai and Vietnamese originated *A. testudineus*.

Discussion. The present study revealed variations in body shape among *A. testudineus* originated from Bangladesh, Thailand and Vietnam. PC analysis helps to know the body shape variation and the present study revealed that variations in body shape existed among Bangladeshi, Thai and Vietnamese *A. testudineus* using PC analysis. Shape variation of three populations of *Sardinella lemuru* from Mindanao Island, Philippines was observed by using PC analysis (Luceno et al 2014) and the variation between wild and captive lines of rainbow trout *Oncorhynchus mykiss* were also observed by using PC analysis and found significant variation (Pulcini et al 2013). In the present study female populations showed comparatively higher morphometric difference than male. Mohaddasi et al (2013) studied about four populations of *Alburnus chalcoides* and found the similar result where male showed less morphometric differences than female. In the present study, significant differences were also observed among Bangladeshi, Thai and Vietnamese *A. testudineus* populations; the cluster analysis revealed that *A. testudineus* originated from Bangladesh were completely separate from Thai and Vietnamese originated *A. testudineus*. Similar results from the cluster analysis among four populations of *A. chalcoides* in the south Caspian sea was found to form three major groups (Mohaddasi et al 2013). The reasons of morphological differences among three populations of same species are fairly difficult to explain which were previously agreed from the study of Poulet et al (2004), but there is a close relationship with environmental conditions (Wimberger 1992). Habitat variation of fish species was one of the very important factors for morphological variation (Swain et al 1991; Wimberger 1992). The size variation highly depends on environmental factors, where the long time living in a specific environment may cause genetic variation among the geographically isolated population of the same species which may be resulted due to gene-environmental variation. Similar observation was stated by Bagherian & Rahmani (2007).

Conclusions. The study concluded that considerable morphological and shape variation exist among indigenous (Bangladeshi), Thai and Vietnamese *A. testudineus*. The study predicts there may be the chance of speciation among them in future. To protect the purity of our indigenous *A. testudineus* proper conservation steps are highly required. At the same time, to increase the aquaculture production, proper broodstock management and disclose of breeding strategies are essential.

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