Research Article

Geometric morphometric approach to understand the body shape variation in the pony fishes (Leiognathidae) of Odisha Coast, India

Jaya Kishor SETH[*1], Tapan Kumar BARIK[1], Subhrendu Sekhar MISHRA[2]

[1]*Post Graduate Department of Zoology, Berhampur University, Odish, India-760007.*
[2]*Marine Fish Section, Zoological Survey of India, 27-J.N.Road, Kolkata, West Bengal, India- 700016.*
[*]*Email:* jkseth52@gmail.com

Abstract: Landmark-based geometric morphometric (GM) analysis was carried out in the present study on the species of the family Leiognathidae. Pattern of shape variation along the axes of the principal components and canonical variates were analyzed after the General Procrustes analysis. Canonical variates analysis confirmed the occurrence of eleven species of the family Leiognathidae along the Gopalpur-on-Sea, Odisha coast. Further, inter-specific shape variation among the species of the family were carried out using Discriminant function analysis, which would help us to understand the morphological divergence with respect to shape.

Keywords: Principal component analysis, Canonical variates analysis, Discriminant function analysis, Shape variation.

Introduction

Traditional morphometrics which used linear distance like length, width, height etc. are highly correlated with size. Therefore, measurements form two different source may produce same results and same source may produce different results; consequence of which shape analysis becomes very difficult. To overcome this, landmark-based geometric morphometrics (GM) has been used to study the shape variation (Adams et al. 2004). Shape variation using geometric morphometrics can help us to understand phenotypic, ecological, behavioural differences and can elucidate the path of evolution (O'Reilly & Horn 2004; Klingenberg et al. 2003). Further, GM has been used along with phenotype, environment and molecular data to understand adaptation and future speciation. It is also used for predicting inter-specific and intra-specific variation as well (Lorenz et al. 2017).

The fishes belonging to the family Leiognathidae are morphologically very similar, overlapping and have been confusing taxonomically. Again, taxonomy of the pony fishes has been very dynamic in recent decade as many new genera and new species have been described of late (Abraham et al. 2011; Renxie et al. 2010). The family Leiognathidae includes nine genera viz; *Aurigequula*, *Equulites*, *Eubleekeria*, *Gazza*, *Karalla*, *Leiognathus*, *Nuchequula*, *Photopectoralis* and *Secutor*, which includes approximately 47 species (Chakrabarty et al. 2010). Indian costal water is known to harbour around 20 known species of the family Leiognathidae (Abraham et al. 2011), out of which 17 species of the said family were listed from Odisha coast, India (Barman et al. 2007). However, after the recent modification in taxonomy of the family Leiognathidae and elevation of the subgenus *Nuchequula* to generic level, this family need an intensive revision work. Further, along with the morpho-taxonomy, molecular-taxonomy and phylogeny should be given due consideration for better understanding of systematic, diversity,

Fig.1. Gopalpur-on-Sea, Odisha coast with marking on fish landing centre.
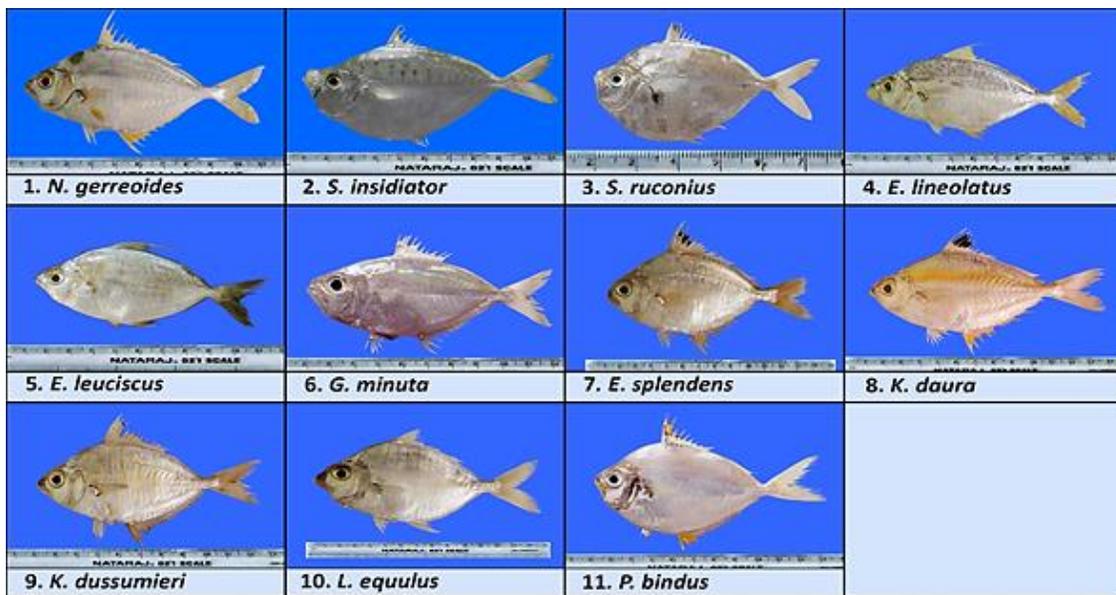

Fig.2. Photographs of the species belonging to the family Leiognathidae collected from Gopalpur-on-Sea, Odisha coast.

distribution and population dynamics of the species belonging to this family. Hence, this paper dealt with the shape variation analysis of the species of the family Leiognathidae collected from Gopalpur-on-sea, Odiah coast, India.

Materials and Methods

Fishes belonging to the family Leiognathidae were collected from Boxipalli and Haripur fish landing centre of Gopalpur-on-sea (Fig. 1) during the period 2015 (post monsoon) to 2018 (post monsoon). The specimens were photographed by placing on a measuring board with 1cm gradation and preserved in 5% formalin in the Zoological Museum, Animal Diversity Laboratory at Berhampur University, Odisha. The specimens have been identified based on keys and features described by Chakrabarty et al. (2008, 2010). A total of 69 unbend digital
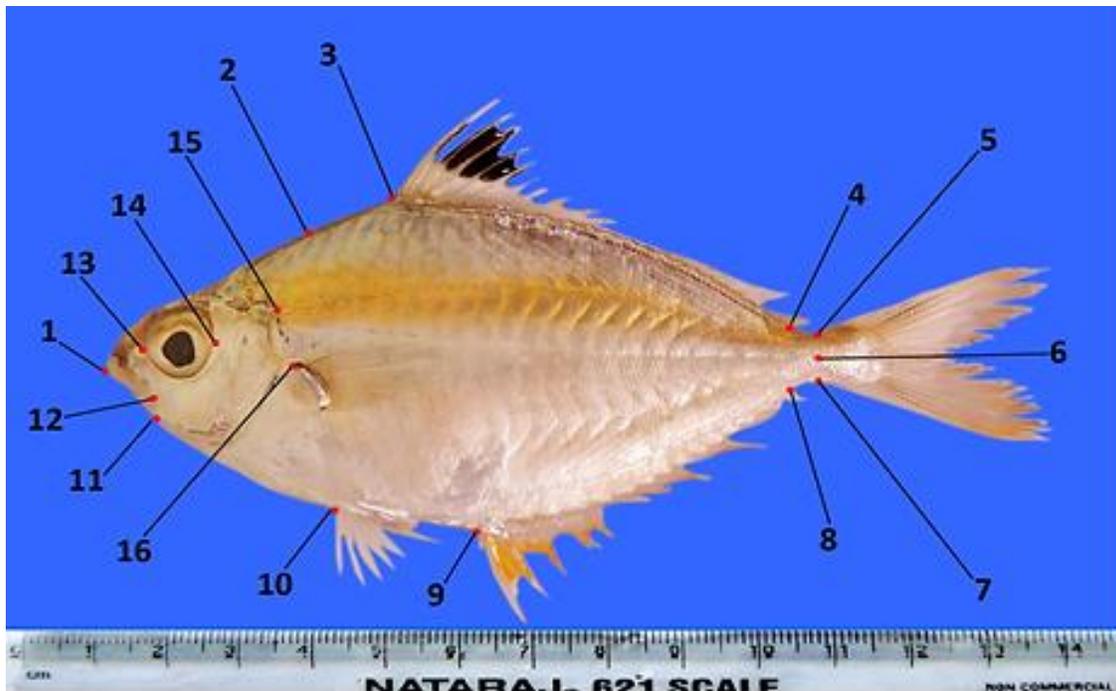
Fig.3. Landmark points used for PCA, CVA and DFA analysis: (1) rostral tip of premaxilla, (2) posterior end of nuchal spine, (3) anterior insertion of dorsal fin, (4) posterior insertion of dorsal fin, (5) dorsal insertion of caudal fin, (6) midpoint of caudal border of hypural plate, (7) ventral insertion of caudal fin, (8) posterior insertion of anal fin, (9) anterior insertion of anal fin, (10) dorsal base of pelvic fin, (11) ventral end of lower jaw articulation, (12) posterior end of maxilla, (13) anterior margin through midline of orbit, (14) posterior margin through midline of orbit, (15) dorsal end of opercle, and (16) dorsal base of pectoral fin.

photographs were chosen and saved as TPS file using the program TPSutil32 (Rohlf 2015); *Nechequula gerreoides* (n=10), *Secutor insidiator* (n=5), *S. ruconius* (n=5), *Equulites lineolatus* (n=7), *E. leuciscus* (n=6), *Gazza minuta* (n=8), *Eubleekeria splendens* (n=9), *Karlla daura* (n=3), *K. dussumieri* (n=8), *Leiognathus equulus* (n=5) and *Photopectoralis bindus* (n=3). The photographs of all the species are provided in Figure 2. The landmark points were digitized on each photograph using the software TPSDig232 (Rohlf 2015). The landmarks used for the analysis were as per Chakrabarty et al. (2008). All the 16 landmark points and their description are provided in the Figure 3. The outliers of the samples were identified and removed. Then procustes superimposition was carried out to have centroid in common and to find out the procustes sum of the squares and tangent sum of the squares. The principal component analysis (PCA), canonical variates analysis (CVA) and discriminant function analysis (DFA) were made to understand the shape

variation by using the programme MorphoJ (Klingenberg 2011). The values of this result were taken up to 3 digits after the decimal point.

Results

The procustes sum of the squares and tangent sum of the squares value were found to be 0.2489 and 0.2475. The 16 landmarks yielded 28 shape variables and accordingly 28 principal components (PCs). Each PCs had progressively less eigenvalues and variance. PC1 and PC2 had eigenvalues 0.0015 and 0.0011, with variance 42.356% and 32.619% respectively representing more than 74% of the total variance. The scatter plot of the first two PCs for the 69 specimens shows 11 distinct clusters. As shown by the lollipop transform grid of PC1; the L2, L3, L9 and L10 shows greater changes, while the lollipop transform grid of PC2; the L1, L9, L10 and L11 shows greater changes (Fig. 4).

The canonical variates analysis (CVA); yielded 10 CVs which also had progressively less eigenvalues

Table 1. Procrustes distance matrix between each species obtained through Discriminant function Analysis (DFA).

	N.g	S.i	S.r	E.l	E. le	G.m	E.s	K.d	K. du	L.e	P.b
N. gerreoides	0	0.089	0.116	0.052	0.041	0.051	0.053	0.039	0.023	0.087	0.054
S. insidiator	0.089	0	0.077	0.082	0.088	0.085	0.117	0.076	0.095	0.143	0.09
S. ruconius	0.116	0.077	0	0.136	0.132	0.12	0.113	0.119	0.119	0.119	0.088
E. lineolatus	0.052	0.082	0.136	0	0.026	0.066	0.1	0.037	0.06	0.135	0.096
E. leuciscus	0.041	0.088	0.132	0.026	0	0.06	0.085	0.036	0.046	0.12	0.087
G. minuta	0.051	0.085	0.12	0.066	0.06	0	0.087	0.057	0.056	0.109	0.076
E. splendens	0.053	0.117	0.113	0.1	0.085	0.087	0	0.08	0.048	0.047	0.049
K. daura	0.039	0.076	0.119	0.037	0.036	0.057	0.08	0	0.048	0.116	0.078
K. dussumieri	0.023	0.095	0.119	0.06	0.046	0.056	0.048	0.048	0	0.083	0.057
L. equulus	0.087	0.143	0.119	0.135	0.12	0.109	0.047	0.116	0.083	0	0.071
P. bindus	0.054	0.09	0.088	0.096	0.087	0.076	0.049	0.078	0.057	0.071	0

N.g=N. gerreoides, S.i=S. insidiator, S.r=S.ruconius, E.l=E. lineolatus, E.le=E. leuciscus, G.m=G. minuta, E.s=E. splendens, K.d=K. daura, K.du=K. dussumieri, L.e=L.equulus, P.b=P. bindus.

Table 2. Mahalanobis distance matrix between each species obtained through Discriminant function Analysis (DFA).

	N.g	S.i	S.r	E.l	E. le	G.m	E.s	K.d	K. du	L.e	P.b
N. gerreoides	0	12.372	18.045	9.486	10.513	8.148	9.396	4.749	6.274	10.139	7.488
S. insidiator	12.372	0	6.233	8.116	12.39	10.132	13.631	6.275	9.608	12.973	6.888
S. ruconius	18.045	6.233	0	15.535	15.846	16.214	23.672	11.562	19.702	17.165	4.887
E. lineolatus	9.486	8.116	15.535	0	6.72	11.167	25.178	4.143	6.673	21.863	10.805
E. leuciscus	10.513	12.39	15.846	6.72	0	11.885	19.965	5.971	9.206	19.438	9.952
G. minuta	8.148	10.132	16.214	11.167	11.885	0	28.436	7.748	13.651	12.655	5.598
E. splendens	9.396	13.631	23.672	25.178	19.965	28.436	0	16.916	10.848	6.766	6.65
K. daura	4.749	6.275	11.562	4.143	5.971	7.748	16.916	0	5.12	9.637	4.375
K. dussumieri	6.274	9.608	19.702	6.673	9.206	13.651	10.848	5.12	0	10.976	6.774
L. equulus	10.139	12.973	17.165	21.863	19.438	12.655	6.766	9.637	10.976	0	9.539
P. bindus	7.488	6.888	4.887	10.805	9.952	5.598	6.65	4.375	6.774	9.539	0

N.g=N. gerreoides, S.i=S. insidiator, S.r=S.ruconius, E.l=E. lineolatus, E.le=E. leuciscus, G.m=G. minuta, E.s=E. splendens, K.d=K. daura, K.du=K. dussumieri, L.e=L.equulus, P.b=P. bindus.

Table 3. T-square value matrix between each species obtained through Discriminant function Analysis (DFA).

	N.g	S.i	S.r	E.l	E. le	G.m	E.s	K.d	K. du	L.e	P.b
N.gerreoides	0	510.324	1085.471	370.566	414.479	295.078	418.199	52.056	174.991	342.687	129.41
S. insidiator	510.324	0	97.134	192.133	418.712	315.89	597.281	73.832	284.076	420.78	88.973
S. ruconius	1085.471	97.134	0	703.818	684.834	808.939	1801.22	250.661	1194.408	736.605	44.79
E. lineolatus	370.566	192.133	703.818	0	145.906	465.617	2496.196	36.06	166.273	1394.238	245.212
E. leuciscus	414.479	418.712	684.834	145.906	0	484.362	1434.978	71.32	290.572	1030.495	198.104
G. minuta	295.078	315.89	808.939	465.617	484.362	0	3424.758	130.98	745.455	492.77	68.383
E. splendens	418.199	597.281	1801.22	2496.196	1434.978	3424.758	0	643.861	498.419	147.166	99.509
K. daura	52.056	73.832	250.661	36.06	71.32	130.98	643.861	0	57.213	174.15	28.719
K.dussumieri	174.991	284.076	1194.408	166.273	290.572	745.455	498.419	57.213	0	370.739	100.116
L. equulus	342.687	420.78	736.605	1394.238	1030.495	492.77	147.166	174.15	370.739	0	170.641
P. bindus	129.41	88.973	44.79	245.212	198.104	68.383	99.509	28.719	100.116	170.641	0

N.g=N. gerreoides, S.i=S. insidiator, S.r=S.ruconius, E.l=E. lineolatus, E.le=E. leuciscus, G.m=G. minuta, E.s=E. splendens, K.d=K. daura, K.du=K. dussumieri, L.e=L.equulus, P.b=P. bindus.

and variance. CV1 and CV2 had eigenvalues 104.2929 and 75.8806, with variance 46.548% and 33.867%, respectively representing more than 80% of the total variance. The scatter plot of the first two CVs for 69 specimens shows 11 distinct clusters; which were more prominent then that of PCA analysis. As shown by the lollipop transform grid of CV1; the L9 and L10 shows greater changes, while the lollipop transform grid of CV2; the L2 and L3 shows greater changes (Fig. 5). The shape variation
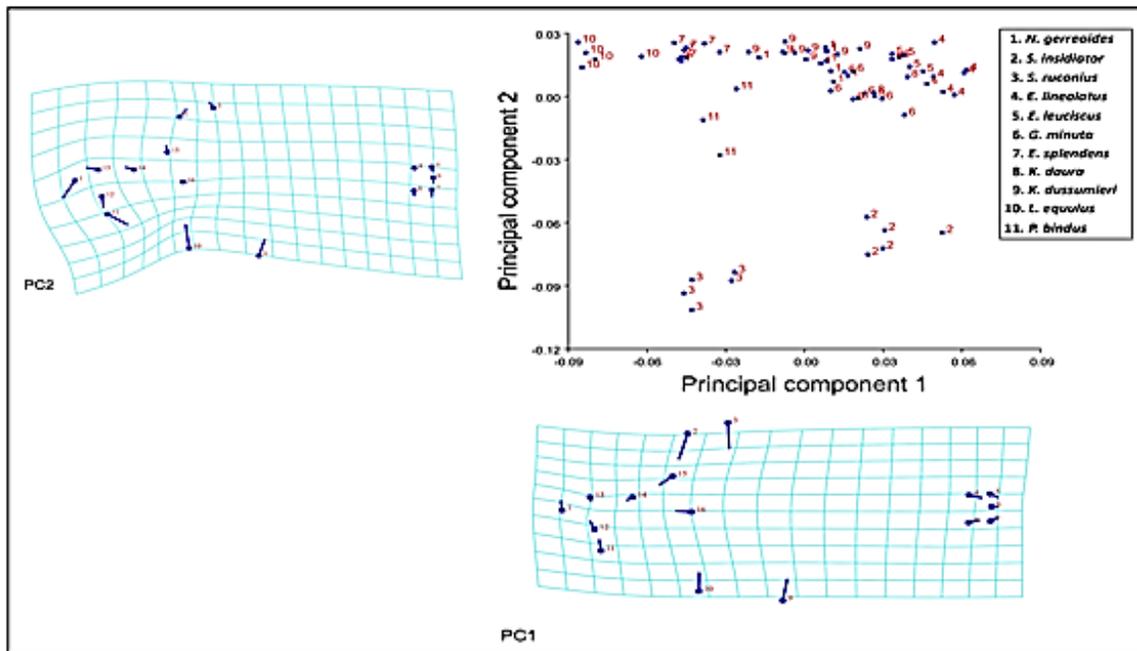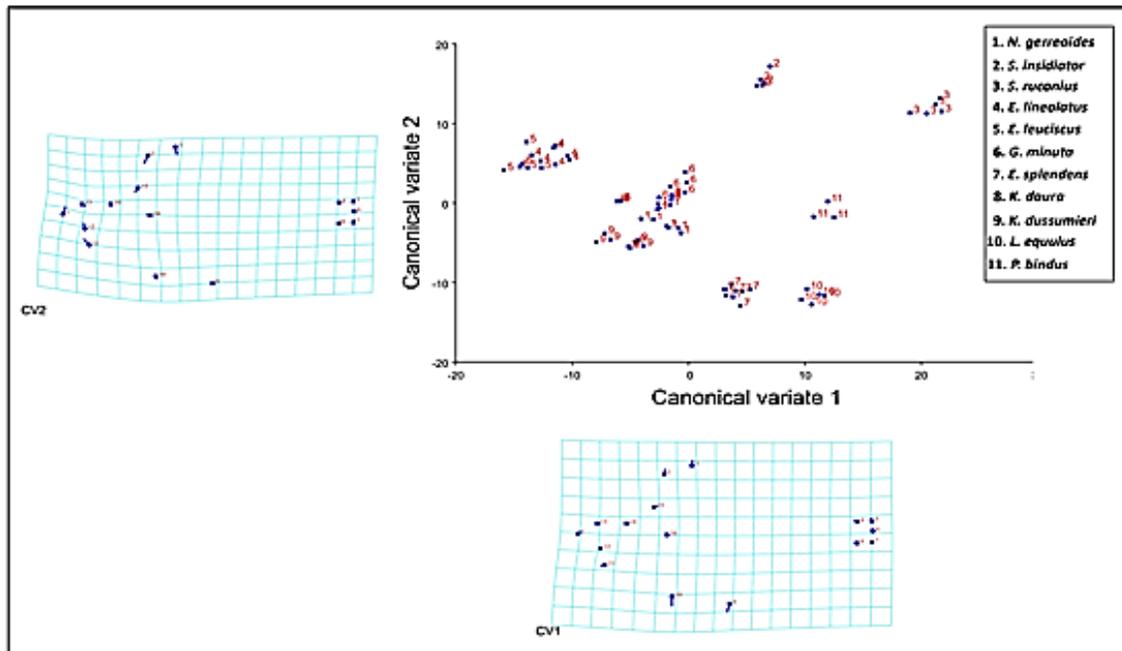
Fig.4. Scatter plot on the first two PCs for the samples of 69 specimens and the pattern of changes along the PC1 and PC2. Circles represent the location of the landmarks in the mean shape of the samples; arrows indicates the changes in the relative positions of the landmark points.

between each pair of species under consideration in the form the Procrustes distance, Mahalanobis distance and T square value between each species obtained through Discriminant Function Analysis (DFA) are presented in the Tables 1-3.

Nechequula gerreoides showed significant shape difference from genus *Secutor* and from species *E. leuciscus;* which is evident from the Procrustes distance, Mahalanobis distance and T-square value; $P<0.5$. (Procrustes distance, Mahalanobis distance and T-square value between *N. gerreoides* and *S. insidiator* were 0.089, 12.372 and 510.324; Procrustes distance, Mahalanobis distance and T-square value between *N. gerreoides* and *S. ruconius* were 0.116, 18.045 and 1085.471; while the Procrustes distance, Mahalanobis distance and T-square value between *N. gerreoides* and *E. leuciscus* were 0.041, 10.513 and 414.479, respectively). However, *N. gerreoides* did not show any significant shape variation with the other species under consideration ($P>0.5$) (Tables 1-3).

In the genus *Secutor*, intra-specific variation was not so significant as the Procrustes distance,

Mahalanobis distance and T-square value between *S. insidiator and S. ruconius* was 0.077, 6.233 and 97.134 ($P>0.5$). Apart from *N. gerreoides*, the species *S. insidiator* also showed significant shape variation from *E. leuciscus* (Procrustes distance: 0.088, Mahalanobis distance: 12.39 and T-square value: 418.712; $P<0.5$), *G. minuta* (Procrustes distance: 0.085, Mahalanobis distance: 10.132 and T-square value: 315.89; $P<0.5$), *E. splendens* (Procrustes distance: 0.117, Mahalanobis distance: 13.613 and T-square value: 597.281; $P<0.5$), *L. equulus* (Procrustes distance:0.143, Mahalanobis distance: 12.973 and T-square value: 420.78; $P<0.5$), *P. bindus* (Procrustes distance: 0.09, Mahalanobis distance: 6.888 and T-square value: 88.973; $P<0.5$) and from both the species of genus *Karalla* (Procrustes distance, Mahalanobis distance and T-square value from *K. daura* were 0.076, 6.275 and 73.832; $P<0.5$; Procrustes distance, Mahalanobis distance and T-square value from *K.dussumieri* was 0.095, 9.608 and 284.076; $P<0.5$; while the other species of the genus i.e. *S. ruconius* showed significant shape variation from all other species with significant

Fig.5. Scatter plot on the first two CVs for the samples of 69 specimens and the pattern of changes along the CV1 and CV2. Circles represent the location of the landmarks in the mean shape of the samples; arrows indicates the changes in the relative positions of the landmark points.

Procrustes distance, Mahalanobis distance and T-square value; $P<0.5$ except *P. bindus*. The Procrustes distance, Mahalanobis distance and T-square value between *S. ruconius* and *P. bindus* was 0.088, 4.887 and 44.79 respectively; $P>0.5$ (Tables 1-3).

The intra-specific variation within the species of the genus *Equulites* was not so significant. The species *E. lineolatus* showed significant shape variation from *G. minuta* (Procrustes distance: 0.066, Mahalanobis distance: 11.167 and T-square value: 465.617; $P<0.5$), *E. splendens* (Procrustes distance: 0.1, Mahalanobis distance: 25.178 and T-square value: 2496.196; $P<0.5$), *L. equulus* (Procrustes distance: 0.135, Mahalanobis distance: 21.863 and T-square value: 1394.238; $P<0.5$) and *P. bindus* (Procrustes distance: 0.096, Mahalanobis distance: 10.805 and T-square value: 245.212; $P<0.5$); while the other species of the genus *E. leuciscus* showed significant shape variation from all the species except from the species of the genus *Kalara* (Procrustes distance, Mahalanobis distance and T-square value between *E. leuciscus* and *K. daura* was 0.036, 5.971 and 71.32 respectively; while between *E. leuciscus*

and *K. dussumieri;* Procrustes distance was 0.046, Mahalanobis distance were 9.206 and T-square value were 290.572, respectively; $P>0.5$) (Tables 1, 2, 3).

Apart from the genus *Secutor*, *Equulites*, the species *G. minuta* also showed significant shape variation from *E. splendens* (Procrustes distance: 0.087, Mahalanobis distance: 28.436 and T-square value: 3424.758; $P<0.5$) and from one species of the genus *kalara i.e. K. dussumieri* (Procrustes distance, Mahalanobis distance and T-square value between *G. minuta* and *K. dussumieri* was 0.056, 13.651 and 745.455 respectively; $P<0.5$) (Tables 1-3).

E. splendens showed significant shape variation from species under consideration except *N.gerreoides* (Procrustes distance, Mahalanobis distance and T-square value between *E. splendens* and *N. gerreoides* was 0.053, 9.396 and 418.199 respectively; $P>0.5$), *L. equulus* (Procrustes distance, Mahalanobis distance and T-square value between *E. splendens* and *L. equulus* was 0.047, 6.766 and 147.166, respectively; $P>0.5$) and *P. bindus* (Procrustes distance. Mahalanobis distance and T-square value between *E. splendens* and *P. bindus* was

0.049, 6.65 and 99.509, respectively; $P>0.5$) (Tables 1-3).

The intra-specific shape variation within the genus *Kalara* was not so significant. Apart from the significance shape difference between *K. dussumieri* and *G. minuta*; both the species of the genus *Kalara* showed significant shape variation from genus *Secutor,* from species *E. splendens* (Procrustes distance, Mahalanobis distance and T-square value between *K. daura* and *E. splendens* were 0.08, 16.916 and 643.861, respectively; $P<0.5$; while the Procrustes distance, Mahalanobis distance and T-square value between *K. dussumieri* and *E. splendens* was 0.048, 10.848 and 498.419, respectively; $P<0.5$) and *L. equulus* (Procrustes distance, Mahalanobis distance and T-square value between *K. daura* and *L. equulus* was 0.116, 9.637 and 174.15, respectively; $P<0.5$; while the Procrustes distance, Mahalanobis distance and T-square value between *K. dussumieri* and *L. equulus* was 0.083, 10.976 and 370.739, respectively; $P<0.5$) (Tables 1-3).

The species of *L. equulus* showed significant shape variation from all the species under considearaion except *E. splendens*. The species *P. bindus* showed significant shape variation from *S. insidiator*, genus *Equulites* and *L. equulus* (Procrustes distance, Mahalanobis distance and T-square value between *P. bindus* and *L. equulus* was 0.071, 9.539 and 170.641, respectively; $P<0.5$) (Tables 1-3).

Discussion

Selection of landmarks in the landmark-based studies relate to the existence of shape variation between species and population (Dujardin 2008). GM based investigations generally used to explore shape variation in an evolutionary pathway that have focused on vertebrates to understand transformations in skull shape, scapula shape, body form and so on (Swiderski 1993; Zelditch et al. 1995; Oettle et al. 2005; Stayton 2005). However, the present investigation highlights the usefulness of the Geometric morphometric based analysis for easy and quick identification of the fishes, which can be beneficial for fisheries recourse management. Further, GM based shape variation analysis can be used as first-hand information in understanding the adaptation and to elucidate the path of evolution.

The present study of shape variation analysis is the first report in the family leioghtidae from Indian water. The 11 distinct clusters obtained in the CVA analysis confirm the occurrence of the 11 taxonomic groups of the family Leiognathidae along the Gopalpur-on-Sea coast. In the principal components and canonical variates analysis; significant differences were found among species with respect to extension of the nuchal spine, anterior insertion of dorsal fin, anterior insertion of anal fin, dorsal base of pelvic fin and ventral end of lower jaw articulation, which reveals that interspecific morphological divergence might had happened with respect to these point in the course of evolution.

The discriminant function analysis uncovered the variety of body shape variation in the species of the family leiognathide. This test reveals that these species under consideration are morphological related (Tables 1-3). The finding of the study further unfold that there is no apparent shape difference within the same genus which are consistent with the taxonomic treatment of the group (Chakrabarty et al. 2008, 2010) and 16s rRNA based molecular phylogeny study (Seah et al. 2012).

Moreover, shape variation between some species belonging to different genus were found to be very significant, if not all. But, it is very difficult to correlate the inter-specific shape divergence between different genus with the molecular phylogeny analysis reported earlier using mitochondrial NADH dehydogenase (ND4 and ND5) (Ikejima et al. 2004) and 16S rRNA gene (Seah et al. 2012). In the molecular phylogeny analysis based on ND4 and ND5; the species *E. splendens*, the genus *Gazza* and *Secutor* belongs to the same sister clade; but in the present study, both the species of the genus *Secutor* showed significant shape variation from both *E. splendens* and *G. minuta*. Likewise, study based

on 16s rRNA gene; the genetic divergence between *L. equulus* and *E. splendens* is significant but in our present study shape variation between the aforesaid two species is not so significant.

This overlapping and separation of body shape variation might be due to their overall genetics, evolutionary adaptation and differential interaction to their surrounding habitat. Sometime selection pressure like overfishing/overexploitation, geographic range of their migration, temperature range, resources availability, growth pattern and differential phenotypic plasticity of different species may influence differently to the body shape (Echem 2016).

Landmark-based GM is not only able to elaborate shape differences between species, but it also provided a meaningful way to improve the visualization and quantification of these differences at the time of species identification. Further, accurate identification of a particular species and population discrimination are important in the conservation of biodiversity and their management. In conclusion, the result of this study suggest that landmark based GM analysis along with the morpho-taxonomy is useful for specie level confirmation as it can easily discriminate groups. However, for robust analysis like predicting possible route of speciation and character divergence; GM alone cannot provide all the information. Therefore, GM based studies should be combine with more than one molecular markers for better understanding of evolution and adaptation.

Acknowledgements

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مقاله پژوهشی

روش ریخت‌سنجی هندسی برای درک تنوع شکل بدن در پنجزار ماهیان (Leiognathidae) در ساحل اودیشا، هندوستان

جای کیشور ست*۱، تاپان کومار باریک۱، سوبهرندو سکهار میشرا۲

۱گروه جانورشناسی، دانشگاه برهامپور، اودیش، هندوستان.

۲بخش ماهیان دریایی، مطالعات جانورشناسی هندوستان، کلکته، بنگال غربی، هندوستان.

چکیده: در مطالعه حاضر آنالیز ریخت‌سنجی هندسی لندمارک پایه برروی گونه‌های خانواده پنجزار ماهیان انجام شد. الگوی تغییرات شکل در امتداد محورهای تجزیه به مولفه‌های اصلی و تحلیل همبستگی کانونی بعد از رویهم گذاری به روش پروکراست آنالیز شدند. تحلیل همبستگی کانونی حضور ۱۱ گونه از خانواده پنجزار ماهیان در دریای گوپالپور، ساحل اودیشا را تایید نمود. همچنین تغییرات بین گونه‌ای در گونه‌های این خانواده با استفاده از آنالیز تابع تشخیص انجام شد که می‌تواند به درک واگرایی ریختی مربو به شکل کمک نماید.

کلمات کلیدی: تجزیه به مولفه‌های اصلی، تحلیل همبستگی کانونی، آنالیز تابع تشخیص، تغییرات شکل.