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

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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,
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, Odisha 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...
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 *Photpectoralis 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.
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
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 gerreoide* 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. gerreoide* *and S. insidiator were 0.089, 12.372 and 510.324; Procrustes distance, Mahalanobis distance and T-square value between *N. gerreoide* *and S. ruconius were 0.116, 18.045 and 1085.471; while the Procrustes distance, Mahalanobis distance and T-square value between *N. gerreoide* *and E. leuciscus were 0.041, 10.513 and 414.479, respectively). However, *N. gerreoide* 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. gerreoide,* 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
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 *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 1-3).

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 Leioghnathidae 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 dehydrogenase (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.

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مقاله پژوهشی

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

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چکیده: در مطالعه حاضر آنالیز ریخت‌سنجی هندسی لندمارک پایه بر روی گونه‌های خانواده پنجزار ماهیان انجام شد. انواع تغییرات شکل در امتداد محورهای تجزیه به مولفه‌های اصلی و تحلیل همبستگی کانونی بعد از روزه کناری به روش بروکراس آنتیلیز شدند. تحلیل همبستگی کانونی حضور 11 گونه از خانواده پنجزار ماهیان در دریای گوپالپور، ساحل اودیشآ را تایید نمود. همچنین تغییرات بین گونه‌های در گونه‌های این خانواده با استفاده از آنتیلیز تابع تشخیص انجام شد که می‌تواند به درک واکنش‌های ریختی مربوط به شکل کمک نماید.

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