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Note

Application of scale shape variation in fish systematics - an illustration using six species of the family Nemipteridae (Teleostei: Perciformes)

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ABSTRACT

An attempt was made to analyse the variation in the morphology of the tenth lateral line scale of six nemipterid species (*Nemipterus japonicus, Nemipterus bipunctatus, Nemipterus randalli, Parascolopsis aspinosa, Scolopsis vosmeri* and *Scolopsis bimaculatus*) from Mumbai waters. Outline shape variation of the tenth lateral line scale was subjected to elliptical fourier analysis using 74 fourier descriptors. Images were binarised to generate chain codes using SHAPE 1.3 version. Subsequently, principal component analysis was performed to measure scale shape variation among the six nemipterid species. The study revealed that there is shape variation between the 10th lateral line scale of the six nemipterid species and the investigation reiterated that scale characteristics can provide useful taxonomic information which could be employed in identification of fish species.

Keywords: Chain codes, Elliptical Fourier Analysis, Fourier descriptors, Lateral line scale, Nemipteridae, Principal Component Analysis.

Aquatic species diversity, especially of fish species is on the decline due to overfishing and habitat destruction. and therefore proper management of the resources gains importance. Correct identification of fish species is essential in order to formulate management and conservation measures for vulnerable fish species. Several morphological characters are being used in fish taxonomic studies, of which analysis of scale morphology appears to be promising as it is easy to apply, rapid, inexpensive and does not require animal kill and dissection. Scale shape and scale number have benn used for taxonomical investigations since the first half of the 19th century when Agassiz (1883-1884) used it in fish taxonomy for the first time. During the late 19th century and first half of 20th century, studies on scale morphology have increased dramatically in the field of taxonomy (Williamson, 1851; Baudelot, 1873; Timms, 1905; Cockerell, 1910, 1913, 1914, 1915; Chu, 1935; Lagler, 1947; Kobayasi, 1951, 1952, 1953, 1955; McCully, 1961). With the invention of scanning electron microscopy (SEM), fish species have been differentiated based on scale characteristics (De Lamater and Courtenay, 1973, 1974; DeLamater *et al*., 1972; Hughes, 1981; Roberts, 1993; Lippitsch, 1990, 1992, 1993, Jawad and Jufaili, 2007, Reza *et al*., 2009). Ichthyologists have been using fish scale to determine the age and growth for a long time (Hickman *et al*., 1993). Fish scale characteristics are very much useful in the identification as they tend to change from species to species. Circuli, radii, ctenii, lateral line canal and other structures associated with scales have been used authentically for classification (Hughes, 1981; Hollander, 1986; Dicenzo and Sellers, 1998; Kaur and Dua, 2004).

So far no attempt has been made to differentiate the species of Nemipteridae using scale shape variation. So, this study was undertaken to analyse the outline shape variation of the tenth lateral line scale of six nemipterids species (*Nemipterus japonicus, N. bipunctatus, N. randalli, Parascolopsis aspinosa, Scolopsis vosmeri* and *S. bimaculatus*).

Samples of six nemipterid species were collected from Versova fish landing centre of Mumbai as well as from the research vessel, "MFV Narmada" of Central Institute of Fisheries Education operating along Mumbai waters. Species selected were *N. delagoae* (111-211mm total length, TL), *N. japonicus* (111-164 mm TL), *N. randalli* (84-154 mm TL), *P. aspinosa* (85-125 mm TL), *S. bimaculatus* (106-210 mm TL) and *S. vosmeri* (85-150 mm TL). Scales were extracted from lateral line of each species as per Patterson *et al.* (2002) using a flat end forceps. The 10th lateral line scale on the fish body surface was selected for the analysis. Thirty two (32) lateral line scales were taken from each species of fish. Extracted lateral line scales were imaged using Leica stereo-zoom microscope under x2 magnification.

The 24-bitmap images generated were processed into gray scale binary images using Shape Version 1.3 software (Iwata and Ukai, 2006), to which a one-step noise filter was applied in order to erase grainy marks on the surface of the scales. After noise reduction, contours of the scales were extracted *via* edge detection and the contour was stored as chain codes (Freeman, 1974; Dalayap *et al.*, 2008). Normalised elliptical fourier descriptors (EFD) obtained from the chain codes were measured using elliptic fourier transformation (Kuhl and Giardina, 1982; Ferson *et al*., 1985; Rohlf, 1990). Normalisation of data obtained from chain codes used the first harmonic ellipse as a base, which was corresponding to the first fourier approximation and utilised the later 19 harmonics to be calculated as suggested by Iwata and Ukai (2006).

Fourier descriptors generated were then subjected to principal component analysis (PCA) (Jolliffe, 1986) by SHAPE 1.3 version software. Visualisation of PCA diagram provided a better understanding of variation among the species. Later the variations in shape of the tenth lateral line scale among six different species of Nemipteridae were analysed using the scatter plots of principal component scores generated using the SAS software platform (SAS version 9.2, 2008). The principal components were checked for significance employing Kruskal-Wallis one way ANOVA test (Kruskal and Wallis, 1952) using SPSS software (PASW Statistics v. 16.0). PC scores of the first two components were used to analyse the amount of variation between the six species of Nemipteridae. Principal component scores were subjected to cluster analysis using the method of squared distance with the help of software platform SAS version 9.2 (SAS version 9.2, 2008). Dendrogram was also generated to visualise the clustering pattern between nemipterid species under study.

Generally elliptic, oblong, pentagonal, rectangular, square, triangular and cycloid shaped scales are found in bony fishes (Jawad, 2005), while the nemipterid species under investigation revealed 4 types of scales *viz*., elliptical (*N. randalli, N. bipunctatus* and *P. aspinosa*), round (*N. japonicus*), square (*S. bimaculatus*) and rectangular (*S. vosmeri*). Elliptical fourier analysis could derive 74 normalised elliptical fourier coefficients using 20 harmonics of chain code data. Outline of lateral line scale (Fig. 1) under study have shown similarity in genus *Nemipterus* and *Parascolopsis* which were oval to round in outline. The shape was found to be similar in all the species studied under the genus *Nemipterus* (*N. japonicus, N. bipunctatus,* and *N. randalli*) while the two species under the genus *Scolopsis* (*S. bimaculatus* and *S. vosmeri*) were distinct with rectangular and square outlines respectively (Fig. 2). Elliptical fourier analysis of the 10th lateral line scale of six nemipterids (Fig. 3) showed significant difference ($p \leq 0.001$) in scale outline shape. Principle component analysis has described five effective principle components for fourier descriptors of scale outline, which accounts for 82.42% total significant variation. PC1 contributed to the variation in length-width ratio (31.27%) while PC2 contributed to the variation in anterior ends away from midpoint of scale (14.19%) (Table 1). The scatter plot of first and second principal components (Fig. 4) was successful in separating the species by scale outline shape as these have high contribution to scale shape. In the scatter plot, genus *Nemipterus* seems to be well separated from genus *Scolopsis* and genus *Parascolopsis* (which seems intermediate in position, closer to *Scolopsis*), Genus *Scolopsis* possesses square and rectangular scale shape in outline that demarcated this genus from the genera *Nemipterus* and *Parascolopsis* which have round to elliptical scale shapes..

Dendrogram of cluster analysis (Fig. 5) using principal component scores have illustrated significant difference ($p \leq 0.001$) among the six species under study. It was observed that genus *Nemipterus* has shown remarkable separation from the other two genera (*Scolopsis* and *Parascolopsis*). *N. bipunctatus* and *N. japonicus* formed a single cluster while *S. vosmeri* and *S. bimaculatus* formed a separate cluster. *N. randalli* was separated from *N. japonicus* and *N. bipunctatus*. Similarly *P. aspinosa* was distinct from the cluster formed by

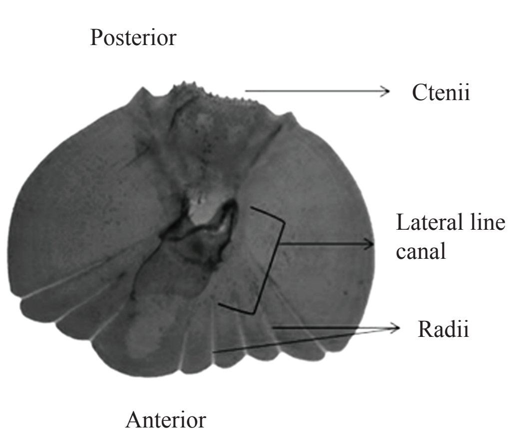

Fig. 1. Morphology of lateral line scale

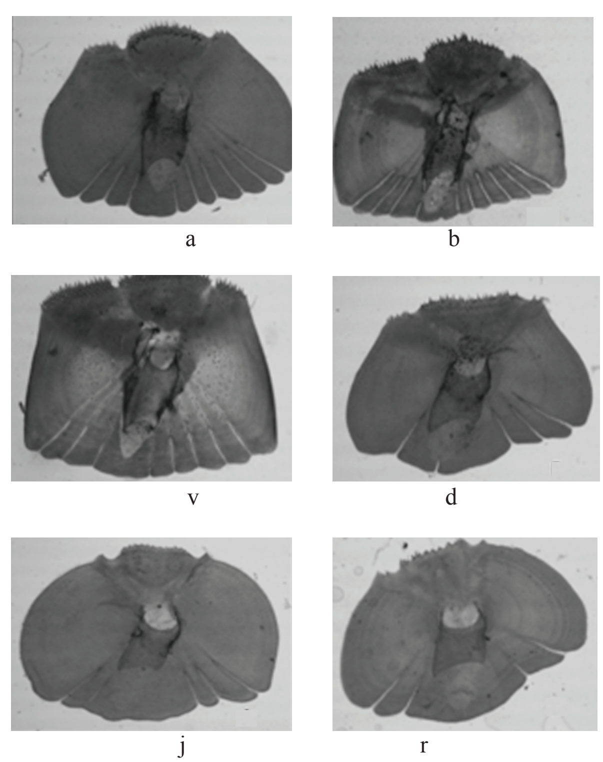

Fig. 2. Morphological comparison of lateral line scales from six species of nemipterids
A : *P. aspinosa;* B : *S. bimaculatus*; V : *S. vosmeri*
D : *N. bipunctatus*; J : *N. japonicus;* R: *N. randalli*

S. vosmeri and *S. bimaculatus*. Cluster analysis supported the results of general description of scale shape outline and the principal component analysis using fourier descriptors. Kruskal Wallis test (Table 2) has shown that PC1, PC2, PC3, PC4 and PC5 were significant ($p \leq 0.001$) in contributing effect to scale shape variation among the six different species of Nemipteridae, while remaining PCs were of limited contribution to shape variation.

Along with morphometry and meristic characters, variation in hard parts such as scale can be used as a tool for differentiation of fish species. As the scale being a unique part of fish body, the study reveals that scale shape can be used successfully to discriminate between species especially at juvenile stages. The results of the present study has clearly indicated the potential application of scale shape variation in identification of species of nemipterids. There is further scope for analysng the differences based on number of ctenii and arrangement, position of lateral line canal and angle of alignment. Use of more precise imaging tools like scanning electron microscopy can produce images with more details that may give more accurate results.

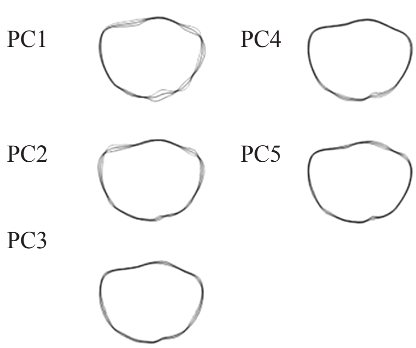

Fig. 3. Contour shape of scales generated using elliptic fourier descriptors from five PC scores explaining shape variation

Table 1. Reconstructed scale outline of lateral line scale from six species of nemipterids illustrating the five effective principal components with their contribution to the total variation

Principal components	Eigen value (10^4)	Proportion (%)	Cumulative %	Contribution to variation
PC1	10.68	31.27	31.27	Length width ratio
PC2	4.85	14.19	45.47	Anterior end away from midpoint
PC3	2.51	7.34	52.81	Anterior tip and lateral margins
PC4	2.30	6.73	59.54	Remaining variation at posterior margin
PC5	2.17	3.94	77.79	All remaining variations

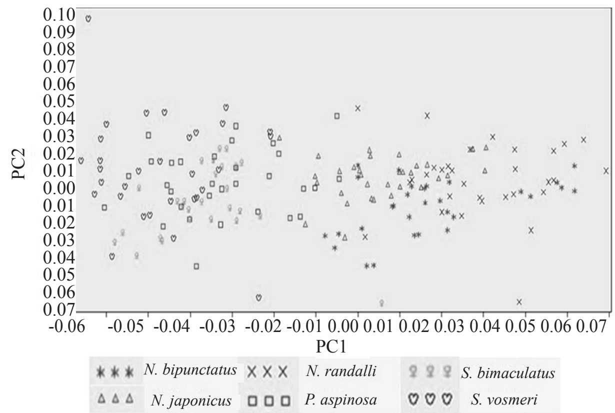

Fig. 4. Scatter plot of first and second principal components generated from fourier descriptors

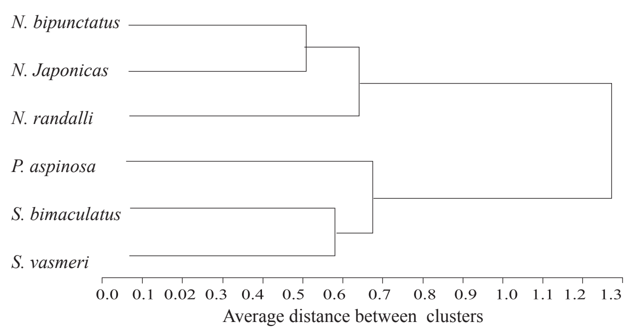

Fig. 5. Dendrogram of clusters eluted from PC scores.

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