GENERAL METHODOLOGY
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Samples of *Channa punctatus* were obtained live from two ecologically distinct regions of the country, namely, Aligarh in the north and Vishakhapatnam in the south.

All measurements were made to the nearest 0.1 mm with the help of a manual and digital caliper. Before analyzing the data, the measurements were transformed and standardized in order to minimize the variability resulting from allometric growth (Beacham, 1985; and Reist, 1985).

Fish were measured for their morphometric and meristic characteristics, using the method employed by the Hubbs and Lagler (1947). Specimens showing excessive curvature were discarded. The morphometric and meristic parameter chosen for the study were:

**Total Length (TL):** distance between the anterior most part of the snout and tip of the caudal fin.

**Standard Length (SL):** distance in a straight line between the anterior most part of the snout or the upper lip which makes the anterior most extremity of the body and the base of the caudal fin, where the median fin meets the hypural plate.

**Predorsal Length (PDL):** distance before the dorsal fin up to the tip of the anterior most part of the snout.

**Head Length (HL):** distance in a straight line between the anterior most part of the snout or the upper lip/ whichever
extends the farthest forward and the posterior most edge of the operculum bone.

**Caudal Fin Length (CFL):** length of the caudal fin from base to tip.

**Depth through dorsal fin (DDF):** distance between the dorsal and ventral surface at the deepest point.

**Orbit Diameter (OD):** diameter of eye orbit.

**Scale Count:** formula expressing the number of scales along the lateral line and the transverse row.

**Lateral Line Scale Count (LLS):** denotes the maximum number of scales in the lateral line.

**Scales above the lateral line (SAL):** scales falling along the line starting from the origin of the dorsal fin and extending downward and backward to meet the lateral line.

**Scales below the lateral line (SBL):** scales falling along the line that starts from the origin of the anal fin and running upward and forward to reach the lateral line. Any scale encroaching both above and below the line was counted among the scales from below the lateral line.

**Scales before the dorsal fin (SBD):** scales present in a row before the dorsal fin.

**Gill raker count (NGR):** number of gills raker present on the first gill arch.

**Fin ray count:** number of fin rays on each fin, viz. dorsal (DFR), ventral (VFR), pelvic (P1FR), pectoral (P2FR) and caudal (CFR).

Besides conventional measurements, truss network system was also employed (Fig.1) for measuring fish in vertical, horizontal and oblique manner between anatomical landmarks
There are several biases and weaknesses inherent in traditional character sets, where most of the characters tend to align with one of the very few axis such as longitudinal with only scant sampling of depth and breath. Thus, large amount of information on variation in oblique direction remains lacking. Besides, some morphological landmark, such as tip of the snout and posterior end of the vertebral column are used repeatedly. Thus, truss body form reconstruction method has been considered more reliable in such studies. The procedure for morphometric measurements were the same as those described by Winans (1984) and Swain and Holtby (1989). Each specimen was kept in its natural position on a water resistant paper. Landmark positions were recorded by making holes with a dissecting needle in a water resistant paper alongside the location of each landmark. Distance between each landmark was then measured with the help of an electronic digital caliper nearest to 0.1 mm (Fig 2). Ten landmarks were chosen to form the truss box, producing 21 homologous distances. The distance between each landmark measured was as follows:

Maxillary length (ML), Dorsal Head Length (DHL), post dorsal extension of the neurocranium to pectoral fin origin (PDENPO), end of jaw to pectoral fin origin (EJPO), snout to pectoral fin origin (SNPO), the post dorsal extension of neurocranium to end of lower jaw (PDENEJ), the post dorsal extension of neurocranium to dorsal fin origin (PDENDO), dorsal fin origin to ventral fin origin (DFOVFO), post dorsal extension of neurocranium to ventral fin origin (PDENVO), dorsal fin origin to
pectoral fin origin (DFOPFO), dorsal fin origin to dorsal fin insertion (DFODFI), dorsal fin origin to ventral fin insertion (DFOVFI), dorsal fin insertion to ventral fin origin (DFIVFO), dorsal fin origin to ventral fin insertion (DFOVFI), dorsal fin insertion to dorsal caudal fin origin (DFIDCFO), dorsal caudal fin origin to ventral caudal fin origin (DCOVCO), ventral fin insertion to caudal fin origin (VFICFO), dorsal caudal fin insertion to caudal fin origin (DCICFO), dorsal caudal fin origin to ventral fin insertion (DCOVFI).

**Slab gel electrophoresis**

A polyacrylamide slab gel electrophoresis (PAGE) was performed following the method as described by the Laemmli (1970). The following stock solutions were prepared.

**Acrylamide bisacrylamide** (30:0.8): 30 g of acrylamide and 0.8 g of bisacrylamide were dissolved in a total volume of 100 ml of distilled water. The solution was filtered and stored at 4°C in an amber coloured bottle in order to prevent photopolymerization.

**Resolving gel buffer:** Tris (36.3 g, 3M) was dissolved in 48 ml of IN HCL, the pH adjusted to 8.8 and final volume brought to 100 ml.

**Stacking gel buffer:** Tris (6.05 g 0.5M) was dissolved in 40 ml of distilled water, titrated to pH 6.8 with IN HCL, and the final volume adjusted to 100 ml with distilled water.

**Tank buffer:** 0.02 M tris and 0.192M glycine (pH 8.3) containing 0.1% SDS.
Sample buffer: 6 g tris was dissolved in 80 ml of distilled water and its pH was adjusted to 6.8 with ortho-phosphoric acid. The final volume was brought to 100 ml with distilled water. The samples were electrophoressed for 8-10 hours at room temperature at 80 volts or for 3-4 hours at room temperature at 20 mA, using mini slab gel electrophoresis apparatus (Genei, Banglore).

The data on morphological characteristics were computed for univariate and multivariate analysis. The data collected were transformed and standardized and subjected to Principal Component Analysis (PCA), and Discriminant Function Analysis (DFA). PCA, a multivariate ordination method of analysis, is useful in distinguishing morphological differences between groups (Winans, 1987). It attempts to express the whole variational pattern of the data in terms of few variates compounded from those measured originally, thus reducing the data and expressing almost all the information more concisely in fewer numerical entities (Finney, 1980). PCA has been preferred over univariate and bivariate analysis. Gould and Johnston (1972) have also emphasized the use of multivariate approach for morphological variations. PCA computes a set of uncorrelated composite variables called principal components from various covariance or correlation matrix.

DFA uses measurement of individuals from two or more groups within the species and determines from a linear function or other compound of measurement that best distinguishes one group from another.
All statistical analyses were performed on Compaq Presario (Pentium III), Laptop on SPSS 7.5 version of windows XP.
Fig 1. The location of the 10 homologous landmarks, recorded on each individual, are shown as • and the 21 distance measure. The landmarks refer to: 1, the anterior point of the snout on the upper jaw; 2, the end of jaw; 3, the posterior dorsal extension of neurocranium; 4, pectoral fin origin; 5, dorsal fin origin; 6, ventral fin origin; 7, dorsal fin insertion; 8, ventral fin insertion; 9, dorsal caudal fin origin; 10, ventral caudal fin insertion.
Fig 2. Digital Caliper used for measurement