Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(5): 940-944

Received: 06-07-2016
Accepted: 07-08-2016

Verma J
Fish Biogenetics Lab
Department of Zoology
University of Lucknow,
Lucknow, India

Serajuddin M
Fish Biogenetics Lab,
Department of Zoology,
University of Lucknow,
Lucknow, India

Correspondence
Verma J
Fish Biogenetic Lab
Department of Zoology
University of Lucknow,
Lucknow, India.

Study of Phylogeny based on Truss Analysis and Molecular Characterization in Freshwater Catfish, *Eutropiichthys vacha*

Verma J and Serajuddin M

Abstract
The present study was designed to investigate the morphological variations between three different stocks and analysis of nucleotide sequences based on major (45S) and minor (5S) ribosomal DNA in freshwater catfish, Eutropiichthys *vacha*. In truss analysis, univariate analysis of variance showed all significant morphometric measurements ((P<0.05) in 78 characters studied in three sub populations of *E. vacha*. In linear discriminant function analysis (DFA), the overall assignment of individuals into their original groups was 91.7%. The proportion of individuals correctly classified into their original groups was 88%. The principal component analysis (PCA) scatter plot and dendrogram also showed high degree of variation among three different stocks (sub populations) of *E. vacha*. The molecular characterization of each stock of *E. vacha* was done using the sequence length range and GC % of different primers. Findings of the present study can be utilized to understand the morphological and molecular variations in thre stocks of *E. vacha* of IUCN listed species.

Keywords: *E. vacha*, morphological variations, discriminant function analysis, major and minor rDNA.

Introduction
E. vacha is a freshwater and brackish-water subtropical species which is commonly known as 'river cat- fish'. It is a commercially important food fish in Asian countries and has gained popularity among consumers due to its high nutritional value and good taste [1]. This fish is an important target species for small-scale fisheries. It is commonly known as Bacha and River catfish in Bangladesh [2], Batchwa or vacha in India [3], Cherki in Nepal [4] and Challi in Pakistan [5]. The populations of *E. vacha* from rivers, streams, canals, reservoirs, lakes and swamplands have seriously declined. *E. vacha* is widely distributed in Asia, throughout the Indian subcontinent including Bangladesh, India, Pakistan, Nepal, Myanmar and Thailand [6]. Morphometric and meristic characters of fish are the measurable and countable characters respectively common to all fishes. Landmarks refer to some arbitrarily selected points on a fish's body and with the help of these points; the individual fish body shape can be analyzed. In other words, a landmark is a point of correspondence on an object that matches between and within populations [7-8]. Truss network systems constructed with the help of landmark points are powerful tools for stock identification. A sufficient degree of isolation may result in notable morphological, meristic and shape differentiation among stocks of a species which may be recognizable as a basis for identifying the stocks. The characteristics may be more applicable for studying short term, environmentally induced disparities and the findings can be effectively used for improved fisheries management [9-12]. The conservation status of *E. vacha* should be developed through effective habitat protection, public awareness programs and ranching.

Studies of ribosomal RNA (rRNA) genes used especially for identification of evolutionary relationships and the characterization of genome structure animals and plants. In higher eukaryotes, rRNA genes are organized as two distinct multigene families comprised of tandem arrayed repeats composed of hundreds to thousands copies. One class is represented by the 45S rDNA which consists of a transcriptional unit that code for 18S, 5.8S and 28S rDNAs separated by internal transcribed spacers (ITSs) and surrounded by intergenic spacers (IGS). The other class codes for 5S rRNA gene that consists of a highly conserved coding sequence of 120 base pairs (bp), which is separated from each transcriptional unit by a NTS. Unlike the 45S rDNA, the 5S rDNA is not normally associated with nucleoli formation [13].

Although the 5S rDNA coding sequences are highly conserved even between non-related species, the variations in the non transcribed spacers (NTS) owing to insertions/deletions, mini-repeats and pseudo genes have been frequently characterized in several organisms [14-16]. This variation in the NTS of 5S rDNA has been useful for evolutionary studies and served as species or population-specific markers [17-18]. The arrangement of 5S rRNA genes have been extensively studied in plants and animals [19-22] and yielded information about the evolution of this gene cluster as well as the species. However, in most eukaryotes, the 5S rDNA is normally detected in areas of the genome distinct from the 45S rDNA and histone gene clusters [22]. Due to attractive properties of fast evolving and repetitive nature, the rDNA is widely utilized for examining genetic variability and divergence among the species.

The purpose of this study was to collect the morphological and genetic information about this threatened fish species, *E. vacha*. The results will provide useful information for species identification and genetic diversity assessment.

Materials and Methods

The specimens of *E. vacha* used for this study were randomly collected in period of two year during August 2014 to April 2016 from river Ganga (Allahabad), Gomti river at Lucknow (Uttar Pradesh) and Son river (Rewa) with the help of local fishermen. The geographical coordinates of the collection sites were taken by GPS: Ganga river- Allahabad site (Latitude-25^0 25'17.31"N, Longitude- $81^0$50'50.25"E, Altitude 285ft), Gomti river-Lucknow site (Latitude- 26^0 51'36.83" N, Longitude- $80^0$55'46.28" E, Altitude- 375ft), Son river- Betwa site (Latitude-24^0 00'44.76" N, Longitude-$80^0$53'28.59" E, Altitude-1267ft). A total of 41 specimens were used to study the morphological variations. The 13 landmarks used to infer morphological differences among populations: 1 Tip of snout; 2 center of eye;3 forehead (end of frontal bone); 4 end of operculum; 5 dorsal origin of pectoral fin; 6 origin of dorsal fin; 7 origin of pelvic fin; 8 termination of dorsal fin; 9 origin of anal fin; 10 termination of anal fin; 11 dorsal side of caudal peduncle; 12 ventral side of caudal peduncle; 13 end of lateral line adapted as adoted by Strauss & Bookstein, 1982 [23].

A total of 78 distance measurements between 13 landmarks were surveyed using the truss network system according to Strauss & Bookstein, 1982 [23] with minor modifications for this species. Truss network measurements are a series of measurements calculated between landmarks that form a regular pattern of connected quadrilaterals or cells across the body form [25]. The fish were placed on a graph paper with dorsal and anal fins. The right body profile of each fish was photographed with digital camera. Images were saved in jpg format and analyzed with TPS dig [25] to coordinates of 13 landmarks. A box truss of 26 lines connecting these landmarks was generated for each fish to represent the basic shape of the fish [26] All measurements were transferred to a spreadsheet file (Excel 2007), and the X–Y coordinate data transformed into linear distances by computer (using the Pythagorean Theorem) for subsequent analysis [24]. ANOVA was used to test for the significant differences in the morphometric characters.

Size dependent variation was corrected by adapting an allometric method as suggested by Elliott et al. [27]

$$Madj = M (Ls/Lo)b$$

where M is original measurement, Madj is the size adjusted measurement, Lo is the standard length of the fish, Ls the overall mean of standard length for all fish from all samples in each analysis, and b was estimated for each character from the observed data as the slope of the regression of log M on log Lo using all fish from the five groups. The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length [24]. Univariate analysis of variance (ANOVA) was performed for each morphometric character to evaluate the significant difference between the five locations [28], and those morphometric characters which showed significant variations (P>0.05) in the present study, linear discriminant function analyses (DFA), principal component analysis (PCA) and cluster analysis (CA) were employed to discriminate the five populations. Principal component analysis helps in morphometric data reduction [29] in decreasing the redundancy among the variables [30] and in extracting a number of independent variables for population differentiation [31]. The DFA was used to calculate the percentage of correctly classified (PCC) fish. Statistical analyses for morphometric data were performed using the SPSS version 16 software package.

For molecular study, muscle samples were used for DNA isolation from fish specimens. The genomic DNA was also extracted from the whole blood using the standard phenol-chloroform–isoamylalcohol method as described by Sambrook and Russell, 2001 [32]. The primers for 18S amplification were designed from *Cyprinus carpio* sequence (NCBI accession no. AF133089) of Singh *et al*. 2009 [33]. The 5S and 5.8 S amplification were carried out from Moran *et al*. 1996 [34]. The primers for 28S were taken from Zardoya *et al*. 1996 [35]. PCR reaction mixture contained 10× Taq buffer 10µl, 4µl dNTPs mix, 400ng of each primer, 1µl Taq DNA polymerase (3U/□µl), and 1µl genomic DNA in a final reaction volume of 100 µL with water. The PCR cycling conditions for each ribosomal genes were: initial denaturation at 94 ºC for 5 min; followed by 35 cycles of denaturation at 94 ºC for 30 sec; primer annealing at 55 ºC for 30 sec; primer extension at 72 ºC for 1min with post cycling extension at 72 ºC for 5 min. Amplified products were run on 1.5% agarose gel stained with ethidium bromide. The PCR products were custom sequenced and the sequences were submitted to NCBI database.

Results

The schilbid catfish, *Eutropiichthys vacha* (IUCN Red List) are commercially important inland water teleostean fish which are quite palatable as a table fish. The present study reaffirms that the taxonomy and the phylogeny possesses the general trend of variability between the species as well as the conservedness in same family. The species mostly displaying the conservedness belong to the same geographical locations with diverse ecological conditions but here the fishes of Ganga River and Son River were closely related to each other. On the other hand the 45S (18S, 5.8S and 28S) and 5S (coding and NTS) rDNA units were amplified, sequenced, analyzed in subject species. This is the first study in the endangered species i.e. *E. vacha*. For amplification of divergent domain of 28S (D9, D11), partial 18S rDNA, 5.8S, 5S suitable primers were used (Table 1). The length size (bp), GC % and accession numbers of internal transcribe spacer 1 and 2, 18S rDNA, different divergent domains of 28 S rDNA, 18S, 5.8S and 5S rDNA are given in Table 2. In *Eutropiichthys vacha*, all characters were found to be significant in Anova.

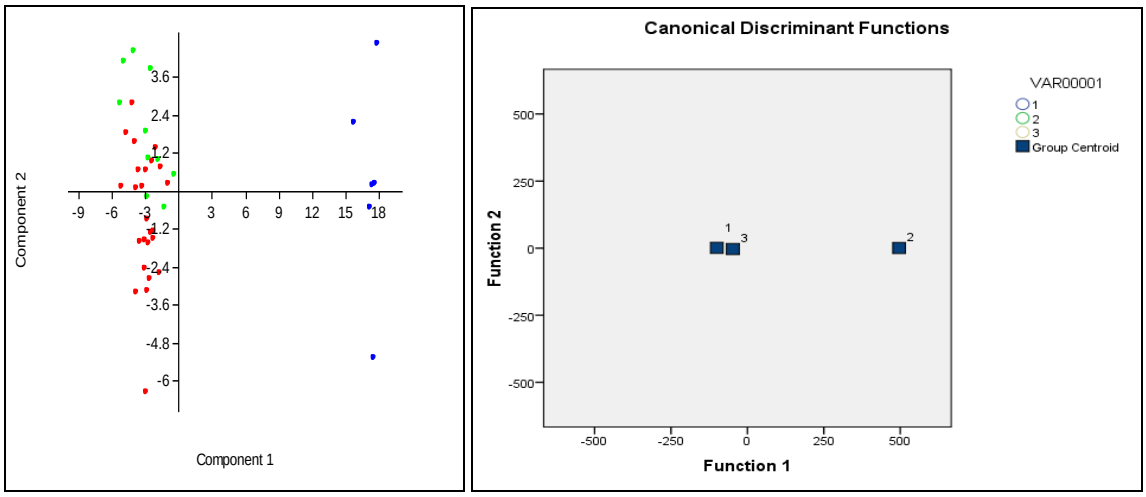

Fig. 1: PCA plot of 3 populations of *Eutropiichthys vacha* of river Ganga (Red), Gomti River (Blue), Son River (Green) and canonical discriminant function

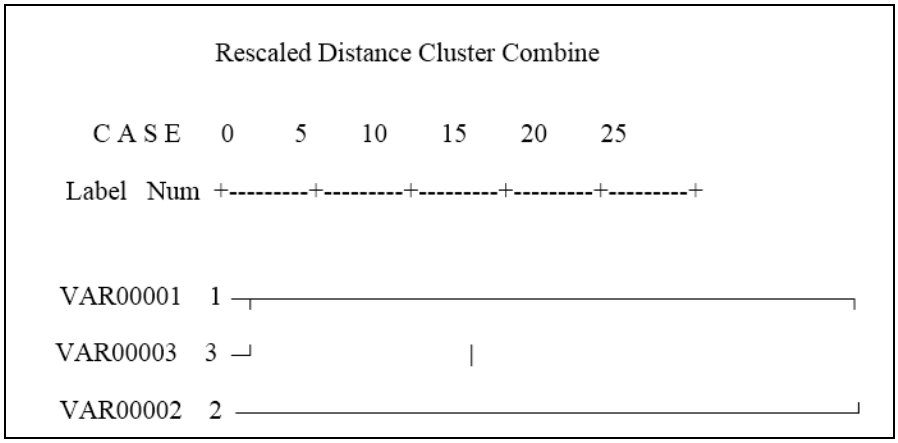

Fig. 2: Dendrogram based on morphometric characters and landmark distances of three populations of *E.vacha* i.e. Ganga (VAR00001), Gomti (VAR00002) and Son river (VAR00003)

Table 1: Primer sequences of 45S and 5S rDNA used in the study.

S. No.	Code	Primer sequences
1	D9 F	5'-CGGCGGGAGTAACTATGACTCTCTTAAGGT-3'
2	D9 R	5'- CCGCCCCAGCCAAACTCCCCA-3'
3	D11 F	5'- TGAAATACCACTACTCTTATCGTT-3'
4	D11 R	5'- GGATTCTGACTTAGAGGCGTTCAG-3'
5	18 S F	5'- GTAGTCATATGCTTGTCTC -3'
6	18 S R	5'- GAAACCTTGTTACGACTT-3'
7	5.8 F	5'- CAACTCTTAGCGGTGGATCA-3'
8	5.8 R	5'- AGCGACCCTCAGACAGGCGTGG-3'
9	5SF	5'-TACGCCCGATCTCGTCCGAT-3'
10	5SR	5'-CAGGCTGGTATGGCCGTAAGC-3'

Table 2: GenBank accession numbers, Gene name, Species name, length (bp) and GC% of the sequences of the subject species used in the study.

S. No.	Accession numbers	Gene name	Species name and length (bp)	length (bp)	GC%
1.	KX827804	Divergent domain 9 of 28S rDNA	*E. vacha*	465 bp	49%
2.	KX827805	Divergent domain 11 of 28S rDNA	*E. vacha*	531bp	48%
3	KX827801	Partial 18S rDNA	*E. vacha*	1657bp	55%
4	KX827802	5.8S rDNA	*E. vacha*	370bp	63%
5	KX827803	5S rDNA	*E. vacha*	428bp	48%

4. Discussion

This is the first report on nucleotide composition of 45S major and 5S minor rDNA. The G+C contents were lower than A+T contents in the divergent domain 9 and 11 of 28S rDNA. The 28S rDNA genes in fishes are slightly shorter than mammals. Hassouna *et al.* 1984 [36], while working with mouse, suggested that major variations in 28S rRNA gene size during the evolution have been restricted to a unique set of a few sites within a largely conserved secondary core structure. The divergent domains, responsible for the large increase in size of the molecule, from prokaryotes to higher eukaryotes represent half of the mouse 28S rRNA length.

The sequence length of partial 18S rDNA and 5.8S rDNA are highly conserved. According to Singh *et al.*, 2009 [33] the 18S

rDNA sequence showed 94% identity among the various species of *Tor*. In 5S region, the sequence length was much higher than that of other species. The sequence length of 5S was slightly similar to that of the different species of *Tor*. The variations in nucleotide sequences could be used for the understanding the genetic diversity. These results, in general, would enhance the value and interpretation of ecological assessment data for conservation of various species belonging to the family schilbeidae. The studies on ribosomal DNA gene activities have gained importance in a wide range of organisms especially for species/ population characterization as well as phylogenetic and evolutionary relationships [37].

5. Conclusion

The aim of this study was to follow a series of investigations to understand the morphological characters like morphometric measurements and molecular characterization. Results of the present study may lead to understand the nucleotide sequence length since the sequence of variable region remain species specific, therefore, these segments can be used to study short term evolution. They can be used to measure gene variation at interspecies level and can be used extensively for purpose of taxonomic identification.

6. Acknowledgement

The first author would like to express sincere thanks to the Department of Science and Technology (DST), New Delhi for providing fund for carrying out the research work and also thankful to the HOD, Department of Zoology, University of Lucknow, Lucknow for permitting to undertake research work in departmental lab (Fish Bio-Genetic Research Lab).

7. References

1. Hasan MF, Molla AH, Ahsan MS, Alam MT. Physicochemical properties and fatty acids distribution pattern in lipids of *Eutropiichthys vacha* Hamilton-Buchanan (Family Schilbeidae). Pakistan J Biol Sci. 2002; 5:696-698
2. Rahman AKA. Freshwater Fishes of Bangladesh. Zoological Society of Bangladesh. Department of Zoology, University of Dhaka, Dhaka. 1989; 1000:364.
3. Talwar PK, Jhingran AG. Inland Fishes of India and Adjacent Countries. A.A. Balkema, Rotterdam. 1991; 2:541.
4. Shrestha J. Fishes, fishing implements and methods of Nepal. Smt. M.D. Gupta, Lalitpur Colony, Lashkar (Gwalior), India. 1994, 1-150
5. Soomro AN, Baloch WA, Jafri SIH, Burdi GH, Fulanda B. Reproduction and feeding habits of the River Catfish *Eutropiichthys vacha* (Hamilton, 1822) (Siluriformes: Schilbidae) in an impacted habiat: Kotri hydrodam, River Indus, Paksitan. Our Nature. 2012; 10:269-280
6. Talwar PK, Jhingran AG. Inland Fishes of India and Adjacent Countries. A.A. Balkema, Rotterdam. 1991; (2):541.
7. Swain DP, Foote CJ. Stocks and chameleons: the use of phenotypic variation in stock identification. Fish Res. 1999; 43:113-128.
8. Ihssen PE, Evans DO, Christie WJ, Reckahnand JA, Desjardine RL. Life history, morphology, and electrophoretic characteristics of five allopatric stocks of lake whitefish (*Coregonus clupeaformis*) in the Great Lake region. Can J Fish Aquat Sci. 1981; 38:1790-1807.
9. Templeman W. Stock discrimination in marine fishes. NAFO Sci Counc Stud. 1983; 6:57-62.
10. Smith PJ, Jamieson A. Stock discreteness in herrings: a conceptual revolution. Fish Res. 1986; 4:223-234
11. Turan C, Erguden D, Turan F, Gurlek M. Genetic and morphologic structure of *Liza abu* populations from the Rivers Orontes, Euphrates and Tigris. Turk J Vet Anim Sci. 2004a; 28:729-734.
12. Long EO, David ID. Repeated genes in eukaryotes; Annu. Rev. Biochem. 1980; 49:727-764.
13. Nelson DW, Honda BM. Genes coding for 5S ribosomal RNA of the nematode *Caenorhabditis elegans*. Gene. 1985; 38:245-251.
14. Leah R, Frederiksen S, Engberg J, Sorensen PD. Nucleotide sequence of a mouse 5s rRNA variant gene. Nucl. Acids Res. 1990; 18:7441.
15. Sajdak SL, Reed KM, Phillips RB. Intra individual and interspecies variation in the 5S r DNA of coregonid fish. J Mol. Evol. 1998; 46:680-688.
16. Suzuki H, Moriwaki K, Sakurai S. Sequences and evolutionary analysis of mouse 5S rDNAs. Mol. Biol. Evol. 1994; 11:704-710.
17. Pendas AM, Moran P, Martinez JL, Garcia-Vasquez E. Applications of 5S rDNA in Atlantic salmon, brown trout, and in Atlantic salmon x brown trout hybrid identification. Mol. Ecol 1995; 4:275-276.
18. Vitelli L, Batistoni R, Andronico F, Nardi I, Barsacchi-Pilone G. Chromosomal localization of 18S+28S and 5S ribosomalRNA genes in evolutionary divergence anuran amphibians; Chromosoma. 1982; 84:475-491.
19. Lomholt B, Frederiksen S, Jensen LR, Christensen K, Hallenberg C. 5S rRNA genes in Macaca fascicular is map to chromosome in three loci; Mamm. Genome. 1996; **7**:451-453.
20. Prado EA, Faivre-Rampant P, Schneider C, Darmency M A. Detection of a variable number of ribosomal DNA loci by fluorescent in situ hybridization in Populus species. Genome. 1996; 39:1020-1026.
21. Adacchi J, Watanabe K, Fukui K, Ohmido N, Kosuge K. Chromosomal localization and reorganization of the 45S and 5S rDNA in the *Brachyscome lineariloba* complex (Asteraceae). J. Plant Res. 1997; 110:371-377.
22. Drouin G, Moniz de Sa M. The concerted evolution of 5S ribosomal genes linked to the repeat units of other multigene families. Mol. Biol. Evol; 1995; 12:481-493.
23. Strauss RE, Bookstein FL. The truss: body form reconstruction in morphometrics. Systematic Zoology. 1982; 31:113-135.
24. Turan C. A note on the examination of morphometric differentiation among fish populations: the truss system. Turk. J Zool. 1999; 23:259-263.
25. Rohlf FJ. TPS Dig, Version 2.04. Department of Ecology and Evolution, State University of New York, Stony Brook, 2005.
26. Cardin SX, Friedland KD. The utility of image processing techniques for morphometric analysis and stock identification. Fish Res. 1999; 43:129-139.
27. Elliott NG, Haskard K, Koslow JA. Morphometric analysis of orange roughly (*Hoplostethus atlanticus*) off the continental slope of Southern Australia. J Fish Biol. 1995; 46:202-220.
28. Zar JH. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, NJ.52 Hydrobiologia. 1984; 673:41-52.
29. Veasey EA, Schammass EA, Vencovsky R, Martins PS, Bandel G. Germplasm characterization of Sesbania accessions based on multivariate analyses. Genet. Resour. Crop Evol. 2001; 48:79-90

30. Samaee SM, Mojazi-Amiri B, Hosseini-Mazinani SM. Comparison of Capoeta capoeta gracilis (Cyprinidae, Teleostei) populations in the south Caspian Sea River basin, using morphometric ratios and genetic markers. Folia Zoologica. 2006; 55:323-335.
31. Samaee M, Patzner RA, Mansour N. Morphological differentiation within the population of Siah Mahi, Capoeta capoeta gracilis, (Cyprinidae, Teleostei) in a river of the south Caspian Sea basin: a pilot study. J Appl Ichthyol. 2009; 25:583-590.
32. Sambrook J, Russell I. Molecular cloning: a laboratory manual. 3rd edition. Cold Spring Harbor Laboratory Press, Plainsveiw; New York USA, 2001.
33. Singh M, Kumar R, Nagpure NS, Kushwaha B, Gond I, Lakra WS. Chromosomal localization of 18S and 5S rDNA using FISH in the genus Tor (Pisces, Cyprinidae). Genetica. 2009; 137:245-252
34. Moran P, Martinez JL, Garcia-Vazquez S, Pendas AM. Sex linkage of 5S rDNA in rainbow trout (*Oncorhynchus mykiss*). Cytogenet Cell Genet. 1996; 75:145-150.
35. Zardoya R, Meyer A. Evolutionary relationships of the coelacanth, lungfishes, and tetrapods based on the 28S ribosomal RNA gene. Proc Natl Acad Sci. 1996; 93:5449-5454.
36. Hassouna N, Michot B, Bachelleire JP. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. Nucleic Acids Res. 1984; 12:3563-3583
37. Martins C, Galetti PM. Conservative distribution of 5S rDNA loci in *Schizodon* (*Pisces*, *Anastomidae*) chromosomes. Chromosome Res. 2000; 8:353-355