ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(3): 22-26

www.fisheriesjournal.com
Received: 05-03-2016
Accepted: 06-04-2016

Shalu Thomas

National Institute of Malaria Research (ICMR), IDVC Field Unit, NIE Campus, 2nd Main Road, TNHB, Ayapakkam, Chennai- 600 077, India.

Lijo John
Export Inspection Agency-Kochi Panampally Nagar South, Kochi- 682 036, Kerala, India.

Alex Eapen
National Institute of Malaria Research (ICMR), IDVC Field Unit, NIE Campus, 2nd Main Road, TNHB, Ayapakkam, Chennai- 600 077, India.

Correspondence
Alex Eapen
National Institute of Malaria Research (ICMR), IDVC Field Unit, NIE Campus, 2nd Main Road, TNHB, Ayapakkam, Chennai- 600 077, India.
Email: alexeapen@yahoo.com

Biometric variations among populations of Carnatic ricefish (*Oryzias carnaticus*, Jerdon, 1849), a native larvivorous fish of South India

Shalu Thomas, Lijo John, Alex Eapen

Abstract

The possibility of any biometric variations in *Oryzias* (ricefish) populations present in three geographically isolated drainages around the city of Chennai, India were studied. Though biometric characters were insufficient to differentiate the three populations as distinct species, the dorsal and anal fin ray counts had shown considerable variation from the baseline information reported earlier besides, certain scale counts were significantly different among as well as between the populations. Branched pelvic fin ray counts (6-7) of Sriperumbudur population was different from the baseline information (6). The anal fin rays of saline inhabitant population were higher compared to those in freshwater. Furthermore, as most of the baseline meristic variables overlap from one species to another, identification of *Oryzias carnaticus* and *Oryzias dancena* need not be solely based on the meristic characters. The application of biometrics has limitations in identifying this species and molecular techniques may resolve the taxonomic ambiguity of *Oryzias* species in the Indian subcontinent.

Keywords: Oryzias, ricefish, biometrics, taxonomical ambiguity

1. Introduction

Ricefishes belonging to genus *Oryzias* (Beloniformes: Adrianichthyidae: Oryziinae) are found in fresh and brackish waters from India to Japan along the Indo-Australian archipelago, China and many parts of South Asia [1]. Until recently, genus *Oryzias* was represented by a single species in peninsular India namely '*Oryzias melastigma*', widely distributed in Bengal and Tamil Nadu, primarily in estuarine, brackish as well as freshwater habitats [2]. A revision of genera by Roberts opined that the species available in India are *O. dancena* and *O. carnaticus* but not *O. melastigma*, mainly based on minor variations in meristic characters like anal and dorsal fin ray counts [3]. However, detailed study on the comparative morphology of all the 28 known ricefish species by Parenti, revised its taxonomy with a few variations in meristic characters viz., anal fin rays, pectoral fin rays and number of vertebrae, many of which are overlapping between the above mentioned Indian species [1].

Morphological variability among spatially separated fish populations are reported to be induced by genetic as well as environmental factors, while phenotypic plasticity in fish morphology has been widely documented. Many of the earlier classifications of fishes including *Oryzias* are mainly based on biometric data employing morphometric and meristic variables. But it is evident from the findings of Roberts (1998) [3] and Parenti (2008) [1] that the biometric characters, especially the meristic variables that define *Oryzias* species, have changed over the years. Insufficient information on intraspecific variation was reported resulting in ambiguous species identifications especially when biometrics is used as an identification tool [4]. Ever since the taxonomic revisions of tropical Asian medakas or rice fishes were attempted, there has been a considerable taxonomic ambiguity on the diversity of different species of *Oryzias* in India. Hence this study was undertaken to find out the possibility of any biometric variations of *Oryzias* species present in three geographically isolated drainages around the city of Chennai, India.

2. Materials and methods

Live specimens (N - 59) collected from January to March, 2010 using eco-friendly methods like cast and scoop nets were subsequently identified as *O. carnaticus* using available literature [3, 5]. 20 specimens of *O. carnaticus* each from a pond in Perungalathur (12^0 54'N, 80^0 05'E), canal in Pulicat (13^0 24'N, 80^0 19'E) and 19 from a rivulet in Sriperumbudur (13^0 01'N, 80^0 04'E), geographically distinct areas around Chennai, India, were used for the biometric study. 19 morphometric characters of *O. carnaticus* were selected for the analysis. Allometric growth *i.e*., heterogeneity in body size, among samples was reported to result in heterogeneity of shape among populations [6]. Morphometric variations were analysed following the formula of Lleonart *et al* [7].

$$M_{adj} = M\ (L_s/L_o)^b$$

wherein, M is the original morphometric measurement, M_{adj} the size adjusted measurement, L_o the standard length of fish and L_s the overall mean of standard length for all fishes from all samples. The parameter b was estimated for each character from the observed data as the slope of the regression of log M on log Lo, considering all specimens. The efficiency of size adjustment transformations was assessed by testing the significance of correlation between transformed variables and standard length. Both univariate and multivariate analysis of variance were carried out to test the significance of morphometric differences among populations. The descriptive statistics *viz*, minimum, maximum, mean and standard deviation for morphometric traits (raw measurements converted into % SL and % HL) were estimated using SPSS (ver. 10.0) statistical package. The coefficient of variation (CV) was computed for each character using the formula adopted by Van Valen, 1978 [8].

$$CV = (100\times SD)/X_m$$

wherein, *SD* is the standard deviation and X_m is the mean of the transformed measurements of species-specific characters. In each sample group, morphological variability was estimated by the multivariate generalization of the coefficient of variation (CVp) using the formula of Van Valen, 1978 [8].

$$CV_p = 100 \times \sqrt{\Sigma\ S_x\ /\Sigma M_x},$$

wherein, S_x is the variance of each morphometric variable and M_x is square of the mean.

Further, 13 meristic characters were selected to find out any variations among the three populations of *O. carnaticus*. The meristic characters included in the present study were unbranched and branched rays of dorsal fin; unbranched and branched rays of anal fin; branched pectoral and pelvic fin rays; lateral line scale rows, lateral line transverse rows (dorsal origin to anal), circumpeduncular scales, scale row at the anal fin base, pre- anal scales and principal caudal fin rays. The counts were routinely taken from the right side of the fish by observing it under a binocular microscope (WILD M38, Switzerland).

In order to identify any statistically significant differences among and between the populations for each biometric character, one-way analysis of variance (ANOVA) was performed [9] with Bonferroni correction, using SPSS (ver. 10.0) statistical package. In addition, size-adjusted data was standardized and computed to Principal Component Analysis (PCA) scores programmed with TPS software (ver. 1.40). The approach on PCA was to reduce dimensions by calculating the Eigen values and Eigen vectors of the covariance or correlation matrix and project the data orthogonally into space spanned by the Eigen vectors corresponding to the largest Eigen value. Clustering and scatter plots were generated using PAST (ver. 1.89) statistical package following Hammer *et al.* (2001) [10].

3. Results

The detailed analyses of the morphometric traits of the three populations indicated that the coefficient of variation (CV) varied from 5.70 to 35.68 (Table 1). Comparatively low values observed for the multivariate generalized coefficient of variation (CVp) in each population (Table 1) indicated a relatively low intra-population variation within the species. On comparison of all the three populations of *O. carnaticus*, the univariate analysis of variance (ANOVA) of specimens showed significant differences in seven morphometric traits i.e., BD - Body Depth; PL - Peduncle Length; AFH - Anal Fin Height; DFL - Dorsal Fin Length; UJL - Upper Jaw Length; HD - Head Depth and GW - Gape Width (Table 1). PCA performed with seven significant morphometric variables factoring the correlation matrix yielded four principal components accounting for 80.2% of the total variation in the original variables. The PCA of morphometric variables and the bivariate scatter plot indicated morphological homogeneity among the populations (Figure 1).

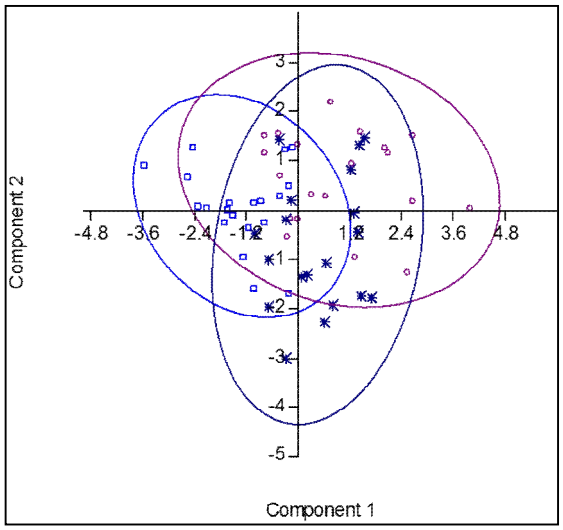

Fig 1: Scatter diagram based on PCA of significant morphometric variables among populations of *O. carnaticus* (Perungalathur- Blue squares; Pulicat- Violet Circles; Sriperumbudur- Blue Stars; Component 1- 30.04%; Component 2- 19.32%).

Table 1: Analysis of morphometric variables of different populations of *O. carnaticus* in Chennai, India

	Sriperumbudur (n = 19)	Pulicat (n = 20)	Perungalathur (n = 20)	F value
Variables+	CV	CV	CV	
SL	8.04	12.15	8.10	
%SL				
HL	11.58	8.25	5.70	0.74 NS
BD	5.78	6.50	7.28	3.42*
PL	18.54	15.84	13.15	5.01*
AFH	32.86	35.68	17.67	21.55**
CFL	8.43	8.01	8.13	0.70 NS
DFL	13.75	20.22	21.48	9.12 **
PcFL	8.20	12.75	7.67	3.05 NS
PlFL	9.97	15.80	13.20	0.61 NS
PD	7.16	6.51	6.74	1.81 NS
DBPcV	17.51	24.09	18.20	1.43 NS
%HL				
UJL	22.63	24.80	17.10	7.06 **
SntL	35.08	17.98	12.59	2.89 NS
HD	16.38	11.55	14.84	14.59 **
OW	15.34	12.06	8.18	2.11 NS
IOW	21.31	19.65	12.48	2.33 NS
HW	12.46	12.34	7.48	0.16 NS
GW	14.23	9.54	8.28	4.80 *
LJI	15.13	12.25	13.33	0.76 NS
CVp	10.98	10.72	10.07	

** Significant with Bonferroni corrected alpha value (F value- 6.62); * marginally significant (NS- not significant at 5% level)

(+ SL- Standard length; HL- Head Length; BD- Body Depth; PL- Peduncle Length; AFH- Anal Fin Height; CFL- Caudal Fin Length; DFL- Dorsal Fin Length; PcFL- Pectoral Fin Length; PlFL- Pelvic Fin Length; PD- Peduncle Depth; DBPcV- Distance between Pectoral fin and Vent; UJL- Upper Jaw Length; SntL- Snout Length; HD- Head Depth; OW- Orbit Width; IOW- Inter Orbital Width; HW- Head Width; GW- Gape Width; LJI- Lower Jaw to Isthmus)

The univariate analysis of variance (ANOVA) showed significant differences in 6 out of 13 meristic characters on comparison with the populations of *O. carnaticus* (Table 2). PCA of the significant variables of *O. carnaticus* yielded four principal components accounting for 82.88% of the total variation from the original variables. The fin ray counts indicated minor variation among the populations of *O. carnaticus*. Besides, bivariate scatter plot based on PCA revealed that meristic characters overlapped and the populations are not distinctly separated despite different ecological habitats (Figure 2). Careful examination of the vertebrae of *O. carnaticus* revealed the specimens were having a vertebral count of 28.

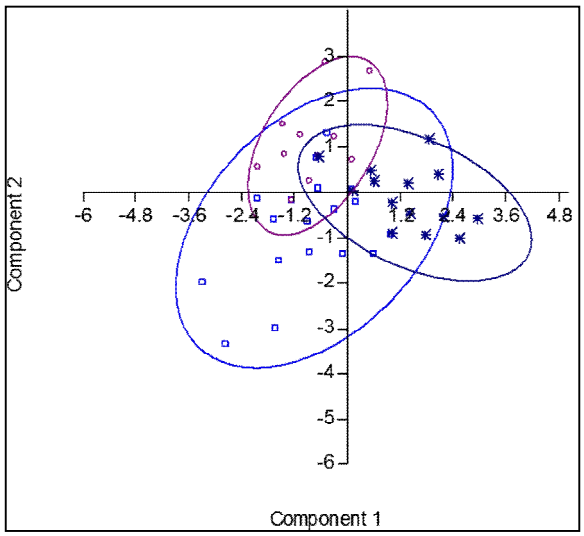

Fig 2: Scatter diagram based on PCA of significant meristic variables among populations of *O. carnaticus* (Perungalathur- Blue squares; Pulicat- Violet Circles; Sriperumbudur- Blue Stars; Component 1- 32.47%; Component 2- 21.82%).

Table 2: Analysis of meristic variables of the different populations *of O. carnaticus* in Chennai, India

Parameters	Sriperumbudur	Pulicat	Perungalathur	F value
Unbranched Dorsal Fin Rays	1.00±0.00(1-1)	1.00±0.00(1-1)	1.00±0.00(1-1)	Nil
Branched Dorsal Fin Rays	5.21±0.42(5-6)	6.00±0.00(6-6)	5.65±0.49(5-6)	22.12 **
Unbranched Anal Fin Rays	1.26±0.45(1-2)	2.15±0.37(2-3)	1.55±0.51(1-2)	20.12 **
Branched Anal Fin Rays	20.00±0.00(20-20)	19.95±0.69(19-22)	19.55±0.60(19-21)	4.23 **
Branched Pelvic Fin Rays	6.05±0.23(6-7)	6.00±0.00(6-6)	6.00±0.00(6-6)	1.05 NS
Branched Pectoral Fin Rays	11.47±0.51(11-12)	11.40±0.50(11-12)	11.20±0.52(10-12)	1.49 NS
Lateral Scale Rows	27.89±0.31(27-28)	27.95±0.22(27-28)	28.00±0.00(28-28)	1.10 NS
Upper Transverse Rows	3.95±0.23(3-4)	4.00±0.00(4-4)	4.15±0.37(4-5)	3.48 *
Upper Transverse Rows (Anal)	8.21±0.42(8-9)	8.00±0.00(8-8)	8.00±0.46(7-9)	2.23 NS
Circumpeduncular Scales	9.53±0.61(8-10)	9.45±0.51(9-10)	8.50±0.51(8-9)	21.71**
Anal Scale Rows	18.84±0.76(18-20)	17.95±0.39(17-19)	17.85±0.59(17-19)	16.12**
Pre Anal Scales	18.05±0.70(17-19)	17.60±0.59(16-18)	17.75±0.44(17-18)	2.96 NS
Caudal Fin Rays	12.89±0.31(12-13)	13.00±0.00(13-13)	13.00±0.00(13-13)	2.23 NS

* ($p < 0.05$); ** ($p < 0.01$) (NS- not significant at 5% level)

4. Discussion

Morphometric and meristic variations were reported in geographically separated populations belonged to the same species of fishes [3]. In the present study, though biometric characters were insufficient to differentiate the three populations as distinct species as per ANOVA, the PCA scatter plot indicated population overlap in meristic characters and the probable chances of inter mixing of populations due to flooding of drainages which cannot be ruled out. Watershed boundaries can be an organizing factor isolating genetic diversity in fishes, resulting in intra- species variation [11]. Further, the scale counts in upper transverse rows, circumpeduncular and anal scale rows in the present study, were found to be significantly varying between the three populations (Table 2), which were not reported earlier by Roberts (1998) [3] and Parenti (2008) [1] and hence could be included along with other variables for describing and distinguishing *Oryzias* species.

The seven morphometric traits with significant differences among the three populations in this study may be interpreted as the reflection of differences in habitats [12]. Further, the anal fin ray count range (Table 2) of Pulicat (highly saline habitat) population were higher compared to the other two populations (fresh water habitats). This could be a geographical variation as in the case of number of anal fin rays of *O. latipes* [13] or due to the influence of biotic and abiotic factors such as salinity which is unclear, since the study was restricted to biometrics.
The low CV value indicted low intra population variation in morphological characters and each population is homogenous. Similarly, Mamuris *et al*. (1998) [14] reported that the low CV value indicates high inheritability and consequently a limited influence of environmental variations on morphological variability. However, the dorsal and anal fin ray counts of the studied populations had shown considerable variation in the meristic variables from the findings of Roberts (1998) [3] and Parenti (2008) [1]. Parenti (2008) [1] had distinguished almost all *Oryzias* species based on meristic variables namely fin ray, vertebrae and scale counts, but most of these counts overlap from one species to another and doesn't aid much in species discrimination. Hence, identification of *Oryzias* species viz., *O. carnaticus* and *O. dancena* need not be solely based on the meristic characters reported by Roberts (1998) [3] and Parenti (2008) [1].

Also, the specimens from Sriperumbudur had an extended range of branched pelvic fin ray counts (6-7) while the baseline information restricted them to 6 in *O. carnaticus* as well as *O. dancena* (Table 2) [1,3]. Further, Parenti considered the number of dorsal fin rays as one of the strong candidate for species differentiation [1]. On the contrary, in our study, we have found significant differences in the counts of branched dorsal fin rays (Table 2) among the three populations which could be regarded as a suggestive evidence of variation among the three populations.

The study revealed that it is difficult to confirm *Oryzias* species found in India based on phenotypic characters, as some of the meristic characters such as anal and pectoral fin rays overlapped with *O. carnaticus* and *O. dancena* [1]. Besides, it clearly indicated that phenotypic characters alone will not be sufficient enough to confirm the identity of a species.

5. Conclusion

To conclude, the study clearly pointed out the variations in the biometric data of the populations of *O. carnaticus* present in and around Chennai within and between the populations as well as from the existing base line information. The findings/inferences highlight the limitations of biometric system as a standard method for classification of fishes. As the occurrence of certain variations were obvious among the variables distinguishing *O. carnaticus* from *O. dancena*, it is important to carry out extensive, periodic sampling comparing the species from different locations with biometrics and application of molecular markers comprising mitochondrial DNA, nuclear genes to confirm/resolve the taxonomic ambiguity and phylogeny of *Oryzias* species in the Indian subcontinent. It is pertinent to note that *Oryzias carnaticus* known to breed and thrive in rice field agro-ecosystem are larvivorous in nature and therefore, can act as an efficient bio-control agent against mosquito vectors transmitting Japanese encephalitis (JE) and malaria in rural areas. Hence, they play a crucial role in mosquito control/integrated vector management programme.

6. Acknowledgements

We thank the Director, National Institute of Malaria Research (ICMR) for providing the facilities and staff of IDVC Field Unit, NIMR Chennai for assistance in collection of the specimens. We also thank Dr. K. Rema Devi, Retired Scientist of Zoological Survey of India, Chennai in identifying the specimens.

7. References

1. Parenti LR. A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Beloniformes, Adrianichthyidae). Zoological Journal of the Linnean Society. 2008; 154: 494-610.
2. Menon AGK. *Indigenous larvivorous fishes of India*. Malaria Research Centre. Delhi, India, 1991, 66.
3. Roberts TR. Systematic observations on tropical Asian medakas or ricefishes of the genus *Oryzias*, with descriptions of four new species. Ichthyological Research. 1998; 45:213-224.
4. Gunawickrama KBS. Intraspecific variation in morphology and sexual dimorphism in *Puntius singhala* (teleostei: cyprinidae). Ceylon Journal of Science (Biological Sciences). 2008; 37(2):167-175.
5. Jayaram KC. The Freshwater Fishes of the Indian Region. Second Edition. Narendra Publishing House, Delhi, India, 2010, 616.
6. Reist JD. An empirical evaluation of several univariate methods that adjust for size variation in morphometric variation. Canadian Journal of Zoology. 1985; 63:1429-1439.
7. Lleonart J, Salat J, Torres GJ. Removing allometric effects of body size in morphological analysis. Journal of Theoretical Biology. 2000; 205:85-93.
8. Van Valen L. The statistics of variation. Evolutionary Theory. 1978; 4:35-43.
9. Zar JH. *Biostatistical Analysis*. 2nd edition, Prentine-Hall, Englewood Cliffs, NJ, 1984, 718.
10. Hammer Ø, Harper DAT, Ryan PD. PAST: Palaeontological Statistics Software Package for

education and data analysis. Palaeontologia Electronica. 2001; 4(1):1-9.
11. Loxterman JL, Keeley ER. Watershed boundaries and geographic isolation: patterns of diversification in cutthroat trout from western North America. BMC Evolutionary Biology. 2012; 12:38. doi:10.1186/1471-2148-12-38.
12. Mohaddasi M, Shabanipour N, Abdolmaleki S. Morphometric variation among four populations of Shemaya (*Alburnus chalcoides*) in the south of Caspian Sea using truss network. The Journal of Basic and Applied Zoology. 2013; 66(2):87-92.
13. Egami N. Studies on the variation of the number of anal-rays in *Oryzias latipes*. I. Geographical variation in wild populations. Japanese Journal of Ichthyology. 1953; 3(1, 2):33-35; 87-89.
14. Mamuris Z, Apostolidis AP, Theodorou AJ, Triantaphyllidis C. Application of random amplified polymorphic DNA (RAPD) markers to evaluate intraspecific genetic variation in red mullet *(Mullus barbatus)*. Marine Biology. 1998; 132:171-178.