Morphometric and meristic variation of congeneric sciaenid fishes *Otolithes cuvieri* Trewavas, 1974 and *Otolithes ruber* (Schneider, 1801) from Maharashtra, west coast of India

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Two closely related species *Otolithes cuvieri*, Trewavas, 1974 and *Otolithes ruber*, (Schneider, 1801) have been differentiated based on morphometric and meristic traits. A simple yet useful criterion based on a pair of canine teeth present on the upper and lower jaw as well as position of the mouth is currently used to differentiate two congeneric sciaenid fish species the *O. cuvieri* and *O. ruber*. Findings of the present study indicated that simply two morphometric and meristic characters are sufficient to differentiate these two species. MANOVA (Multivariate analysis of variance) and stepwise discriminant function were used to decide the morphometric traits, significant for differentiation of the species of family Sciaenidae. Discriminant function analysis revealed that 98 % of the species were correctly classified based on five morphometric characters namely Prepectoral fin length (PPFL), Pre-anal fin length (PAL), Post orbital head length (POHL), Post anal fin length (POAL) and Body depth (BD). The m-transformed morphometric traits were found to be useful tools in generating canonical variables in differentiating the species. The first canonical variables showed altogether 98 % variance. The scatter plots by first three canonical variables have well differentiated the species. Two meristic characters such as the number of gillrakers present on lower limb of first gill arch and figure of arborescent appendages on the swim bladder are important in differentiation of these species.

[**Keywords**: Congeneric; Morphometric; Multivariate analysis; Sciaenid; *Otolithes cuvieri; Otolithes ruber,*]

Introduction

Identification of a species is a primary step towards any research work. Morphological characters such as morphometric measurements with meristic counts are well defined valid tools for identifying the fish specimen known as morphological systematics[1]. Morphometric traits are measurements of different external body parts of an organism and meristic counts mean anything that can be counted[2]. Morphometric and meristic features of fish have been used in morpho-taxonomic identification, stock identification and also in differentiating two closely related species[3-5].

The family Sciaenidae consists of a diverse group of species with a total of 70 genera and 270 species[6] that are scattered along the Indian, Pacific as well as Atlantic Oceans[7]. In Indian Ocean, there are reports of 48 species of sciaenids belonging to 27 genera[8]. Majority of taxonomic studies of the sciaenids have been made based on the external features such as the position of mouth, body form, size of second anal spine, dentition pattern and sensory pores on snout and lower jaw[9]. Chao[10] utilized morphology of swimbladder and otolith to establish evolutionary groupings. Trewavas[11] identified and differentiated the sciaenids found in the west coast of Africa based on morphology of swimbladder and otoliths. Chu *et al.*[12] also reported sciaenid species found in China and its adjacent sea and identified based on the swimbladder structure, sensory pore on snout and hard parts such as otolith morphometry, lower jaws shape and dentition pattern of jaws. Mohan[13,14] have studied a few sciaenid collected from Indian water and clarified some of taxonomic ambiguities based on otoliths shape and swimbladders structure and arborescent appendages. Currently, species are differentiated based on morphometrics, meristic characteristics and swimbladder structure, where variability within the characters leads to significant overlap among species and increases complexity of differentiation. Therefore, statistical tools are used to differentiate the species or group based on the morphometric traits. In view of this, present investigation was aimed at studying the

morphometrics and meristics of the *O. cuvieri* Trewavas, 1974 and *O. ruber* (Schneider, 1801), to (i) examine intra-specific variations and (ii) identify the most significant morphometric and meristic traits for species differentiation.

Materials and Methods

Sampling

In the present study, specimens of *Otolithes cuvieri* and *O. ruber* were collected from by-catch of commercial fish landings at New Ferry Wharf, Sassoon Docks, Versova in Mumbai, and Mirkarwada in Ratnagiri coast of India. The specimens collected at landing centers were transported to laboratory in ice. At laboratory, twenty morphometric and four meristic variables were measured in each specimen, after thawing. The morphometric traits were measured using a digital caliper nearest to 0.1 mm while meristic characters were counted by simple visual inspection under magnifying lens. The meristic traits were count of dorsal fin soft rays and spiny rays, count of gillrakers on the ceratohypobranchial portion of the primary gill arch (ascending) and count of arborescent appendages present on the swimbladder.

Statistical analysis

Morpho-taxonomic traits were subjected to various statistical analytical tools by using statistical software SAS[15]. Since the morphometric characters are different in origin (such as continuous) and biologically more susceptible to the environment, while meristic characters are discrete and fixed early in development, they were analysed separately[16]. Statistical analysis showed that the size of fish and morphometric measurement were highly correlated. Therefore, to avoid error in differentiation of species due to size dependent variables, the morphometric data was transformed before further analysis. The variations in morphometric traits due to allometric growth were corrected as per Reist[17]. The formula used for sample data transformation:

$$M_{trans} = \log M - \text{ß} (\log SL - \log SL_{mean})$$

Where, M_{trans}: transformed measurement, M: original measurement, SL: standard length of fish, SL_{mean}: location wise mean SL, ß: within group slope regressions of the logM against logSL.

To ascertain effective removal of influence of size dependent morphometric variables such as standard length of fish, transformed variable was observed and correlation coefficient was estimated for each species. MANOVA (Multivariate analysis of variance) was used to assess the morphological divergence between the groups. To test if there is any difference among the pre-defined groups, a SDA (stepwise discriminant analysis) was employed with the selected traits after factor analysis. A stepwise MDA (multivariate discriminant analysis) was used separately for morphometric and meristic data to categorize the group of variables that best separate the groups by using SAS. CDA (Canonical discriminant analysis) is a dimensional reduction tools related to the SDA. The pooled within canonical structure and pooled within class standardized canonical coefficient were used to find out the contribution of each variable to discriminant function. Three canonical variables were generated to illustrate the separation of the species. SDA and CDA were performed with the program SAS 9.2 STEPDISC and DISCRIM procedures.

Results and Discussion

The observed descriptive analysis of each morphometric for the species is elaborated in Table 1. Correlation between SL and other morphometric characters such as TL: Total length; CFL: Caudal fin length; HL: Head length; OD: Orbit Diameter: SNL: Snout length; POHL: Post orbital head length; DFBL: Dorsal fin base length; PDFBL: Pre-dorsal fin base length; PODFBL: Post dorsal fin base length; PFL: Pectoral fin length; PPFL: Pre-pectoral fin length; POPFL: Post-pectoral fin length; PAL: Pre-anal fin length; POAL: Post anal fin length; CPL: Caudal peduncle length; CD: Caudal depth; BD: Body depth; IOD: Inter-orbital distance; IINDASL: Second anal fin spine length for these two species were studied, which were positive and highly significant. Hence transformation of morphometric characters was done to avoid influence of size on species differentiation. The proportion (%) of head length, orbit diameter, snout length, caudal depth, caudal peduncle length, body depth, inter orbital distance and second anal spine length to the standard length or head length of all eleven proportions between the two species were overlapping (Table 2). MANOVA suggested that there were significant difference (MANOVA, Wilks' Lambda < 1.00, df =18, 69.8; P< 0.001) in at least one of the morphometric traits of these two species (Table 3).

Stepwise discriminant analysis identified 5 of the initial 20 morphometric characters as the most important characters for differentiating species;

therefore these characters were incorporated into Discriminant Function analyses (DFA). The most important variables for discriminating intra specific variation of *O. cuvieri* and *O. ruber* was PPFL with

partial r^2 0.9475, followed by PAL, POHL, POAL and BD (Table 4). Therefore, only PPFL, PAL, POHL, POAL and MBD were used to produce canonical variables. Five canonical variables were produced to

Table 1— Descriptive statistics of 20 Morphometric traits of *O. cuvieri* and *O. ruber*

	O. cuvieri (N = 74)				*O. ruber* (N = 27)			
Traits*	Min	Max	Mean ± SE	CV %	Min	Max	Mean ± SE	CV %
TL	12.5	27.2	16.9 ± 0.5	17.01	13.2	28.1	19.7 ± 2.0	27.15
SL	10.2	22.8	14.1 ± 0.4	17.72	11.0	24.5	16.8 ± 1.8	28.57
CFL	2.2	4.4	2.8 ± 0.1	15.40	2.2	3.7	2.9 ± 0.2	19.88
HL	3.3	6.6	4.3 ± 0.1	16.26	3.1	7.0	4.8 ± 0.5	27.67
OD	0.7	1.6	1.0 ± 0.0	17.45	08	1.2	1.0 ± 0.6	17.15
SNL	0.6	1.3	0.8 ± 0.0	18.94	0.7	1.5	1.0 ± 0.1	30.36
POHL	1.6	3.7	2.4 ± 0.1	18.52	1.7	4.0	2.8 ± 0.3	27.64
DFBL	5.4	12.7	7.7 ± 0.2	18.12	5.9	14.2	9.4 ± 1.1	31.13
PRDFL	3.3	9.8	4.9 ± 0.2	29.49	3.5	7.5	5.3 ± 0.5	28.00
PODFL	1.5	3.2	2.1 ± 0.1	19.11	1.6	3.6	2.3 ± 0.3	34.08
PFL	2.0	4.7	2.9 ± 0.1	21.00	2.2	4.8	3.8 ± 0.4	29.03
PPFL	3.5	7.2	4.5 ± 0.1	15.50	3.4	6.2	4.7 ± 0.4	22.12
POPFL	6.7	15.2	9.3 ± 0.3	18.90	7.2	16.5	11.4 ± 1.3	30.83
PAL	7.1	15.8	9.9 ± 0.3	17.93	7.4	17.2	11.8 ± 1.3	29.90
POAL	2.5	5.7	3.5 ± 0.1	20.48	2.6	6.0	4.1 ± 0.4	28.27
CPL	1.0	2.0	1.3 ± 0.1	17.89	0.9	2.2	1.5 ± 0.2	27.95
CD	1.0	2.2	1.3 ± 0.0	20.34	0.9	2.2	1.4 ± 0.2	31.19
BD	2.1	6.1	3.8 ± 0.1	18.75	2.8	7.3	4.4 ± 0.6	34.58
IOD	0.9	1.7	1.8 ± 0.0	12.75	0.9	1.7	1.2 ± 0.1	27.74
IINDASL	0.8	1.1	0.8 ± 0.0	8.93	0.7	1.1	0.9 ± 0.0	14.29

*TL: Total length; SL: Standard length; CFL: Caudal fin length; HL: Head length; OD: Orbit Diameter: SNL: Snout length; POHL: Post orbital head length; DFBL: Dorsal fin base length; PDFBL: Pre-dorsal fin base length; PODFBL: Post dorsal fin base length; PFL: Pectoral fin length; PPFL: Pre-pectoral fin length; POPFL: Post-pectoral fin length; PAL: Pre-anal fin length; POAL: Post anal fin length; CPL: Caudal peduncle length; CD: Caudal depth; BD: Body depth; IOD: Inter-orbital distance ; IINDASL: Second anal fin spine length.

Table 2— Proportion (%) of head length, orbit diameter, snout length, caudal depth, caudal peduncle length, body depth, inter orbital distance and second anal spine length to the standard length or head length for *O. cuvieri* and *O. ruber*

Species	% (HL/SL)	% (OD/SL)	% (OD/HL)	% (SNL/SL)	% (CD/SL)	% (CPL/SL)	% (BD/SL)	% (IOD/SL)	% (IOD/HL)	% (IIASL/SL)	% (IIASL/HL)
O. cuvieri	28-33	6-9	22-26	5-7	8-10	8-10	20-31	7-9	23-31	6-7	16-21
O. ruber	27-32	5-7	17-25	5-6	8-9	8-9.5	24-30	6-7	21-29	4-6	16-23

Table 3 — Multivariate analysis of variance of m-transformed morphometric data for twelve different species of Sciaenidae

Statistics	Value	F-Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.000177536	62.57	18	69.8	<.0001
Pillai's Trace	1.982862552	58.40	18	20	<.0001

Table 4 — Stepwise selection summery Intra-specific classification of *O. cuvieri* and *O. ruber* (STEPDISC Procedure)

Step	Characters*	Partial R-Square	F Value	Pr > F	Wilks'Lambda	Pr<Lambda	Average Squared Canonical Correlation	Pr>ASCC
1	PPFL	0.9475	1569.25	<.0001	0.05252840	<.0001	0.94747160	<.0001
2	PAL	0.3470	45.70	<.0001	0.03430108	<.0001	0.96569892	<.0001
3	POHL	0.1679	17.16	<.0001	0.02854076	<.0001	0.97145924	<.0001
4	POAL	0.2216	23.91	<.0001	0.02221714	<.0001	0.97778286	<.0001
5	BD	0.0648	5.75	0.0187	0.02077662	<.0001	0.97922338	<.0001

know the optimal combination of variables required to differentiate between the species. The pooled within canonical structure and pooled within-class standardized canonical coefficients were also listed (Table 5). Canonical variable 1 had the highest correlation with PPFL (3.5214) followed by PAL (2.1401), POAL (1.3011), BD (0.9546) and POHL (-2.2367); therefore, differentiation of the species on the canonical variable 1 was mainly due to differences in the PPFL followed by PAL, POAL, BD and POHL. Canonical variable 2 had the maximum correlation with BD (0.9123), followed by PPFL (0.4926). Canonical variable 3 was highly correlated with PPFL (1.9561) followed by PAL (1.0304). The total variance of first three canonical variables explains 98 % of variance between the species (Table 6). The both species were separated based on these three selected canonical variables shown in Figures 1, 2 and 3, both have distinctly separated *O. cuvieri* and *O. ruber*. The

number of dorsal spiny rays was found to be same (11) in both the species studied while, number of dorsal soft rays varied from 29-31 in *O. cuvieri* while 29-30 in *O. ruber* (Table 7). Number of gillrakers present on lower limb of first arch was 12-15 in *O. cuvieri* but only 10 in *O. ruber* (Table 8). Both the species have similar carrot shaped swimbladder but presence of number of arborescent appendages on the swimbladder ranged between 32-35 and 28-29, respectively (Table 9). Thus, these characters do not contribute to differentiate these species.

Morphometric character analysis demonstrated that although the two species are less distinct, discriminant function analysis reveals that 98 % of species were correctly classified. The PPFL, PAL, POHL, POAL and to some extent BD were found to be important discriminating morphometric characters in the present study with *O. cuvieri* having paired canine teeth on

Table 5— Canonical structure and standardized canonical coefficients

	Pooled Within Canonical Structure			Pooled Within-Class Standardized Canonical Coefficients		
	Can1	Can2	Can3	Can1	Can2	Can3
PPFL	3.5214384	0.4926813	1.95611151	0.889000412	0.11356513	0.45089189
PAL	2.1401358	-0.9423030	1.03047409	0.483332800	-0.23512291	2.00375958
POHL	-2.2367192	-3.2732344	0.58364886	-1.663728506	2.43471484	0.43413282
POAL	1.3011849	0.40816898	0.01857270	0.747935860	0.23462013	0.01067582
BD	0.9546768	0.91239085	0.28897871	0.748333618	0.71518728	-0.22651908

Table 6 — Canonical discriminant analysis of the DISCRIM procedure

Canonical variables	Canonical Correlation	Adjusted Canonical Correlation	Approximate Standard Error	Squared Canonical Correlation	Eigenvalue	Difference	Proportion	Cumulative
1	0.982599	0.981332	0.001572	0.975253	66.8127		1.0000	1.0000
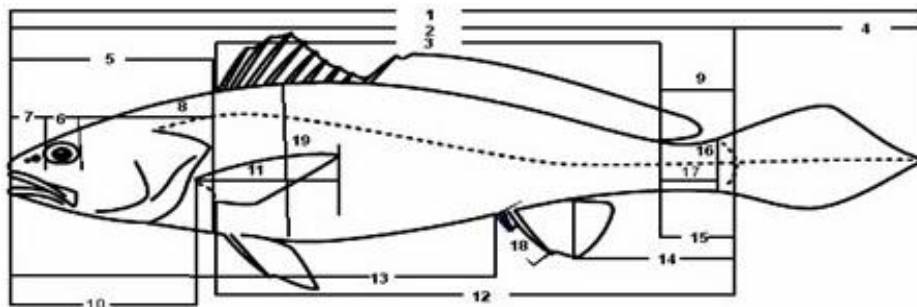
1. Total length (TL)
2. Standard length (SL)
3. Dorsal fin base length (DFBL)
4. Caudal fin length (CFL)
5. Head length (HL)
6. Orbit diameter (OD)
7. Snout length (SnL)
8. Post orbital head length (POHL)
9. Pre-dorsal fin base length (PDFBL)
10. Pre-pectoral fin length (PPFL)
11. Pectoral fin length (PFL)
12. Post pectoral fin length (PoPFL)
13. Pre-anal fin length (PAL)
14. Post anal fin length (POAL)
15. Post dorsal fin base length(PODFBL)
16. Caudal depth (CD)
17. Caudal peduncle length (CPL)
18. Second anal fin spine length (IINDASL)
19. Body depth (BD)
20. Inter-orbital distance (IOD)

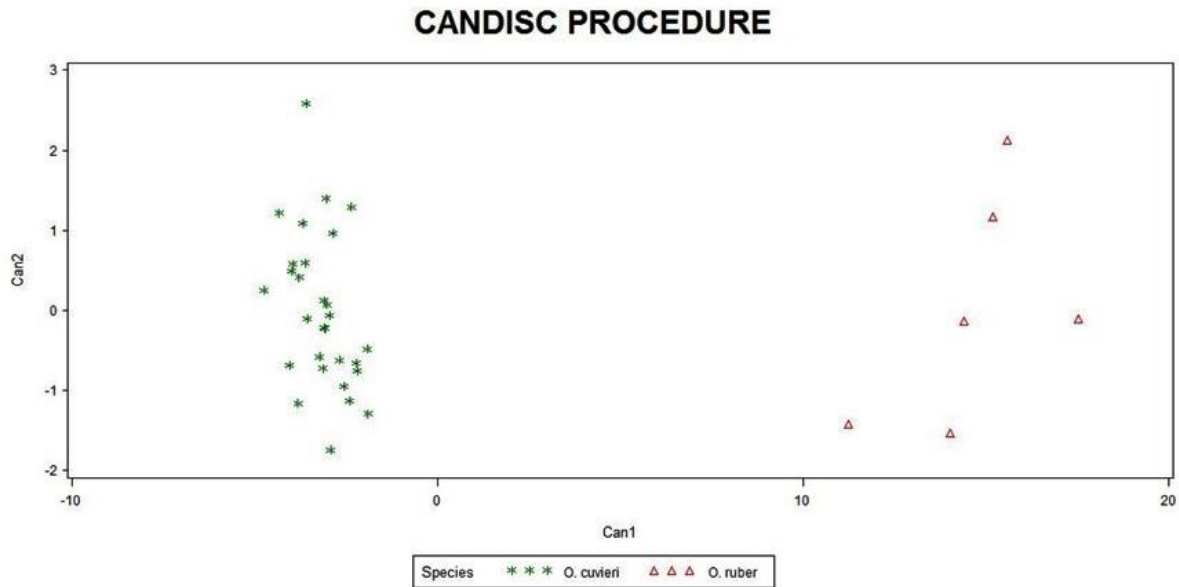Fig. 1 — Measurement of morphometric traits of fish

CANDISC PROCEDURE

Fig. 2— Plot of canonical variables 2 and 1 of *O. cuvieri* and *O. ruber*
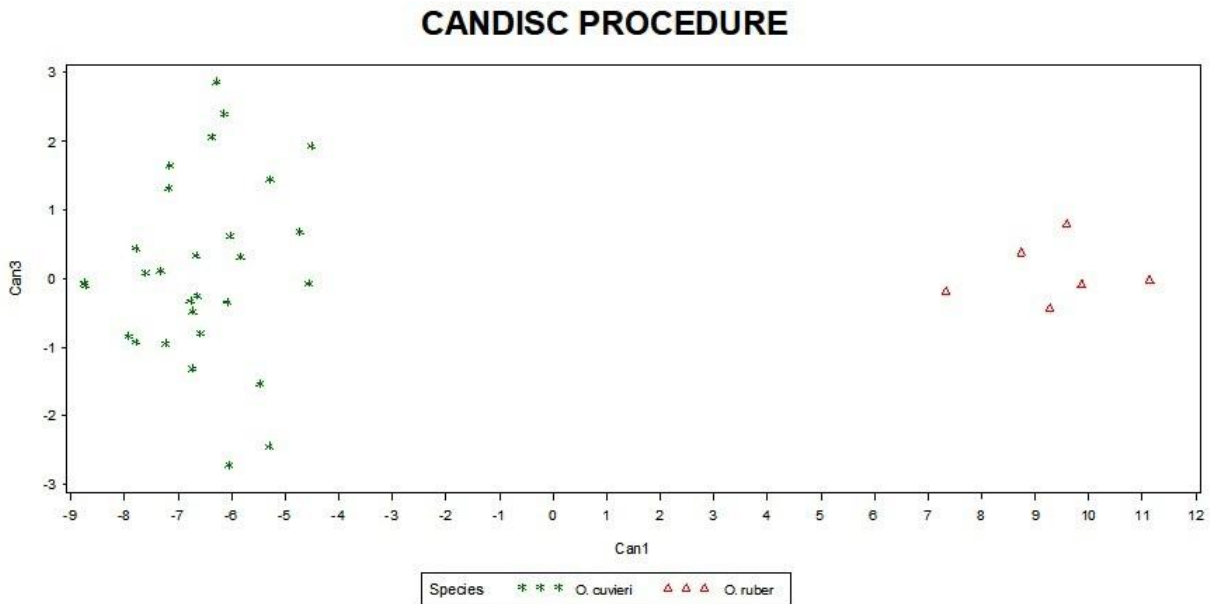
CANDISC PROCEDURE

Fig. 3— Plot of canonical variables 3 and 1 of *O. cuvieri* and *O. ruber*

Table 7— Frequency distribution of number of dorsal spiny and soft fin rays

Species	Dorsal spine		Dorsal soft rays										
	10	11	22	23	24	25	26	27	28	29	30	31	32
O. cuvieri	0	32100	0	0	0	0	0	0	0	2990.63	26.25	13.13	0
O. ruber	0	7100	0	0	0	0	0	0	0	457.14	342.86	0	0

upper and lower jaw with terminal mouth and *O. ruber* with upturned mouth. Morphometric characters are frequently aaplied in fish biology, systematic level, to quantify discreteness and establish relationship among taxonomic groups[18]. Both taxonomic classification of organisms and understanding of species diversity were historically based on morphometric analysis[19]. According to

Table 8 — Frequency distribution of number of gillrakers present on lower limb of first arch

Species	Number of Gillrakers present on lower limb of first arch					
	10	11	12	13	14	15
O. cuvieri	0	0	13.13	1134.38	1856.25	26.25
O. ruber	7100	0	0	0	0	0

Table 9 — Frequency distribution of number of arborescent appendages on the swim bladder

Species	Number of arborescent appendages present on the swimbladder							
	28	29	30	31	32	33	34	35
O. cuvieri	0	0	0	0	13.13	1134.38	1856.25	26.25
O. ruber	685.71	114.29	0	0	0	0	0	0

Ihssen *et al.*[16] morphometric analysis is basically based on continuous set of measurement of size and shape variable data whereas meristics are discrete.

Analyses of meristic characters revealed that *O. cuvieri* and *O. ruber* can be differentiated based on count of gillrakers on lower limb of first gill arch and count of arborescent appendages present on the swimbladder, also supported by Trevawas[19,20]. Trevawas[20] observed 29-32 dorsal soft rays for *O. cuvieri* while 27-30 for *O. ruber;* similar result was also observed by FAO[21]. Swimbladder has been found to be of taxonomic interest[20,22]. Morphometric traits in contrast with meristic were found higher ability to differentiate correctly the species, similarly many authors have found discriminant function analyses is suitable for correctly classifying the species[23-26]. The present study has uncovered some morphological (i.e., morphometric and meristic) distinctions between the two closely related sciaenid fishes, using multivariate techniques as reported for other marine vertebrates and invertebrates[24,27-29,]. The biometric analysis including morphometric and meristic traits, has been used by several authors[30-32]. This study demonstrates that *O. cuvieri* and *O. ruber* from Maharashtra coastal waters were differentiated based on both morphometric as well as meristic characters.

Conclusion

The present investigations confirm the authenticity of biometric approach in species identification as well as differentiation between the species. This study has demonstrated that both morphometric and meristic variations exist between the two species. The morphometric and meristic traits of the species indicate the trait should be used as complementary not alternative, to the same ambiguity. There is also possibility of variability among these traits in different habitat and prey predatory relationship.

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