

Species discrimination of clupeoid fishes (Pisces: Clupeiformes) from Andaman waters based on morpho-meristic characters

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Analysis of morphometric and meristic traits of 11 clupeoids occurring along Andaman coast showed successful differentiation of them based on morphological variables. Higher F-ratio for body depth, mouth length and dorsal fin base signifies their role in species separation either alone or in combination with other morphometric characters. Stepwise discriminant function analysis included all ten considered variables showing their taxonomic significance with body depth, mouth length and dorsal fin base, as the most important discriminating characters. The first two discriminant functions accounted for the explanation of 85.01 % of total variation. Cumulative figure reaches 92.02% when third root is also considered. Means of canonical scores signify the sufficiency of Root 1, Root 2 and Root 3 in discriminating all the considered species except the two members of genus *Sardinella*. S DFA gives correct classification in 94.60% cases. Meristic features were found to be overlapping except in few instances and are found to discriminate only higher taxa.

[**Key words:** Clupeoids, morphometry, meristic, taxonomy, Andaman]

Introduction

Clupeoids fishes are among most dominant species which are landed in huge quantity throughout the year in Andaman Island. These groups of fishes are locally called as “tarni” and preferred for fresh consumption due to its all-time availability and low price. Andaman seas remain rough for 8-9 months, out of this reason very few fishermen goes for fishing in deeper water. Clupeoids group of fishes inhabits shallow water and are easily accessible by local fishermen. In view of major contribution of clupeoids to fish landings, established taxonomy of this group is important.

Importance of taxonomy lies in the fact that it makes diversity accessible to other biological disciplines¹. In addition to that, understanding α -taxonomy is imperative to resource management and biodiversity conservation². Fascinating diversity of fishes coupled with morphological switches across sexes

and geographic regions makes their identification and taxonomy challenging. Despite several attempts made in past^{3,4,5,6,7,8,9,10,11,12}, the taxonomic clarity of these group of fishes is still lacking.

For the differentiation of taxa several features are being used and are referred as taxonomic characters. Characters are variation among homologous features and it is taxonomically important only if it shows variation among taxa under consideration¹³. Morphometric and meristic characters were among the easiest recordable features and form important characters for field identification. Meristic characters correspond to countable features of a taxon whereas morphometric characters refer to the features capable of being measured. Though several morphometric characters featured in descriptions of Clupeoid fishes, a dedicated attempt exploring the possibility of discrimination of members of this group solely morphometric

features are scanty. A present study is an attempt to assess the utility of selected morphometric and meristic characters in species discrimination of clupeoids from waters of Andaman.

Materials and Methods

The present study was based on samples collected from Junglighat & Dignabad Fish Landing Centre of Port Blair, the major landing centres of Andaman & Nicobar Islands (Fig 1). Fish markets were also explored for samples to increase the coverage of species and specimen of different size range which is probably the most important aspect of morphological studies. Specimens belonging to 2 (two) families Clupeidae and Pristigasteridae and seven genera were collected during August 2014 to July 2015. A total of 13 species belonging to the above

mentioned families were collected during the sampling period. Since *Sardinella brachysoma* Bleeker, 1852 and *S. gibbosa* (Bleeker, 1849) were collected in fewer numbers, they are not included in statistical analysis. 48 *Sardinella albella* (Valenciennes, 1847), 21 *S. fimbriata* (Valenciennes, 1847), 31 *Herklotsichthys quadrimaculatus* (Ruppell, 1837), 40 *Amblygaster sirm* (Walbaum, 1792), 14 *A. leiogaster* (Valenciennes, 1847), 31 *Dussumieria acuta* Valenciennes, 1847, 68 *Anodontostoma chacunda* (Hamilton-Buchanan, 1822), 15 *Anodontostoma selangkat* (Bleeker, 1852), 23 *Ilisha melastoma* (Schneider, 1801), 13 *I. sirishai* Seshagiri Rao, 1975 and 13 *Pellona ditchela* Valenciennes, 1847 specimens were analysed for current study.

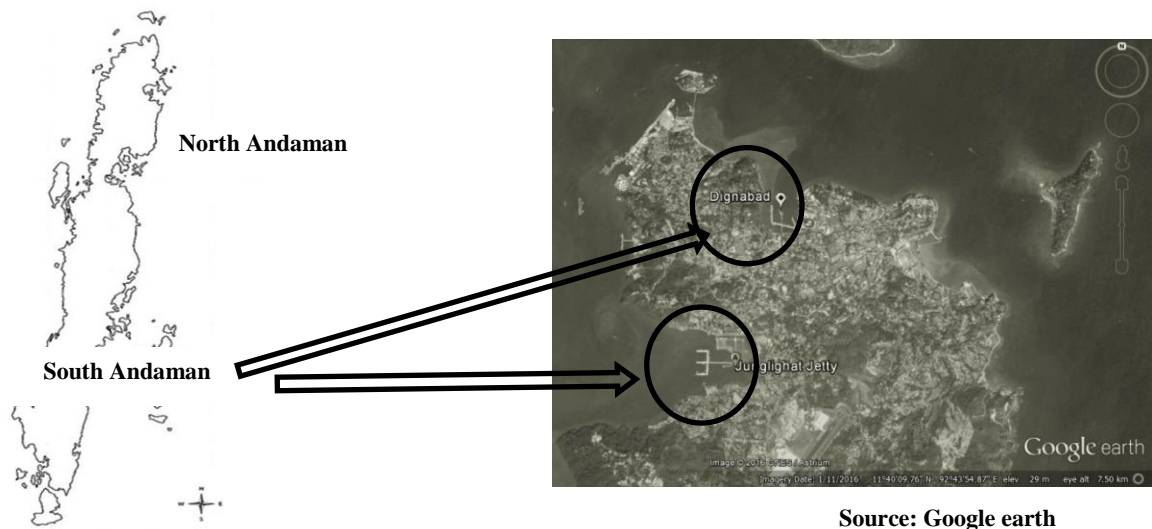


Fig. 1. Dignabad and Junglighat fish landing centres of Andaman, India (major sampling sites)

Stepwise discriminant function analysis (SDFA) forms an ideal multivariate approach for species differentiation based on morphometric variables and exploration of taxonomically important variables. For the current study, 18 morphometric variables were recorded, scaled to standard length and log transformed. Maximum number of variables which can be used in SDFA should be one less than minimum number of cases among all groups (12 in present). Hence, the log transformed variables were subjected to factor analysis to shortlist the important variables based factor loading of more than 0.7 on at least one of the factors.

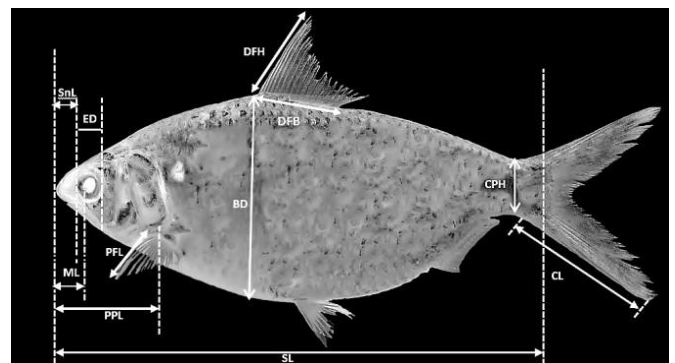


Fig. 2. Morphometric measurements of Clupeoid Fishes

A total of 10 variables namely snout length (SNL), eye diameter (ED), body depth (BD), mouth length (ML), pre pectoral length (PPL), caudal length (CL), caudal peduncle height (CPH), dorsal fin height (DFH), dorsal fin base (DFB) and pectoral fin length (PFL) (Fig: 2) were selected. An average or mean for morphometric variables were calculated and tabulated. The very purpose of doing this is to facilitate comparison with past literatures and form baseline

information for subsequent workers. An ANOVA was also performed for all the selected morphometric ratios to explore difference in means of the variables across different genera and species. Number of rays on dorsal, pectoral, ventral, anal and caudal fin, gill rakers on first gill arch, belly scutes and branchiostegal rays were also accessed for possibility of discriminating the species. All statistical analysis was performed using statistical software package Statistica ver. 8.0¹⁴.

		Table: 1. Means of morphometric proportions of eleven studied clupeoid fishes											F-Val.	Sig.
		A	B	C	D	E	F	G	H	I	J	K		
SNL/SL	Mean	0.0703	0.0692	0.0842	0.0827	0.0830	0.0944	0.0585	0.0745	0.0646	0.0898	0.0833	102.93	<0.001
	Std. Er	0.0009	0.0014	0.0012	0.0010	0.0017	0.0012	0.0008	0.0017	0.0014	0.0018	0.0018		
ED/SL	Mean	0.0703	0.0697	0.0912	0.0636	0.0699	0.0776	0.0718	0.0768	0.0945	0.0964	0.0952	94.845	<0.001
	Std. Er	0.0009	0.0013	0.0011	0.0010	0.0016	0.0011	0.0007	0.0016	0.0013	0.0017	0.0017		
BD/SL	Mean	0.3027	0.3128	0.2657	0.2332	0.2302	0.2429	0.4378	0.4442	0.3619	0.3346	0.3777	482.84	<0.001
	Std. Er	0.0029	0.0044	0.0036	0.0032	0.0054	0.0036	0.0024	0.0052	0.0042	0.0056	0.0056		
ML/SL	Mean	0.1025	0.1049	0.1339	0.0911	0.0812	0.0908	0.0907	0.0926	0.1216	0.1481	0.1430	294.74	<0.001
	Std. Er	0.0009	0.0014	0.0011	0.0010	0.0017	0.0011	0.0008	0.0016	0.0013	0.0017	0.0017		
PPL/SL	Mean	0.2488	0.2402	0.2731	0.2470	0.2461	0.2744	0.2407	0.2765	0.2488	0.2849	0.2831	64.041	<0.001
	Std. Er	0.0016	0.0024	0.0020	0.0017	0.0029	0.0020	0.0013	0.0028	0.0023	0.0030	0.0030		
CL/SL	Mean	0.2909	0.2794	0.2747	0.2185	0.2328	0.2734	0.3213	0.3472	0.2775	0.2934	0.3615	158.91	<0.001
	Std. Er	0.0024	0.0037	0.0030	0.0027	0.0045	0.0030	0.0020	0.0043	0.0035	0.0047	0.0047		
CPH/SL	Mean	0.0959	0.1025	0.0998	0.0759	0.0774	0.1017	0.1308	0.1253	0.1027	0.1065	0.1007	129.32	<0.001
	Std. Er	0.0013	0.0019	0.0016	0.0014	0.0024	0.0016	0.0011	0.0023	0.0018	0.0025	0.0025		
DFH/SL	Mean	0.1480	0.1568	0.1641	0.1275	0.1243	0.1274	0.1908	0.1973	0.1619	0.1743	0.1525	126.00	<0.001
	Std. Er	0.0018	0.0027	0.0022	0.0019	0.0033	0.0022	0.0015	0.0032	0.0026	0.0034	0.0034		
DFB/SL	Mean	0.1520	0.1497	0.1578	0.1158	0.1351	0.1528	0.1989	0.1889	0.1291	0.1629	0.1375	262.79	<0.001
	Std. Er	0.0014	0.0021	0.0017	0.0015	0.0025	0.0017	0.0011	0.0024	0.0020	0.0026	0.0026		
PFL/SL	Mean	0.1714	0.1717	0.1850	0.1496	0.1473	0.1479	0.2310	0.2214	0.1727	0.1588	0.1560	179.60	<0.001
	Std. Er	0.0019	0.0029	0.0023	0.0021	0.0035	0.0023	0.0016	0.0034	0.0027	0.0036	0.0036		

Note: *S. albella* (A), *S. fimbriata* (B), *H. quadrimaculatus* (C), *A. sirm* (D), *A. leiogaster* (E), *D. acuta* (F), *A. chacunda* (G), *A. selangkat* (H), *I. melastoma* (I), *P. ditchela* (J), *I. sirishai* (K)

Results

Means and standard errors for the ten selected morphometric ratios are tabulated for comparison across species (Table: 1). F-values and significance values (Sig.) based on univariate ANOVA is also presented to highlight the relative taxonomic importance of the morphometric variables. Highest F-value was observed for BD/SL (482.84) followed by ML/SL (294.74) and DFB/SL (262.79) reflecting their taxonomic utility in separating species. Species belonging to genus *Sardinella* are characterized by distinctly smaller snout length (SNL/SL) compared to species belonging to genus *Herklotsichthys*, *Amblygaster*, *Dussumeria* and *Pellona*. For most of the characters, *S. albella* and *S. sirm* showed close resemblance and no single character was found to be potent enough in clearly discriminating these two species. Further

Herklotsichthys quadrimaculatus is distinguished from the members of genus *Ilisha* and *Pellona* in having smaller body depth (BD/SL) of 0.265 compared to more than 0.330 for the members of other two genera. Distinctly higher mean value for DFB/SL (0.163) has been recorded for *P. ditchela*, separating the species from the members of genera *Ilisha* (<0.137). Two members of genus *Ilisha*, *I. melastoma* and *I. sirishai* showed prominent difference in several morphometric characters with higher values for SNL/SL (0.083>0.064), ML/SL (0.143>0.121) and CL/SL (0.361>0.277) in *I. sirishai*. Genus *Anodontostoma* is probably the easiest one to separate out from the rest based on significantly higher values for BD/SL (>0.43), CL/SL (>0.32), CPH/SL (>0.125), DFH/SL (>0.19) and DFB/SL (>0.188).

Two members of this genus, *A. chacunda* and *A. selangkat* showed close resemblance for all the considered characters except SNL/SL and PPL/SL for which smaller values of 0.058 and 0.240 is recorded for *A. chacunda* against 0.074 and 0.276 for its counterpart. A combination of longer snout length (SNL/SL=0.094), smaller body depth (BD/SL=0.242) and shorter dorsal fin height (DFH/SL=0.127) gives *D. acuta* a distinct morphometric signature. Smaller body depth (BD/SL<0.266) and shorter Caudal peduncle height (CPH/SL<0.078) and dorsal fin height (DFH/SL<0.128) describes the genus *Amblygaster*. The two considered species belonging to this genus shows high degree of similarity for assessed characters. Nevertheless, longer dorsal fin base and caudal length for *A. leiogaster* could be a discriminating factor.

Though means of morphometric characters are easiest for face to face comparison between species, it does not reflect the true discriminating power of the variables. In most of the cases, a single character is not sufficient to separate a group of species. A matrix or combination of characters is generally required for satisfactory taxonomic resolution of closely

resembling members of a family or group. A multivariate approach like stepwise discriminant function Analysis (SDFA) is tailor-made for such situation.

Results of forward stepwise discriminant function analysis (FSDFA) showed the sufficiency of considered morphometric features in successful discrimination of the concerned species in most of the cases. All the considered morphometric variables are included in the model with BD/SL entering first followed by ML/SL, DFB/SL, PPL/SL, PFL/SL, CL/SL, ED/SL, CPH/SL, SNL/SL and DFH/SL. A total of 10 discriminant functions (roots) were generated with first two roots accounting for the explanation of 85.01% of the total variation. The cumulative figure reaches 92.02% when third root is also considered. A distinct canonical mean of a species for a particular root shows the sufficiency of the root in separating that species. Distinct mean values for *H. quadrimaculatus*, *A. chacunda*, *A. selangkat*, *I. melastoma* and *P. ditchela* were observed for Root 1. Similarly, a distinct mean value for Root 2 was observed in case of *I. sirishai* (Table: 2).

Table 2. Means of canonical variables of 11 species of marine clupeoids in stepwise (forward) discriminant analysis

Species	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6	Root 7	Root 8	Root 9	Root 10
<i>S. albella</i>	-0.75324	0.04309	0.28775	0.42673	1.43812	0.08198	0.39236	0.153051	-0.10319	0.190465
<i>S. fimbriata</i>	0.07961	0.11024	1.24642	-0.13048	1.32376	0.32312	-0.50403	-0.58393	0.438981	0.011907
<i>H. quadrimaculatus</i>	-3.67797	3.05064	-1.02477	-3.48974	-0.02336	-0.40437	0.66782	-0.01526	0.028025	-0.08536
<i>A. sirm</i>	-7.06564	-2.79045	3.11725	0.32532	-0.40358	-0.5353	-0.20012	-0.2334	-0.10918	-0.07054
<i>A. leiogaster</i>	-6.74491	-4.43031	0.25187	0.2992	-0.85029	0.75365	0.05266	1.523813	0.252358	-0.02952
<i>D. acuta</i>	-6.17493	-2.6822	-4.16266	0.97135	-0.53737	0.6037	-0.08071	-0.44558	-0.04144	0.00198
<i>A. chacunda</i>	8.62966	-2.40421	0.01457	-0.48408	0.01532	0.25279	-0.13212	0.029821	-0.06444	-0.0804
<i>A. selangkat</i>	6.68075	-1.39238	-1.56586	1.8459	-1.35713	-2.2998	0.49069	0.011138	0.190531	0.139274
<i>I. melastoma</i>	2.41338	6.36966	1.95094	0.40451	-2.07764	1.0172	0.19046	-0.1834	0.003783	0.147238
<i>P. ditchela</i>	-1.59669	6.40278	-1.78809	-0.93655	0.1234	-0.81093	-2.11954	0.477869	-0.10642	0.092776
<i>I. sirishai</i>	-0.36929	8.65166	-0.5017	3.94043	1.08446	-0.12194	0.43199	0.231985	-0.01499	-0.36825

Hence, majority of species were found to be successfully separated by Root 1 and Root 2 which can be easily visualized in a scatterplot of canonical scores for respective Roots (Fig. 3). Means for *A. sirm*, *A. leiogaster* and *D. acuta* are distinct from each other on Root 3 and hence can be separated by Root 3.

None of the roots were found to satisfactorily separate out two species of genus *Sardinella*.

Nevertheless, the best possible separation was given by Root 3 (Fig. 4). The high degree of morphometric resemblance between *S. albella* and *S. fimbriata* is further emphasized by near zero values of squared Mahalanobis distance (3.67). Morphometric similarity between *A. sirm* and *A. leiogaster* and between two species of genus *Anodontostoma* was also supported by lower values (<22.00).

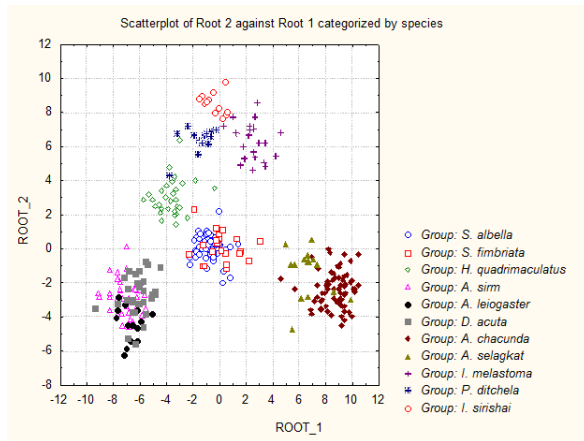


Fig. 3. Scatterplot of canonical scores for Root 1 and Root 2 of the morphometric variables of 11 clupeoid fishes.

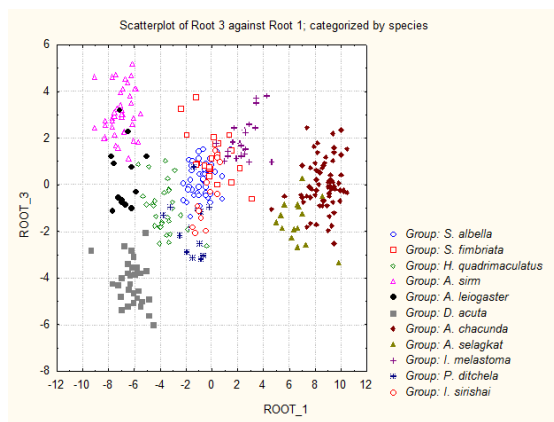


Fig. 4. Scatterplot of canonical scores for Root 1 and Root 3 of the morphometric variables of 11 clupeoid fishes.

A cluster diagram based on squared Mahalanobis distance is presented to show morphometric resemblance and divergence between considered species (Fig. 5). Factor structure matrix shows the contribution of the considered morphometric variables to the respective Roots. As Root 1, 2 and 3 explains the major proportion of variation and distinguishes most of the considered species, the relative loadings of the variables on these Roots explains the relative importance of the morphometric ratios in taxonomic terms.

Factor structure matrix of variables on these Roots reveal higher contribution (>0.35) of BD/SL, DFB/SL, PFL/SL, and CPH/SL on Root 1, ML/SL and ED/SL on Root 2 and PPL/SL,

CL/SL and CPH/SL on Root 3 (Table: 3). The classification matrix generated by FSDFA successfully classifies the specimens in 94.6 % cases, with 17 instances of misclassification.

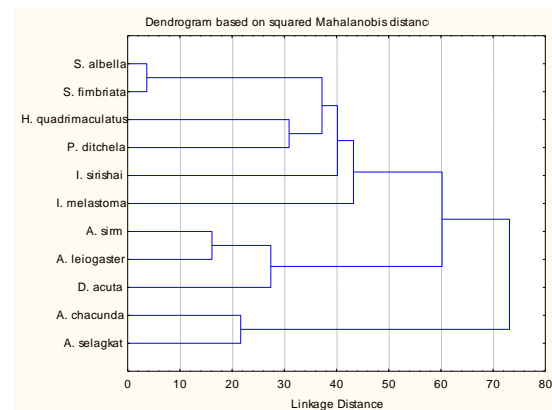


Fig. 5. A dendrogram based on squared Mahalanobis distance matrix of 11 clupeoid fishes

Table: 3. Factor Structure Matrix (First three Roots) for 11 Clupeoid species in discriminant analysis

Variable	Root 1	Root 2	Root 3
BD/SL	0.750173	0.258754	-0.00496
ML/SL	-0.045462	0.804255	-0.05325
DFB/SL	0.429481	-0.10235	-0.6587
PPL/SL	-0.077128	0.260372	-0.39371
PFL/SL	0.37446	-0.04441	0.006385
CL/SL	0.335584	0.253252	-0.46082
ED/SL	0.020653	0.453571	-0.2992
CPH/SL	0.351902	0.065959	-0.40587
SNL/SL	-0.270002	0.098498	-0.29737
DFH/SL	0.329844	0.144568	-0.03813

Highest rate of misclassification was recorded for *S. fimbriata* where only 66.7 % of pre-classified specimens found agreement with the generated matrix and the rest were predicted as *S. albella* by the model (Table: 4).

Discussion

Meristic and morphometric features being most easily recordable, find wide application in fish taxonomic studies and field identification.

Table: 4. Classification Matrix of different species in stepwise (forward) discriminant analysis

Species	Percent	A	B	C	D	E	F	G	H	I	J	H
<i>Sardinella albella</i> (A)	93.7	45	3	0	0	0	0	0	0	0	0	0
<i>Srdinella fimbriata</i> (B)	66.7	7	14	0	0	0	0	0	0	0	0	0
<i>Herklouthy quadrimaculatus</i> (C)	100	0	0	31	0	0	0	0	0	0	0	0
<i>Amblygaster sirm</i> (D)	97.5	0	0	0	39	1	0	0	0	0	0	0
<i>Amblygaster leiogaster</i> (E)	85.7	0	0	0	2	12	0	0	0	0	0	0
<i>Dussumeiria acuta</i> (F)	100	0	0	0	0	0	31	0	0	0	0	0
<i>Anodontostoma chacunda</i> (G)	94.1	0	0	0	0	0	0	64	4	0	0	0
<i>Anodontostoma selangkat</i> (H)	100	0	0	0	0	0	0	0	15	0	0	0
<i>Ilisha melastoma</i> (I)	100	0	0	0	0	0	0	0	0	23	0	0
<i>Pellona ditchela</i> (J)	100	0	0	0	0	0	0	0	0	0	13	0
<i>Ilisha sirishai</i> (K)	100	0	0	0	0	0	0	0	0	0	0	13
Total	94.6	52	17	31	41	13	31	64	19	23	13	13

Meristic characters can be recorded with ultimate precision and interspecies comparison is also easy and direct. But during current study, meristic features viz. number of rays on dorsal, pectoral, ventral, anal and caudal fin, gill rakers on first gill arch, belly scutes and branchiostegal rays were found to insignificant in species level resolution. They were found to be useful in separating higher taxa only. Similar overlapping ranges were also reported in previous literatures^{5,11,12}. Morphometric measurements are continuous features and can be easily recorded from specimens but with varying degree of precision. In addition, analysis and interpretation of morphometric variables is not always as straight forward as in case of discrete meristic features. But impotency of meristic characters in present case, made it imperative to study morphometric variables in detail to find its possible application in species discrimination. Comparisons of morphometric ratios are among the most common way to uncover the size independent shape variation among the closely related fish species. As means of morphometric variables are available even in older taxonomic literatures, it is most suitable for comparison between earlier and current works and also form baseline information for subsequent workers. In present study, the comparison of means (ANOVA) of morphometric ratios of 11 species revealed BD/SL as the most significant in species differentiation supported by highest F-Value (Table: 1). Current finding is in congruence with earlier taxonomic works where, Body depth was among the most featured morphometric variable in description and discrimination of clupeoid fishes^{4,5,7,8,11,12}. Whitehead¹¹ catalogued clupeoid fishes of the world in which a single morphometric character,

body depth in percentage of standard length was mentioned among the diagnostic feature of all the species, emphasizing the importance of the same. Losse⁵ also used percentage body length with respect to standard length as a character for generation of identification key for genus *Sardinella*. He even used upper jaw length (same as mouth length in present study) as a descriptive feature which is found to be the second most important morphometric variable in the present study. In addition Dorsal fin base length (DFB/SL) is also found to be among the important discriminating feature which is poorly explored in past from taxonomic angle. The discriminating power of the above mentioned three variables (BD/SL, ML/SL and DFB/SL) is further emphasized by FSDFA where they entered in the model earlier than rest of the incorporated variables. Higher loading of variables like BD/SL, DFB/SL, ML/SL and ED/SL on Root 1 and Root 2 (Table: 3), the roots explaining 85.01% of total explained variance justifies the use these variables in past and current studies for generic and specific discrimination.

Lower value of squared Mahalanobis distance (3.67) and similar values of means of canonical variables (Table: 2) for *S. albella* and *S. fimbriata* showed the insufficiency of the selected variables in clear discrimination of the species which is visualized by greater degree of overlap in scatterplot (Fig: 2 and 3). The generated SDA models shows 100% successful classification in 5 species and more than 85% in another 5 species. A successful classification percent of mere 66.7% for *S. fimbriata* with 7 out of 21 predicted as *S. albella* (Table: 4) by the function showed the morphometric similarity between the species

which is in agreement with the findings of Whitehead¹¹ and Stern¹².

Conclusion

The morphometric variables do have the potential to successfully discriminate most of species. Body depth emerges out to be the most important morphometric feature supported by both present work and earlier documentation. Present work reveals the potency of dimension of fins as important morphometric variables from taxonomic view point. Insufficiency of meristic characters to delineate species within genera is further affirmed in current study. In few species especially members of genus *Sardinella*, even morphometric features fail to clearly distinguish between species. Clear distinction could possibly be done through exploration of anatomical features and modern molecular approaches.

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