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Article

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Morphological variations in marine pufferfish and porcupinefish (Teleostei: Tetraodontiformes) from Tamil Nadu, southeastern coast of India

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Abstract: In the present study, morphological variations in 14 species of two families, Tetraodontidae and Diodontidae, were examined for individuals collected from five different centres in Tamil Nadu in the southeastern coast of India. Twenty-seven morphological measurements and four meristic characters were taken and used for multivariate analyses such as discriminant function analysis (DFA) & MANOVA. DFA revealed that the first two functions accounted for more than 75% variation between the species. Negative allometric values were observed on head length (HL), orbital length (OL), pupil diameter (PD), interorbital length (IOL), pectoral-fin length (PEL), caudal peduncle depth (CPD), dorsal to pectoral fin distance (DPFD), caudal peduncle length (CPL) and post-pectoral-fin length (POPFL) measurements. Also, MANOVA supported the DFA results. Additions, allometric relationships, and meristic variations were observed for most of these species. Moreover, this is the first attempt to describe a greater number of morphological features of the species belonging to the order Tetraodontiformes.

Keywords: Allometry, Diodontidae, discriminant function analysis, MANOVA, meristics, morphometric variation, porcupinefish, pufferfish, Tetradontidae, trash fish.

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For **Tamil abstract** see end of this article.

Author Details: Mr. K. Kaleshkumar is a PhD research scholar in the Department of Marine Science, whose interest include diversity, distribution, traditional and molecular taxonomy and nutritional evaluation of marine pufferfishes. Currently working on distribution, molecular taxonomy and biomedical applications of marine pufferfishes from Tamil Nadu, south-eastern coast of India. He has seven years of experience in marine pufferfishes. Dr. R. Rajaram is an Assistant Professor in the Department of Marine Science of Bharathidasan University and his research interest include the ichthyotaxonomy, marine natural products and pollution of marine organisms especially fishes. Mr. P. Purushothaman is a PhD research scholar in crustacean fisheries division, whose interest includes marine diversity and evolutionary relationships using novel molecular tools. Mr. G. Arun is currently a PhD research scholar, whose interest include taxonomy and ecology of marine hydrozoans. He is experienced in Island ecosystem health assessment, coral transplantation, SCUBA diving, and coastal survey.

Author Contribution: KK & RR conceived & designed the experiments and analyzed the data; KK performed the sample collections; PP & GA associated the experiments; PP, GA, KK & RR wrote the paper.

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INTRODUCTION

Geographic variation in morphometry has been used to discriminate local forms of fish for over a century (Cadrin 2000). Phenotypic diversities exist in morphological variations within and among populations (Jeffares et al. 2015) and they may be one of the ways to determine the origin of divergence and speciation (Kerschbaumer et al. 2014). Morphometric analysis reveals the differences in body shape between different individuals to discriminate populations of the same species (Hirsch et al. 2013), which can help for the conservation of biodiversity, management of fishery resources, and identification & discrimination of species.

Both pufferfish and porcupinefish belong to the order Tetraodontiformes. Tetraodontidae, the family to which pufferfish belong to which includes 27 genera with 184 species, and which is considered the most important family in this orderthat 27 (Matsuura 2015). Porcupinefish of the family Diodontidae includes 19 species of six genera (Nelson et al. 2016). Some members of the pufferfish and porcupinefish have commercial value in the food industry and in the aquarium trade (Fiedler 1991). The indeterminate consistency of body and loose skin are the great taxonomic features in genera such as *Arothron*, *Chelonodon*, *Lagocephalus*, *Takifugu*, and *Torquigener*. Many species have not been studied taxonomically in detail by using morphological and meristic characters to classify them into appropriate groups (Randall 1985). The detailed counts and measurements were provided for freshwater pufferfish of *Tetraodon* by Dekkers (1975), marine pufferfish of *Canthigaster* by Allen & Randall (1977), *Lagocephalus* by Matsuura (2010) & Matsuura et al. (2011), and *Torquigener* by Hardy (1982a,b, 1983a,b, 1984a,b).

Despite the value and availability of genetic, physiological, behavioral, and ecological data for such studies, systematic ichthyologists continue to depend heavily on morphology for taxonomic characters. Commonly, fish are classified based on the shapes, sizes, pigmentation patterns, disposition of fins, and other external features (Strauss & Bond 1990). Pufferfish have been fatally consumed mainly in Japan, China, and Taiwancausing death (Bragdeeswaran & Therasa 2010; Arakawa et al. 2010; Monaliza & Samsur 2011). A few members of pufferfish are considered as serious hazards to consumers since they contain strong marine toxins that can be lethal to humans. Therefore, misidentification of the species is a major issue in the trade market and clear identifications of pufferfish are a prime need to solve this problem.

Among the different fish products, fresh and dried pufferfish are an important source of animal protein in Tamil Nadu. The preservation process starts when it is harvested and becomes complete when it reaches the consumer's table. According to Immaculate et al. (2015), paralysis resulting from ingestion of pufferfish was reported from southeastern Asia. This kind of study, however, has not been carried out on the Indian species. The improper handling and misidentification of this species can be adverse to human health. Recently, increasing availability and utilization of pufferfish in Tamil Nadu coast has caused health problems to the consumers. The current study deals with understanding the morphological variations of pufferfish and porcupinefish.

MATERIALS AND METHODS

Study area description

The specimens of pufferfish and porcupinefish were collected from five major fish landing centres such as Royapuram (Station I) (13.124^{0}N & 80.297^{0}E), Cuddalore (Station II) (11.716^{0}N & 79.775^{0}E), Nagapattinam (Station III) (10.755^{0}N & 79.849^{0}E), Mandapam (Station IV) (9.276^{0}N & 79.151^{0}E), and Kanyakumari (Station V) (8.0781^{0}N & 77.551^{0}E) located along the Tamil Nadu coast of southeastern India (Fig. 1). The specimens were caught by large fishing boats and small fibre boats with gill nets and trawl nets gear; trawl nets were the main method for collecting pufferfish and porcupinefish.

Sample collection and preservation

The sample collections were carried out for a period of two years from August 2014 to July 2016 by regular visits to the landing centres at monthly intervals. Fourteen species belonging to the families Tetraodontidae and Diodontidae were collected from trash items at all fish landing centres (Image 1). Collected specimens were transported to the laboratory in fresh conditions and stored at -20^{o}C until further analysis. The collected specimens were then thawed at room temperature and weighed. The specimens were identified to species level by referring to standard fishery identification manuals and publications (Fraser-Brunner 1943; Allen & Randall 1977; Leis 1978, 1984; Fischer & Bianchi 1984; Hardy 1982a, b, 1983a, b, 1984a, b; Smith 1958, 1986; Smith & Heemstra 1986; Matsuura 1994, 2002, 2010, 2014; Matsuura et al. 2011; Allen & Erdmann 2012; Randall et al. 2012).

Morphometric features

Morphological measurements were made months

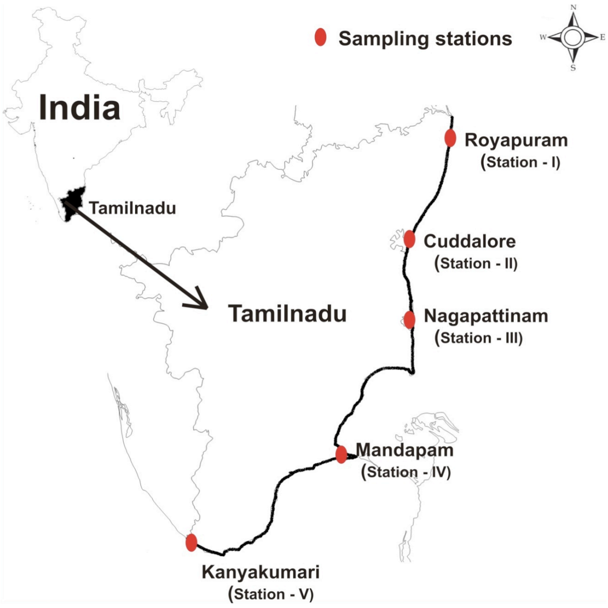

Figure 1. Sampling stations along Tamil Nadu, southeastern coast of India

after fixation in 10% formalin and were taken to the nearest 0.1mm with a dial caliper. In this study, 10 specimens were taken from each species for morphometric and meristic analyses (Table 1). Methods for morphological measurements and fin-ray counts primarily followed Dekkers (1975) and Hubbs & Lagler (1958) with some additional measurements (Fig. 2): standard length (SL), snout length (SNL), mouth gape length (MGL), head length (HL), orbital length (OL), pupil diameter (PD), interorbital length (IOL), pre-nasal length (PRNL), inter nasal length (INL), dorsal-fin base length (DFBL), dorsal-fin length (DFL), pectoral-fin base length (PFBL), pectoral-fin length (PEL), anal-fin base length (AFBL), anal-fin length (AFL), pre-dorsal-fin length (PRDFL), pre-pectoral fin length (PRPFL), pre-anal fin length (PRAFL), post-dorsal-fin length (PODFL), post-pectoral-fin length (POPFL), post-anal-fin length (POAFL), caudal peduncle length (CPL), caudal peduncle depth (CPD), snout to anus distance (SNAD), dorsal to pectoral fin distance (DPFD), dorsal to anus distance (DAD), and depth of body (LDB).

Data analysis

All statistical analyses were performed using the statistical software (SAS 2014). The allometric relationship of all the characters with standard length was estimated using linear regression model and the significance of the allometric coefficient (b) was fixed (b=1: isometry, b>1: negative allometry, b<1: positive allometry).

For multivariate analysis, to remove the effect of size from the data, all the morphometric measurements were transformed to size-independent shape variables using an allometric method as suggested by Reist (1985).

$$M_{trans} = \log M - \beta (\log SL - \log SL\ mean)$$

where M_{trans} is the truss measurement after transformation, M is the original truss measurement, SL is the overall mean standard length of a species, and β is the slope regressions of the log M against log SL. Correlation coefficients were observed between each pair of variables before and after the size effect removal;, the values of which were expected to decrease , after the size effect removal (Murta 2000). Multivariate analysis used in this study consisted of discriminant function analysis (DFA). DFA was run to test the effectiveness of variables in predicting different groups of species (Tomovic & Dzukic 2003; Loy et al. 2007). Finally, multivariate analysis of variance (MANOVA) was performed to see the significant differences between the species.

RESULTS

Morphometric data is provided for the 11 species from six genera (*Arothron*, *Lagocephalus*, *Takifugu*, *Canthigaster*, *Torquigener* & *Chelonodon*) of Tetraodontidae and three species from three genera (*Chilomycterus*, *Diodon* & *Cyclichthys*) of Diodontidae in Table 1 & Image 1. The meristic differences for all the species of both the families are represented in Table 2. The relationship between all morphometric characters and SL has been described and represented in Table 3a, b.

Morphometric data of *Arothron* & *Lagocephalus*

In the present study, four species of *Arothron*, *A. hispidus*, *A. immaculatus*, *A. reticularis* & *A. stellatus*, and three species of *Lagocephalus*, *L. guentheri*, *L. sceleratus* & *L. lunaris*, were investigated by multivariate analyses and exhibited species variation. The results of DFA indicate that the first two components cumulatively explained 85.4% of the total morphometric variation. Some of the morphometric variables (HL, OL, PD, PD, IOL, PEL, CPD & SNAD) loaded heavily on DF, which explained 67.7% of the entire differences and few variables from DF2 (DPFD, CPL, POPFL & PRAFL) with 17.7% (Table 4 & Fig. 3). Additionally, MANOVA analysis also supported and followed the taxonomic status of these species (Table 5). Lower morphometric differences were observed between *A. hispidus* & *A. stellatus* and high differences were noticed in *A. reticularis* to other species of *Arothron* group; *L. sceleratus* & *L. lunaris* showed less variation in *Lagocephalus* group (Fig. 3).

Table 1. Morphometric characters of marine Pufferfish & Porcupinefish from southeastern India.

	Pufferfish											Porcupinefish		
Code	*A. immaculatus*	*A. reticularis*	*A. hispidus*	*A. stellatus*	*L. guentheri*	*L. sceleratus*	*L. lunaris*	*T. oblongus*	*T. brevipinnis*	*C. patoca*	*C. solandri*	*D. holocanthus*	*C. orbicularis*	*C. reticulatus*
SL	14.81±5.49	11.38±8.79	17.71±4.88	25.65±5.18	19.34±1.57	10.85±1.56	10.68±1.96	16.57±4.94	8.15±2.10	12.57±3.04	7.68±2.79	13.57±1.60	14.00±2.29	31.00±9.66
SNL	2.10±0.69	2.03±1.53	3.09±0.85	3.34±0.80	3.18±2.11	1.67±0.47	1.65±0.65	2.19±0.57	1.33±0.48	2.16±0.56	1.28±0.61	1.47±0.35	1.33±0.21	5.23±1.79
MGL	1.79±0.60	1.88±0.81	1.58±0.57	3.46±0.68	1.99±0.98	1.05±0.34	1.05±0.47	1.85±0.62	0.51±0.18	1.79±0.51	0.52±0.22	1.30±0.70	1.73±0.81	3.37±1.10
HL	4.57±2.03	4.25±2.50	4.88±1.34	9.92±2.29	4.96±2.11	2.49±0.38	2.45±0.52	4.31±1.64	1.89±0.63	4.40±1.21	1.80±0.80	4.30±0.61	4.40±0.70	9.03±2.59
OL	1.07±0.37	0.97±0.22	0.88±0.32	2.02±0.46	1.90±0.55	1.44±0.40	1.43±0.54	0.97±0.19	0.89±0.40	1.09±0.30	0.88±0.51	1.37±0.45	1.17±0.25	2.17±0.81
PD	0.83±0.25	0.75±0.18	0.71±0.30	1.14±0.33	1.11±0.36	1.14±0.41	1.18±0.54	0.76±0.10	0.61±0.25	0.84±0.13	0.60±0.32	0.87±0.21	0.77±0.06	1.30±0.53
IOL	2.55±1.02	2.40±1.74	2.42±0.67	4.59±1.35	2.75±1.50	1.60±0.58	1.53±0.79	3.17±1.24	1.42±0.54	2.32±0.48	1.34±0.64	3.13±0.21	3.50±0.72	4.80±1.39
PRNL	1.73±0.65	1.48±0.82	2.11±0.78	3.02±0.69	2.06±1.50	1.38±0.46	1.35±0.65	1.70±0.77	3.59±1.02	2.08±0.49	3.34±1.38	1.33±0.21	1.43±0.35	3.87±1.03
INL	1.37±0.54	1.25±0.77	1.27±0.58	2.16±0.55	2.09±1.00	1.05±0.43	1.08±0.64	1.65±0.71	0.74±0.21	1.64±0.47	0.74±0.27	1.77±0.71	1.40±0.46	2.93±1.07
DFBL	1.23±0.64	1.22±0.88	1.20±0.57	2.78±0.93	1.95±1.22	1.12±0.52	1.13±0.75	1.69±0.65	0.51±0.18	1.05±0.22	0.50±0.21	1.37±0.40	1.17±0.15	3.37±1.31
DFL	2.42±0.85	1.35±1.23	2.73±0.64	3.54±0.90	2.98±1.76	1.97±0.65	1.90±0.88	2.86±0.87	1.58±0.44	1.62±0.51	1.50±0.57	2.03±0.23	2.07±0.29	4.90±1.85
PFBL	1.61±0.83	1.45±0.89	1.64±0.72	3.29±0.78	1.80±1.00	1.36±0.52	1.33±0.74	1.48±0.58	0.72±0.23	1.15±0.38	0.72±0.29	1.87±0.23	1.93±0.31	3.37±0.76
PEL	2.12±0.71	1.52±1.12	2.16±0.66	3.13±0.66	3.24±1.41	2.48±0.69	2.35±0.94	2.61±0.58	1.35±0.69	1.71±0.51	1.32±0.81	2.40±0.50	2.67±0.68	4.27±1.70
AFBL	0.99±0.58	1.12±0.84	1.16±0.68	2.18±0.49	1.70±0.80	0.98±0.51	1.03±0.79	1.71±0.38	0.69±0.42	0.97±0.32	0.54±0.22	1.50±0.66	1.17±0.40	2.53±0.55
AFL	2.09±0.90	1.32±0.93	2.12±0.72	3.34±0.82	3.29±1.80	1.43±0.49	1.45±0.72	2.69±0.50	1.17±0.54	1.60±0.41	1.14±0.65	2.07±0.31	2.03±0.25	4.37±1.87
PRDFL	21.32±5.57	6.98±0.98	13.33±3.62	17.42±2.62	13.29±6.99	8.49±1.59	8.45±2.19	12.51±2.85	5.14±2.96	10.82±2.17	5.78±3.03	11.00±0.95	11.50±1.35	21.20±5.94
PRPFL	4.78±1.15	5.40±1.21	6.47±1.92	6.84±2.33	6.53±4.41	4.61±0.82	4.60±1.22	5.90±1.36	3.08±0.63	5.35±1.62	2.96±0.82	5.47±0.55	5.47±0.55	10.43±4.83
PRAFL	10.45±3.45	7.31±2.04	14.46±3.59	17.95±3.59	13.26±7.63	7.89±1.57	8.00±2.35	12.86±3.04	5.38±2.29	10.98±2.43	5.76±2.68	12.07±0.97	12.80±1.75	21.53±4.80
PODFL	4.84±2.21	3.73±3.40	4.15±1.43	12.77±3.49	6.64±4.03	4.36±1.12	4.28±1.63	5.75±3.69	2.71±0.79	3.22±0.71	2.54±1.03	2.97±0.21	3.23±0.67	11.83±5.40
POPFL	10.21±4.06	5.260±7.75	12.61±4.55	22.99±4.53	13.03±6.98	8.55±2.10	8.50±2.92	13.55±4.20	5.37±1.40	9.94±2.24	5.04±1.87	8.50±0.89	8.50±0.89	23.30±5.48
POAFL	3.81±2.05	2.65±3.03	3.66±1.21	8.29±3.15	6.03±3.34	4.13±1.09	4.15±1.65	6.97±2.32	3.57±1.16	3.95±0.70	3.40±1.54	2.67±0.71	2.30±0.46	9.17±4.01
CPL	3.38±1.06	1.81±2.21	3.25±1.34	6.52±1.63	5.67±2.85	3.47±0.66	3.53±0.99	2.76±0.91	1.65±0.59	3.28±0.55	1.56±0.76	2.63±0.68	2.23±0.15	6.97±2.40
CPD	2.28±0.91	2.30±1.47	2.32±0.90	4.72±1.37	1.76±0.72	1.01±0.43	1.05±0.64	2.30±0.80	0.72±0.24	1.98±0.43	0.68±0.29	1.43±0.85	1.10±0.30	3.83±1.10
SNAD	11.41±2.31	7.729±2.39	14.63±1.92	21.91±2.84	12.24±6.27	7.08±1.46	7.10±2.02	12.49±4.02	6.04±1.14	10.24±2.03	5.64±1.52	11.20±0.80	11.63±1.10	21.93±4.72
DPFD	6.34±2.59	5.0±4.05	7.19±1.80	12.84±3.72	7.29±4.41	4.49±0.93	4.48±1.28	7.77±2.10	3.85±0.68	6.41±1.26	3.70±0.90	6.93±0.83	7.20±1.06	14.43±4.71
DAD	4.18±2.34	3.85±3.77	4.92±1.16	9.84±2.79	4.40±1.93	1.51±0.62	1.58±0.92	4.30±1.75	1.52±0.47	3.96±0.84	1.42±0.60	3.27±0.50	3.53±0.64	8.80±2.61
DB	5.40±2.72	5.02±3.15	6.41±0.92	11.92±2.55	5.53±2.60	3.06±0.97	3.08±1.35	5.30±2.06	2.11±0.54	4.94±0.85	1.98±0.72	4.93±0.25	5.37±0.99	11.07±3.20

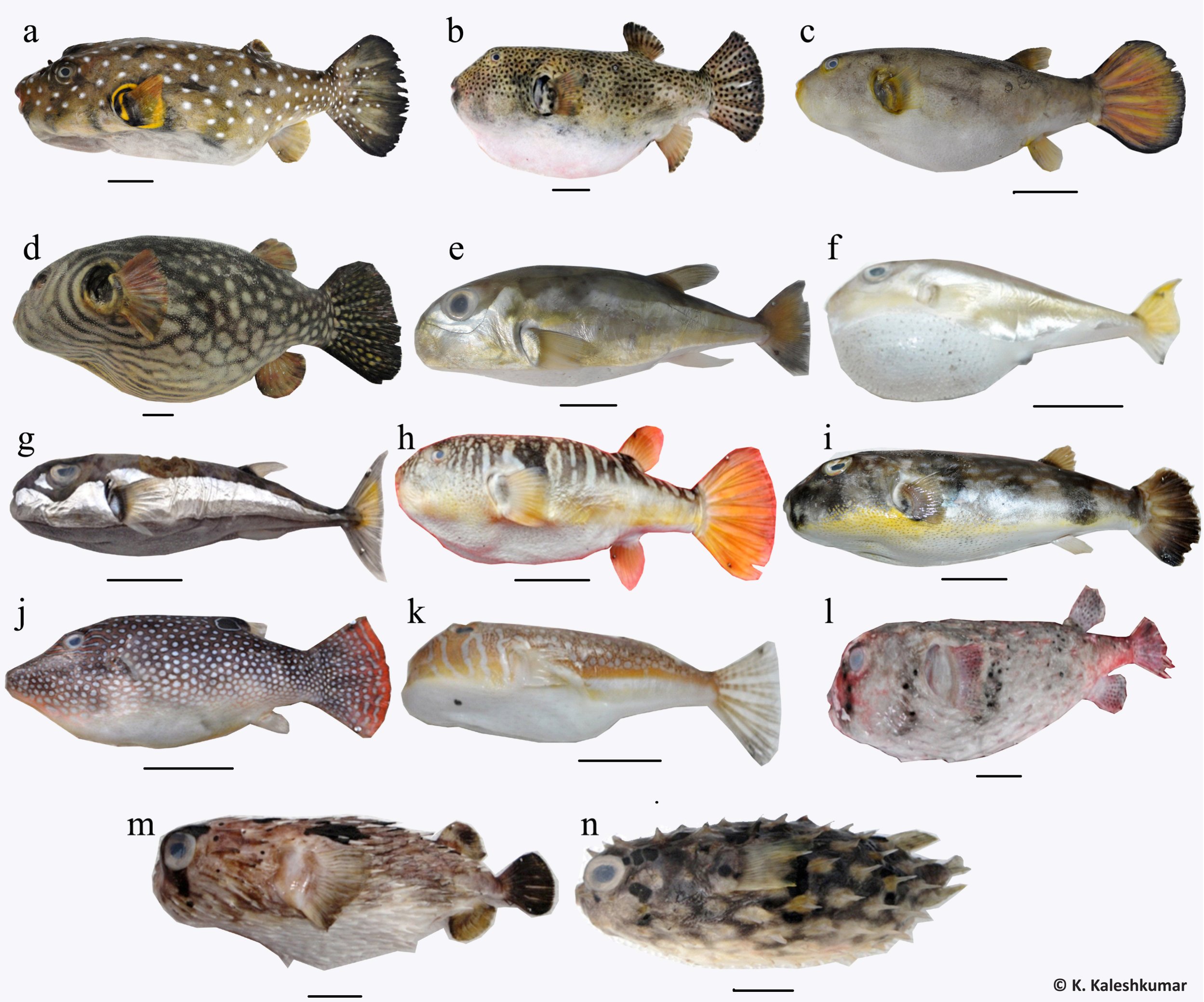

Image 1. List of identified marine Pufferfish & Porcupinefish from southeastern India.
Tetradontidae: a - *Arothron hispidus* (Linnaeus, 1758), b - *A. stellatus* (Anonymous, 1798), c - *A. immaculatus* (Bloch & Schneider, 1801), d - *A. reticularis* (Bloch & Schneider, 1801), e - *Logacephalus guentheri* (Miranda Riberio, 1915), f - *L. lunaris* (Bloch & Schneider, 1801), g - *L. sceleratus* (Gmelin, 1789), h - *Takifugu oblongus* (Bloch, 1786), i - *Chelonodon patoca* (Hamilton, 1822), j - *Canthigaster solandri* (Richardson, 1845) & k - *Torquigener brevipinnis* (Regan, 1903); Diodontidae: l - *Chilomycterus reticulatus* (Linnaeus, 1758), m - *Diodon holocanthus* (Linnaeus, 1758) & n - *Cylichthys orbicularis* (Bloch, 1785). Scale = 10mm.

Morphometric variations of Tetraodontidae & Diodontidae

The first two DF showed a cumulative value (77.7%) of the total morphological variations on the family of Tetraodontidae (Table 6). Moreover, all the loadings on DF1 (50.0 %) showed negative allometry. DF2 described 27.7% of the total variance with negative allometric growth and the characters MGL, HL, PRAFL, PRNL, CPD, SNAD, DPFD, DAD & DB were loaded heavily. Bivariate plot of DF1 and DF2 scores revealed the separation of *Lagocephalus* & *Canthigaster* and close relationship between *Arothron*, *Takifugu*, *Torquigener* & *Chelonodon* (Fig. 4). Also, significant results were observed in MANOVA too (Table 7 & Fig. 4).

Two DF were extracted from the family Diodontidae, exhibiting 95% of the total morphological variation. Probably all the characters show negative allometry and a few characters were noticed heavy loading on DF1 & DF2 (SNL, INL, DFBL, AFBL, POPFL, POAFL, LCPL, CPD & SNAD) (Table 8). Finally, the morphometric characters are showed the ability to discriminate the species in families of Tetraodontidae & Diodontidae. The detailed discriminate function was represented in Table 9 & Fig. 5.

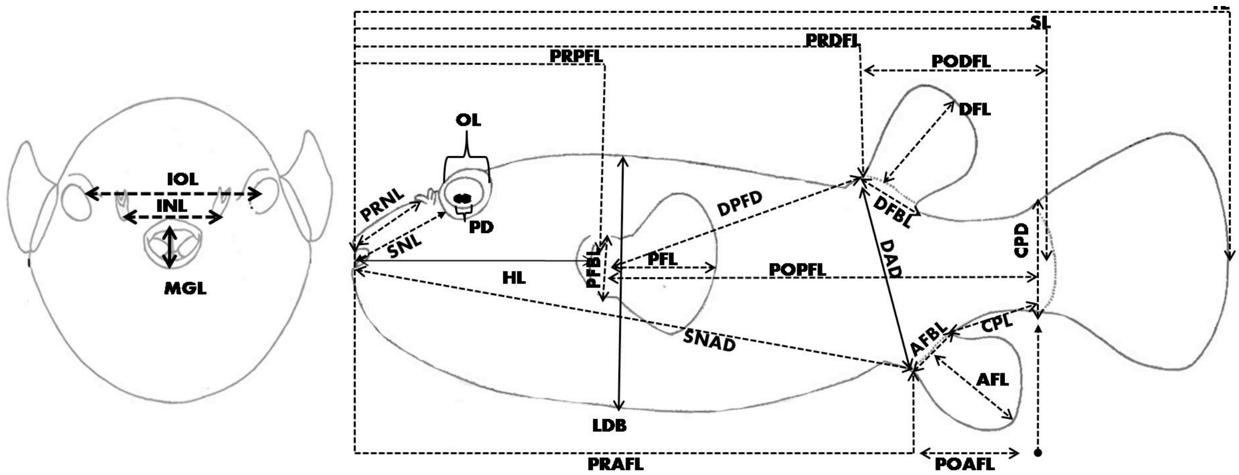

Figure 2. Typical Truss Morphometric Network (TMN) of marine Pufferfish & Porcupinefish.

Table 2. Meristic difference of marine Pufferfish & Porcupinefish from southeastern India.

Species	Meristic characters			
	PFR	DFR	CFR	AFR
Pufferfish (Family: Tetradontidae)				
Arothron immaculatus	21–22	13–14	14–15	12–13
A. reticularis	14–15	10–11	10–11	10–11
A. hispidus	16	11	9	8
A. stellatus	20	12	9	11
Lagocephalus guentheri	21–22	13–14	14–15	12–13
L. sceleratus	15–16	10	11–12	12
L. lunaris	15–16	10	11–12	12
Takifugu oblongus	16–17	12	12	11–12
Torquigenerbrevipinnis	17–18	9–10	7–8	7–8
Chelonodon patoca	15–16	10–11	10–11	10
Canthigaster solandri	17–18	9–10	7–9	7–9
Porcupinefish (Family: Diodontidae)				
Diodon holocanthus	21–22	9–11	8–9	13–14
Cyclichthys orbicularis	21–22	10	9–10	12
Chilomycterus reticulates	19–20	12–13	11	10–11

DISCUSSION

In the present study, the family Tetraodontidae (*Lagocephalus guentheri, L. sceleratus*, *L. lunaris*, *Arothron immaculatus*, *A. reticularis*, *A. hispidus*, *A. stellatus*, *Chelonodon patoca*, *Torquigener brevipinnis*, *Canthigaster solandri* & *Takifugu oblongus*) and Diodontidae (*Diodon holocanthus*, *Cyclichthys orbicularis* & *Chilomycterus reticulates*) were classified based on phenotypic appearance, and morphometric characters were adopted to identify the pufferfish and porcupinefish from the Indian coast. Also, those morphometric characters showed >70% of variation in the morphology. Similarly, Meng & Stocker (1984), Murta (2000) & Simon et al. (2010) noticed that the morphometric discriminant functions effectively classified individuals in fish species. Moreover, the same results were obtained by Mwita (2015). Additionally, these morphometric methods were more popular to reveal the stock differences in fisheries sectors.

The positive and negative values of allometric functions were able to show the taxonomic importance of the intra- and inter-species of the morphology (Meyer 1990; Mekkawy et al. 2002). Similarly, DF results confirmed that specific size and body shapes of various measurements are the determining taxonomic factors in morphometric identification. DF2 relating to the shape of the head regions of the fish separated the species of *Arothron* & *Lagocephalus* and genera of Tetraodontidae except for *Torquigener*. DF1 & DF2 more clearly separated *Cyclichthys* from *Chilomycterus*. The individuals of *Diodon* were not separated clearly, showing the close relationship to *Chilomycterus*. Also, *Torquigener* showed a close relationship to *Arothron* — these two members' results led us to reinvestigate the taxonomic status with molecular studies.

Previously, body shape and colouration characters were frequently used as distinguishing characters of these species. The present study has uncovered some morphological variation between the two closely related families, using multivariate techniques as reported in other marine fish (Pierce et al. 1994; Tudela 1999; Bolles & Begg 2000; Aktas et al. 2006; Mekkawy et al. 2011). This study demonstrates that Tetraodontidae from the southwestern

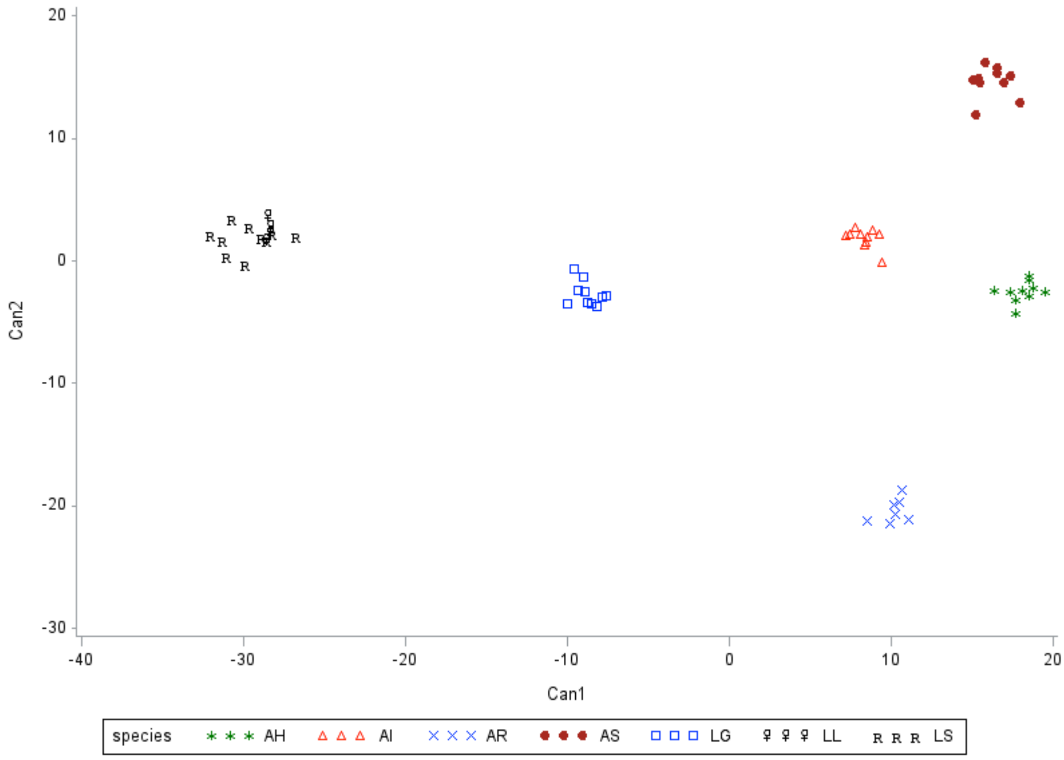

Figure 3. Scatter plot of first two factors from discriminant function analysis for *Arothron* & *Lagocephalus* from southeastern India (cumulative variations: 85%).

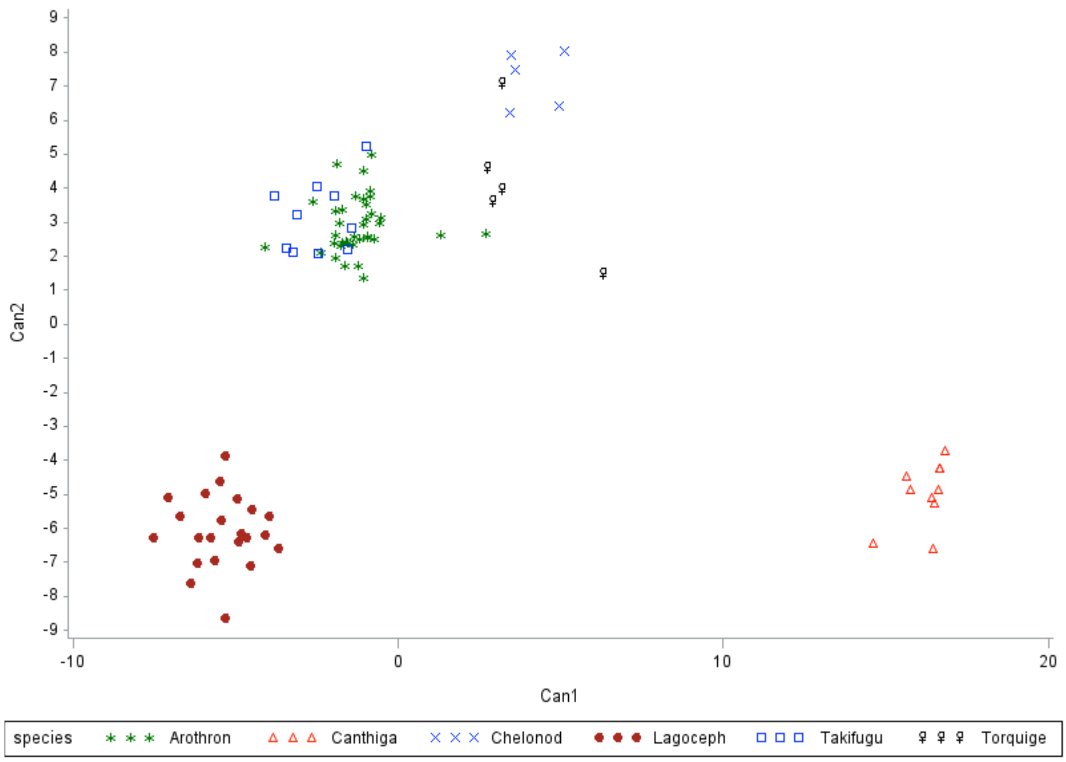

Figure 4. Scatter plot of first two factors from discriminant function analysis for Tetraodontidae from southeastern India (cumulative variations: 77%).

Table 3a. The relationship between all the characters and standard length of Tetradontidae from southeastern India, P<0.01.

Characters	*A. hispidus*			*A. immaculatus*			*A. reticulatus*			*A. stellatus*			*L. guentheri*			*L. sceleratus*		
	b	a	r^2	b	a	r^2	b	a	r^2	b	a	r^2	b	a	r^2	b	a	r^2
SNL	0.97	0.20	0.96	0.80	0.22	0.87	1.40	0.06	0.89	1.20	0.07	0.96	1.00	0.15	0.91	1.20	0.74	0.58
MGL	1.20	0.05	0.91	1.00	0.12	0.94	5.50	0.50	0.88	0.90	0.25	0.95	1.00	0.11	0.91	2.10	0.61	0.82
HL	0.94	0.33	0.96	0.90	0.37	0.90	0.80	0.55	0.99	1.10	0.27	0.95	0.74	0.61	0.84	0.90	0.27	0.85
OL	1.30	0.01	0.86	0.70	0.14	0.53	0.40	0.37	0.82	1.10	0.05	0.91	0.50	0.45	0.94	1.70	0.02	0.86
PD	1.50	0.01	0.81	0.80	0.10	0.84	0.40	0.27	0.80	1.10	0.02	0.02	0.50	0.27	0.78	1.50	0.03	0.56
IOL	1.00	0.15	0.97	1.00	0.15	0.90	0.90	0.25	0.98	1.40	0.04	0.87	1.00	0.15	0.93	2.60	0.00	0.91
PRNL	1.40	0.03	0.94	1.00	0.10	0.79	0.80	0.22	0.94	1.10	0.07	0.93	1.20	0.05	0.93	2.20	0.01	0.86
INL	1.80	0.01	0.89	1.20	0.05	0.79	0.90	0.14	0.91	1.20	0.03	0.91	0.72	0.25	0.91	2.40	0.00	0.71
DFBL	1.90	0.00	0.92	1.10	0.06	0.60	0.60	0.30	0.34	1.60	0.01	0.96	1.60	0.07	0.95	2.80	0.00	0.71
DFL	0.80	0.27	0.96	1.00	0.15	0.88	0.70	0.30	0.33	1.10	0.08	0.87	0.91	0.20	0.35	2.20	0.01	0.87
PFBL	1.80	0.01	0.87	1.40	0.04	0.93	0.80	0.18	0.98	1.10	0.07	0.09	1.03	0.09	0.94	2.60	0.00	0.70
PEL	1.00	0.11	0.94	0.70	0.33	0.66	0.90	0.33	0.96	1.00	0.10	0.97	0.69	0.45	0.91	1.90	0.03	0.86
AFBL	2.61	0.00	0.94	1.40	0.02	0.50	0.90	0.12	0.78	1.10	0.06	0.95	0.85	0.15	0.94	3.30	0.00	0.80
AFL	1.00	0.10	0.95	1.20	0.07	0.82	1.00	0.12	0.98	1.20	0.06	0.94	0.94	0.20	0.91	1.90	0.01	0.84
PRDFL	0.95	0.22	0.98	1.30	0.37	0.30	1.30	0.25	0.48	0.70	0.55	0.95	0.85	0.90	0.95	1.90	0.55	0.88
PRPFL	1.10	0.27	0.92	0.70	0.74	0.96	0.10	0.25	0.40	1.60	0.03	0.98	1.14	0.22	0.97	1.00	0.41	0.88
PRAFL	0.88	1.14	0.99	0.80	0.74	0.96	4.40	0.45	0.46	0.10	0.74	0.98	1.00	0.61	0.98	1.20	0.45	0.84
PODFL	1.30	0.09	0.96	1.10	0.22	0.89	1.10	0.27	0.57	1.30	0.15	0.98	1.04	0.31	0.89	1.70	0.07	0.90
POPFL	1.30	0.27	0.99	1.00	0.61	0.99	1.30	0.20	0.07	0.10	0.90	0.98	1.03	0.64	0.96	1.50	0.20	0.90
POAFL	1.20	0.09	0.94	1.30	0.11	0.85	1.00	0.22	0.50	1.90	0.02	0.99	0.89	0.42	0.84	1.60	0.08	0.95
CPL	1.50	0.03	0.96	0.80	0.41	0.98	1.20	0.10	0.94	1.10	0.15	0.96	0.92	0.37	0.83	1.00	0.30	0.95
CPD	1.20	0.06	0.96	1.00	0.14	0.97	0.90	0.27	0.90	1.40	0.05	0.97	0.71	0.25	0.62	2.60	0.00	0.82
SNAD	0.42	0.25	0.94	0.40	0.33	0.85	0.50	0.45	0.95	0.60	0.41	0.98	0.93	0.79	0.95	1.30	0.27	0.93
DPFD	0.82	2.61	0.91	0.80	0.74	0.83	0.90	0.50	0.89	1.40	0.14	0.98	0.95	0.50	0.95	1.30	0.27	0.92
DAD	0.80	0.50	0.96	1.10	0.18	0.93	1.10	0.25	0.98	1.40	0.09	0.93	0.81	0.43	0.83	2.40	0.00	0.83
DB	0.40	1.58	0.97	1.20	0.20	0.94	0.90	0.61	0.99	1.00	0.41	0.95	0.79	0.56	0.80	2.00	0.03	0.85

Table 3b. The relationship between all the characters and standard length of Tetradontidae from southeastern India, P<0.01.

Characters	*L. lunaris*			*T. oblongus*			*C. solandri*			*T. brevipinnis*			*C. patoca*		
	b	a	r^2	b	a	r^2	b	a	r^2	b	a	r^2	b	a	r^2
SNL	1.40	0.05	0.91	0.80	0.20	0.93	1.30	0.08	0.75	1.10	0.11	0.91	1.30	0.07	0.90
MGL	1.20	0.45	0.70	0.80	0.18	0.49	1.20	0.04	0.76	0.70	0.12	0.80	1.60	0.03	0.89
HL	0.80	0.33	0.88	1.20	0.14	0.91	1.20	0.15	0.95	1.40	0.30	0.96	0.70	0.67	0.92
OL	0.60	0.17	0.62	0.60	0.20	0.36	1.50	0.04	0.08	1.00	0.45	0.30	1.30	0.05	0.74
PD	0.60	0.74	0.71	0.40	0.27	0.67	0.90	0.08	0.25	0.50	0.07	0.93	0.80	0.11	0.95
IOL	0.50	0.74	0.78	1.30	0.07	0.97	1.30	0.08	0.90	0.50	0.25	0.93	1.30	0.07	0.93
PRNL	1.20	0.67	0.97	1.50	0.02	0.98	1.00	0.41	0.90	0.50	0.61	0.04	1.30	0.07	0.94
INL	0.40	0.15	0.63	1.30	0.04	0.95	0.70	0.15	0.67	1.10	0.50	0.44	1.70	0.02	0.89
DFBL	0.90	0.14	0.88	1.40	0.03	0.93	1.10	0.05	0.74	0.80	0.09	0.20	1.70	0.01	0.75
DFL	1.00	0.30	0.98	0.90	0.20	0.95	0.80	0.50	0.68	0.80	0.12	0.55	2.40	0.00	0.09
PFBL	0.70	0.33	0.98	1.20	0.04	0.95	0.70	0.14	0.55	0.90	0.45	0.99	2.00	0.01	0.87
PEL	0.70	0.22	0.85	0.70	0.33	0.93	1.90	0.02	0.08	0.90	0.14	0.99	1.80	0.02	0.89
AFBL	0.70	0.18	0.89	0.60	0.27	0.93	0.00	0.00	0.00	0.50	0.33	0.20	1.40	0.05	0.90
AFL	0.90	0.74	0.57	0.40	0.90	0.32	1.70	0.03	0.85	0.80	0.25	0.90	1.20	0.07	0.91
PRDFL	0.80	0.67	0.93	0.70	0.61	0.94	2.20	0.04	0.52	0.90	0.22	0.45	0.70	0.61	0.77
PRPFL	0.80	0.55	0.97	0.80	0.67	0.99	0.70	0.67	0.94	1.00	0.95	0.95	2.30	0.01	0.79
PRAFL	0.70	0.06	0.85	0.80	0.67	0.96	1.50	0.20	0.73	1.00	0.33	0.94	0.70	0.67	0.83
PODFL	1.50	0.50	0.97	2.10	0.01	0.90	1.10	0.25	0.95	0.70	0.67	0.90	1.40	0.50	0.89
POPFL	1.10	0.06	0.92	0.10	0.90	0.93	1.00	0.61	0.96	1.00	0.50	0.90	0.60	0.82	0.89
POAFL	1.40	0.12	0.94	1.10	0.30	0.98	1.30	0.20	0.96	0.50	0.61	0.53	0.50	0.94	0.88
CPL	1.20	0.07	0.92	0.10	0.14	0.97	1.30	0.09	0.96	0.60	0.97	0.92	0.80	0.45	0.95
CPD	1.20	0.09	0.95	1.10	0.09	0.96	0.50	0.20	0.14	0.80	0.25	0.97	1.30	0.07	0.86
SNAD	0.70	0.67	0.84	0.10	0.74	0.91	0.60	0.74	0.91	0.80	0.82	0.99	0.60	0.50	0.90
DPFD	0.90	0.67	0.93	0.80	0.74	0.93	0.60	0.99	0.95	0.80	0.74	0.84	0.70	0.82	0.86
DAD	0.90	0.20	0.92	1.30	0.11	0.97	1.20	0.12	0.94	0.80	0.50	0.93	1.20	0.18	0.80
DB	0.90	0.50	0.84	1.10	0.18	0.92	0.50	0.74	0.67	0.70	0.67	0.91	0.70	0.82	0.87

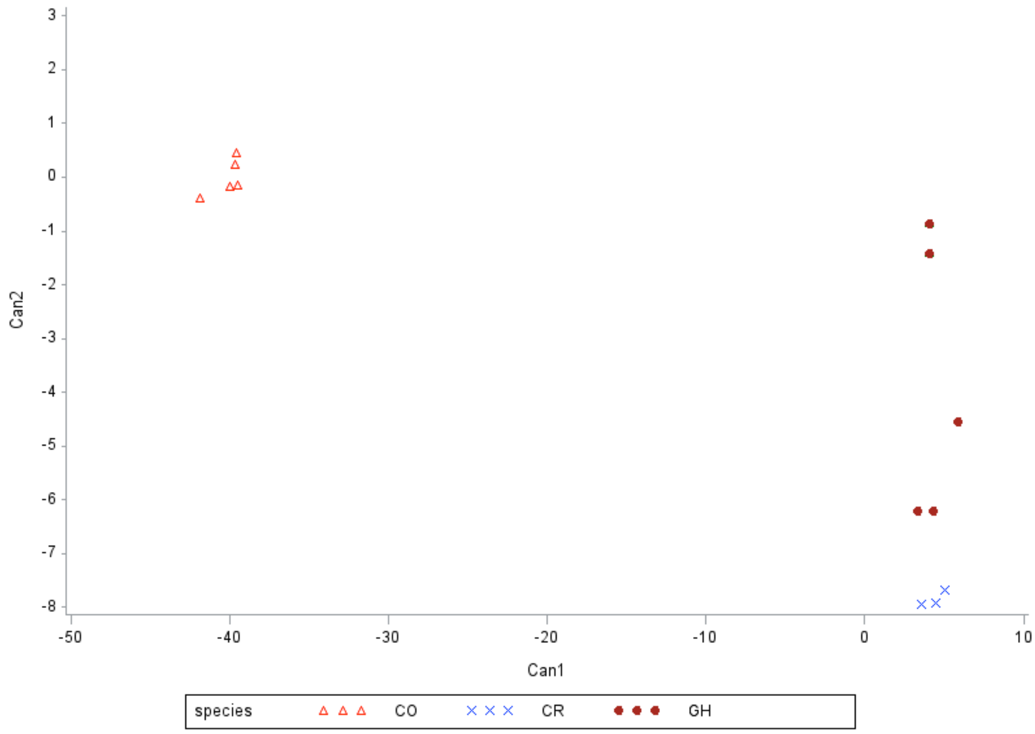

Figure 5. Scatter plot of first two factors from discriminant function analysis for Diodontidae from southeastern India.

Indian coastal waters are different from one another in morphometric characters. Statistical classifications using multivariate discriminant analyses were best for identification of the species of Tetraodontidae while morphometric characters provided comparatively less evidence of differentiation in Diodontidae.

Overall, morphological studies have been valid methods to identify the differences and to find out the relationship between different species and genera of pufferfish and porcupinefish. Also, these analyses will help to produce a better understanding of evolutionary studies with molecular markers.

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Table 4. Discriminant function analysis for *Arothron* & *Lagocephalus* — loading scores on the discriminant functions DF1 & DF2 and discriminatory power of morphometric characters Wilks' lambda (λ), F value & significance.

Variables	DF1	DF2	Wilks' lambda (λ)	F	Sig.
SNL	0.27767	-0.3457	0.577	6.604	0.005
MGL	0.51096	0.0186	0.718	3.527	0.000
HL	0.77027	0.19445	0.452	10.904	0.000
OL	-0.6579	0.19833	0.310	20.011	0.000
PD	-0.6266	-0.0158	0.489	9.403	0.032
IOL	0.48809	0.0053	0.781	2.522	0.000
PRNL	0.26549	0.12757	0.569	6.822	0.006
INL	-0.0079	-0.054	0.725	3.408	0.002
DFBL	0.05109	0.01338	0.684	4.155	0.001
DFL	0.03562	0.1024	0.679	4.262	0.017
PFBL	0.19747	0.16577	0.758	2.870	0.001
PEL	-0.7066	0.15675	0.666	4.515	0.009
AFBL	-0.0343	0.01229	0.738	3.195	0.004
AFL	0.10437	0.25586	0.710	3.679	0.000
PRDFL	0.09368	0.27503	0.600	5.993	0.014
PRPFL	-0.1662	-0.2831	0.751	2.984	0.217
PRAFL	0.38933	0.47538	0.862	1.439	0.000
PODFL	-0.0339	0.52274	0.518	8.373	0.000
POPFL	0.15219	0.66339	0.514	8.494	0.000
POAFL	-0.3403	0.41338	0.538	7.715	0.001
CPL	-0.4434	0.54312	0.653	4.791	0.000
CPD	0.74421	0.03476	0.577	6.607	0.000
SNAD	0.72037	0.52557	0.300	21.015	0.000
DPFD	0.50859	0.3704	0.295	21.483	0.000
DAD	0.83338	0.0651	0.489	9.387	0.000
DB	0.75468	0.16647	0.281	23.006	0.000

Table 5. MANOVA for Arothron & Lagocephalus from southeastern India.

	Multivariate Tests				
	Value	F	Hypothesis df	Error df	Sig.
Pillai's trace	4.944	4.846	174.000	180.000	.000
Wilks' lambda	.000	16.781	174.000	155.572	.000
Hotelling's trace	322.406	43.235	174.000	140.000	.000
Roy's largest root	196.515	203.291[a]	29.000	30.000	.000

Table 6. Discriminant function analysis for Tetraodontidae — loading scores on the discriminant functions DF1 & DF2 and discriminatory power of morphometric characters Wilks' lambda (λ), F value & significance.

Variables	DF1	DF2	Wilks' Lambda (λ)	F	Sig
SNL	-0.1301	0.19393	0.703	7.170	0.000
MGL	-0.4734	0.57841	0.773	4.982	0.000
HL	-0.2756	0.62793	0.489	17.800	0.000
OL	-0.1108	-0.5693	0.631	9.926	0.000
PD	-0.2476	-0.3415	0.818	3.778	0.004
IOL	-0.0282	0.486	0.744	5.851	0.000
PRNL	0.83839	-0.1381	0.717	6.697	0.000
INL	-0.1863	0.16984	0.743	5.889	0.000
DFBL	-0.4264	0.12681	0.799	4.276	0.002
DFL	-0.0065	-0.1592	0.720	6.619	0.000
PFBL	-0.366	0.14915	0.791	4.482	0.001
PEL	-0.4736	-0.3898	0.688	7.695	0.000
AFBL	-0.1924	-0.0013	0.699	7.326	0.000
AFL	-0.2902	0.04044	0.837	3.316	0.009
PRDFL	-0.3733	0.23676	0.775	4.924	0.001
PRPFL	-0.1618	0.09356	0.715	6.775	0.000
PRAFL	-0.3715	0.4519	0.757	5.465	0.000
PODFL	-0.2557	-0.1268	0.649	9.188	0.000
POPFL	-0.3095	0.25291	0.810	3.978	0.003
POAFL	0.06503	-0.2177	0.762	5.296	0.000
CPL	-0.4437	-0.2509	0.888	2.138	0.069
CPD	-0.3471	0.71149	0.708	7.027	0.000
SNAD	-0.1138	0.61576	0.776	4.919	0.001
DPFD	-0.0543	0.50741	0.614	10.699	0.000
DAD	-0.1211	0.70674	0.727	6.370	0.000
DB	-0.2455	0.6481	0.545	14.184	0.000

Table 7. MANOVA for Tetraodontidae from southeastern India.

	Multivariate tTests				
	Value	F	Hypothesis df	Error df	Sig.
Pillai's trace	7.394	5.870	290.000	600.000	.000
Wilks' lambda	.000	14.274	290.000	510.032	.000
Hotelling's trace	151.242	25.659	290.000	492.000	.000
Roy's largest root	44.754	92.595[a]	29.000	60.000	.000

Table 8. Discriminant function analysis for Diodontidae — loading scores on the discriminant functions DF1 & DF2 and discriminatory power of morphometric characters Wilks' lambda (λ), F value & significance.

Variables	DF1	DF2	Wilks' lambda (λ)	F	Sig..
SNL	-0.073	-0.483*	0.652	2.942	0.095
MGL	-.160*	-0.155	0.407	8.026	0.007
HL	-0.204	-0.340*	0.289	13.506	0.001
OL	-0.1	-0.331*	0.598	3.690	0.059
PD	-0.092	-0.275*	0.642	3.063	0.088
IOL	-.209*	-0.109	0.287	13.637	0.001
PRNL	-0.187	-0.392*	0.705	2.300	0.146
INL	-0.079	-0.479*	0.354	10.059	0.003
DFBL	-0.196	-0.509*	0.292	13.320	0.001
DFL	-0.164	-0.395*	0.553	4.443	0.039
PFBL	-0.194	-0.306*	0.221	19.356	0.000
PEL	-.194*	-0.13	0.512	5.249	0.025
AFBL	-0.145	-0.456*	0.337	10.813	0.003
AFL	-0.175	-0.290*	0.478	6.015	0.017
PRDFL	-0.147	-0.179*	0.527	4.940	0.029
PRPFL	-0.057	-0.366*	0.344	10.487	0.003
PRAFL	-0.183	-0.205*	0.386	8.751	0.005
PODFL	-0.298	-0.396*	0.654	2.910	0.097
POPFL	-0.195	-0.448*	0.432	7.235	0.010
POAFL	-0.167	-0.504*	0.779	1.565	0.252
CPL	-0.166	-0.511*	0.473	6.122	0.016
CPD	-0.362	-0.529*	0.454	6.618	0.013
SNAD	-0.239	-0.388*	0.268	15.011	0.001
DPFD	-0.273	-0.381*	0.327	11.317	0.002
DAD	-0.24	-0.356*	0.326	11.356	0.002
DB	-0.286	-0.323*	0.261	15.605	0.001

Table 9. MANOVA for Diodontidae from southeastern India.

	Multivariate tTests				
	Value	F	Hypothesis df	Error df	Sig..
Pillai's trace	1.670	1.013	20.000	4.000	.563
Wilks' lambda	.000	4.447[b]	20.000	2.000	.199
Hotelling's trace	679.540	.000	20.000	.000	.000
Roy's largest root	677.494	135.49[9]c	10.000	2.000	.007

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Tamil abstract:

தற்போதைய ஆய்வில், 14 வகையான மீனினங்கள், 2 குடும்பங்கள் (டெட்ராடொன்டைடிடே மற்றும் டியோடோண்டிடே) ஆகியவைகளை உடல் அளவியல் ஆய்வு செய்யப்பட்டன இதற்கான மாதிரிகளை இந்தியாவின் தென்கிழக்கு கடற்கரையில், தமிழ்நாட்டில் ஐந்து வெவ்வேறு மீன்பிடி மையங்களில் இருந்து சேகரிக்கப்பட்டன. இதற்கான மாதிரி சேகரிப்புகள் இரண்டு வருடங்கள் மேற்கொள்ளப்பட்டன. ஆகஸ்ட் 2014 முதல் ஜூலை 2016 வரை மாதாந்திர இடைவெளியில் அனைத்து மீன்பிடி மையங்கலிருந்தும் எடுக்கப்பட்டன. இருபத்தி ஏழு உடல் அளவீடுகள் மற்றும் 4 மேரிஸ்டிக் எண்ணிக்கைகள் கொண்டு சேகரிக்கப்பட்ட மாதிரிகளில் அளவிடப்பட்டன. மேலும், கணிதவியல் ஆய்வுக்களான Discriminant Function Analysis (DFA) மற்றும் MANOVA பயன்படுத்தப்பட்டன. DFA ஆய்வின்படி, முதல் இரண்டு செயல்பாடுகள் மீனினங்களில் இடையே 75% க்கும் மேற்பட்ட வேறுபாடுகள் கண்டறியப்பட்டன. கூடுதலாக, DFA முடிவுகளை MANOVA ஆதரித்தது. மேலும், இது Tetraodontiformes-யை சேர்ந்த உயிரினங்களின் அதிக எண்ணிக்கையிலான உடற்கூறியல் அம்சங்களை விவரிப்பதற்கான முதல் முயற்சி ஆகும். குறிப்பாக, இந்த ஆய்வு டெட்ராடொன்டைடிடே (Tetraodontidae) மற்றும் டியோடோண்டிடே (Diodontidae) சார்ந்த மீனினங்களை கண்டறிய மிகவும் உறுதுணையாக இருக்ககூடும் என இந்த ஆய்வு பரிந்துரைக்கிறது.

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