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Differentiation of two *Chlorophthalmus* species *Chlorophthalmus corniger* (Alcock, 1894) and *C. acutifrons* (Hiyama, 1940) based on otolith morphometry

R Nikki[a,e] , K V Aneesh Kumar*[,b], K Oxona[b], M P Rajeeshkumar[b], K K Bineesh[c], S H Midhun[d], H Manjebrayakath[b], N Saravanane[b] & M Sudhakar[b]

[a]Kerala University of Fisheries and Ocean Studies (KUFOS), Panangad PO, Kochi, Kerala – 682 506, India

[b]Centre for Marine Living Resources and Ecology (CMLRE), Ministry of Earth Sciences, Govt. Of India, Atal Bhavan, LNG Road, Puthu Vypin South, Ochamthuruthu PO, Kerala – 682 508, India

[c]Zoological Survey of India, Andaman & Nicobar Regional Centre (ANRC), Haddo, Port Blair, Andaman & Nicobar Islands – 744 102, India

[d]School of Marine Sciences, Cochin University of Science and Technology, Foreshore Road, Pallimukku, Kochi, Kerala – 682 016, India

[e]Present address: CSIR-National Institute of Oceanography, Regional Centre – Kochi, Kerala – 682 018, India

*[E-mail: aneeshmenan12@gmail.com]

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A comparative morphometric analysis of otoliths was done to understand the major morphometric characters responsible for differentiating two species of Green-eyes (*Chlorophthalmus corniger* and *C. acutifrons*) which is one of the dominant fish groups caught in the deep-sea trawling during the exploratory surveys as well as in the by-catch of trawlers targeting deep-sea shrimps at a depth range of 300 – 500 m in the Indian waters. A total of 53 intact, right otoliths (25 and 28 for *C. corniger* and *C. acutifrons*, respectively) were considered for the morphometric analysis to differentiate species collected from Andaman Sea. The study extracted four otolith morphometric measurements and five shape indices measured from the otoliths using image analysing software including otolith weight. The otolith morphometric parameters and shape indices showed significant relationship with the fish size were scaled with standard length to remove the influence of fish size from the data. Principal component analysis using scaled otolith morphometric measurements indicated that the first two axes described 84.78 % and 11.80 % of variation, respectively. The PC1 differentiated the species based on ellipticity and otolith weight followed by area and perimeter. *C. acutifrons* is differentiated from their congener with a more elliptic, heavy otolith with more otolith surface area. One-way PERMANOVA confirmed significant difference in otolith morphology between the species. Present study confirmed the suitability of otolith morphometric analysis in differentiating *Chlorophthalmus* species which are quite inevitable for taxonomic studies as well as for the better understanding of the species resolution in diet studies.

[**Keywords**: Andaman Sea, *Chlorophtalmus*, Deep-sea fishes, Otoliths]

Introduction

The genus *Chlorophthalmus* Bonaprte 1840 (Chlorophthalmidae), commonly known as "green-eyes" comprises of small, moderately compressed, fusi-form fishes. They have circum-global distribution with their biogeography extends across the temperate and tropical latitudes[1]. They are benthic fishes inhabiting on the outer continental shelf, slope, rise and abyssal plain. Most species are rare with only a few being locally abundant[2] of which 4 species are reported from India (*C. maculatus*, *C. punctatus*, *C. bicornis* and *C. acutifrons*)[3,4]. *Chlorophthalmus corniger* and *C. acutifrons* are the most dominant species in this genus reported from Indian waters[4]. Both these species differ in their depth preferences as *C. acutifrons* is more pelagic in nature compared to *C. corniger*[5]. They feed mainly on small epibenthic and bentho-pelagic crustaceans[3,6].

Otoliths are used as a potential tool for species identification as they are preferred over molecular analysis which is often time-consuming, expensive and not very accurate when fishes exhibit eco-morphological adaptations[7,8,9]. Otoliths have long been recognised as anatomical structures to identify the fishes in paleo-ichthyology due to their high inter-species morphological variations. Otolith shape indices have been used by many ichthyologists to differentiate between the closely related species[7,10,11] as well as to distinguish populations of a single species or stocks in marine and freshwater environments worldwide[12]. Also, the study of otoliths has many other applications in fish biology, ecology and fisheries science[13]. Mostly, sagittal otoliths, the largest of the three otolith pairs, have been used to study various biological characteristics of fishes such as age and growth pattern, movement and habitat preferences, population structure, and trophic ecology[14,15]. The inter-species variability of otolith shape is extensively used in the prey and predator studies[16]. It is believed that gut content studies in deep-sea fishes gives biased estimations since the fishes show regurgitation which leads to the underestimation of the prey items[17]. However, it is noticed that many hard structures such as bones, scales and otoliths remain in the gut nearly intact or in less degraded form. Among these, otoliths are widely used for the identification of prey and their sizes[9,18,19]. Various factors such as water temperature, diet[16], substrate type and depth of inhabitation can affect fish growth, otolith size and its shape[20,21]. Otolith morphology and morphometric characteristics can vary among the populations of same species in different habitats and regions[22].

Most of the studies carried out in the Indian waters on the genus *Chlorophthalmus* are restricted to taxonomy, food and feeding characteristics and length-weight relationships[23,24]. There is no scientific data available on their stock structure and population characteristics even if it remains as a major species at these depths caught during deep-sea exploratory surveys and also as by-catch in trawlers targeting deep-sea shrimps[3,24]. At present, there is no targeted fishery for this species in India. However, the development of alternative fishery resources is very much important under the purview that most of the coastal fisheries in the country are declining or no scope for the further expansion[24,25]. However, it is important to have thorough knowledge about the stock structure, population characteristics, prey and predator relationships and nutritional value of any non-conventional fauna before going for commercial level exploitation[26]. Hence, accurate identification of fishes up to species level is a major pre-requisite for the assessment of stocks and for formulating adequate management measures for the conservation and management of fishery resources[25].

As a major group at deep sea demersal habitat, the *Chlorophthalmus* fishes perhaps form major diet of many deep-sea fishes. The estimation of the size of *C. acutifrons* (misidentified as *C. nigromarginatus)* using otolith size was reported from the Andaman and Nicobar waters[19]. Better understandings of the otolith morphological variations among *Chlorophthalmus* species are inevitable to differentiate the prey species during the gut content analysis. Hence, the objectives of this study were (1) to understand the efficacy of morphometric variables to differentiate two common species of *Chlorophthalmus* collected from the Andaman Sea, and (2) to seek the influence of major morphometric variables responsible for the difference in the otoliths of *C. corniger* and *C. acutifrons* and their ecological interpretations.

Materials and Methods

Sampling and otolith collection

The sampling was carried out as part of the deep-sea fisheries expeditions onboard FORV *Sagar-Sampada* (Cruise No 349) during April 2016. Trawl operations were carried out in Andaman Sea at three stations (9°.37’529” N – 92°44’179” E; 12°32’54” N – 93°08’77” E; 12°05’107” N – 92°12’539” E) in the depth range of 290 – 360 m using high-speed demersal trawl II (crustacean version) (HSDT-CV). The fishes caught were sorted and the morphometric measurements of all representative species were measured onboard. The samples were identified using standard identification keys[1,27] and specimens were preserved in 5 % formalin and brought to the shore laboratory for further analyses. A total of 53 otoliths (right side) were collected for the study (28 otoliths from *C. acutifrons* (Mean ± SD: 17.84 ± 2.0) and 25 otoliths from *C. corniger* (Mean ± SD: 12.88 ± 0.78), respectively). The sagittal otoliths were collected by making an incision in the cranium after recording all the meristic measurements of the fish. The collected otoliths were cleaned thoroughly with distilled water, and were dried and preserved in glass vials for subsequent analysis. The images of the otoliths (sulcus side) were taken using the stereo zoom trinocular microscope (Leica model No. S8APO: Camera, Leica DFP-425). Five morphometric variables of the otolith (FL, feret length; FW, feret width; area, perimeter, weight) and 5 shape indices (ellipticity, circularity, form factor, rectangularity and roundnes) were measured using the image analysing software ImageJ for differentiating the species[28]. FL (feret length) is the longest dimension between the rostrum and post rostrum, and the FW (feret width) is the dimension from the dorsal to ventral edge taken at right angles to the FL through the focus of the otolith[13]. The otolith weight was measured using Metler, Toledo, ML 503 electronic balance to an accuracy of 0.0001 g. Ellipticity is an indicator of whether the changes in the axis are proportional. Roundness and circularity compares the otolith shape to a perfect circle. Rectangularity indicates the variation in length and width with respect to the area where 1 indicates perfect square. Form factor estimates the irregularity of the otolith margins where 1 is a perfect circle[8]. Shape indices were calculated following specific equations[8]. All the measurements are two-dimensional representations of the otoliths (photograph-based). The summary of the statistics of five otolith morphometric parameters and five shape indices are given in Table 1.

Statistical analysis

The standard length (SL), size parameter used in this study, which is highly correlated with the otolith morphometric measurements (r^2 ranges from 0.3 and 0.94), were confirmed after conducting the linear regressions between otolith measurements and fish size (SL; Table 2). Scaling of the otolith variables is very essential to eliminate the allometric effect of fish size on morphometric variables[8,29]. From the regressions between otolith morphometric measurements and fish size (SL) that have the highest r^2 value, the slope coefficient was used to calculate the standardised (scaled) otolith measurements to remove the allometric influence from the otolith morphometric data[8,29]. Scaled up otolith measurements (M_s) for each fish were calculated by the following equation.

$$M_s = M_o \left(\frac{\bar{x}}{x}\right)^b$$

Where, M_o is the original otolith measurement, $\bar{x}$ is the mean of the size parameter (SL) for all specimens, x is the size parameter (SL) of the individual specimen. The b value was estimated for each otolith measurement as the slope of the regression between log M_o and log x (refs. 8,29). All the otolith morphometric variables were examined for checking normality and homoscedasticity using the Shapiro-Wilk test and Levene test (R Core Team 2014). The principal component analysis (PCA) was employed for scaled up (allometry corrected) values to understand the inter-species otolith morphometric variations[16,30]. Since morphometric measurements were non-normal and heteroscedastic, a non-parametric permutation multivariate analysis, One-way PERMANOVA (distance measure based on Bray-Curtis Similarity Index, 9999 permutations) was performed to understand the species-specific difference in otolith morphology of two *Chlorophthalmus* species using PAST[31,32] (PAlaeontological STatistics, version v1.81).

Results and Discussion

Diagnostic characters of the *C. corniger* and *C. acutifrons*

Chlorophthalmus corniger is easily identified with their lower jaw which possesses a distinct forwardly projecting horizontal plate with strong, spine-like structure directed forward from the corners of the plates; head very large, 34.3 – 40.1 % SL; and eyes large, 29.8 – 40.8 %. While, "hump-like" dorsal profile in large adults and no horizontal forward directed spine-like teeth on lower jaw tip make identification of *C. acutifrons* (Fig. 1). Also, low numbers of 18 – 19 gill rakers on 1st gill arch of *C. acutifrons* compared to 22 – 26 gill rakers in *C. corniger*.

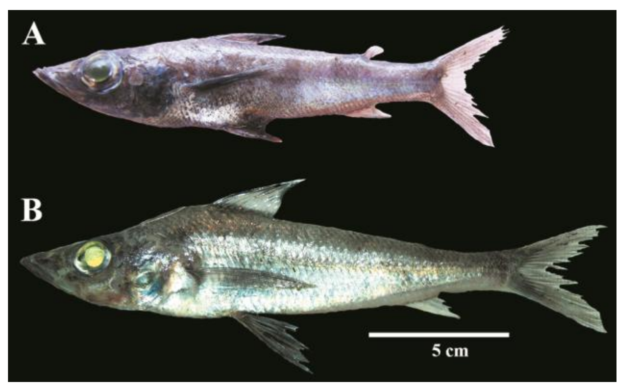

Fig. 1 — Representative images of *Chlorophthalmus corniger* (A) and *C. acutifrons* (B) collected from Andaman Sea during April 2016

Table 1 — Otolith sample size (n), ranges of mean value, standard length and otolith size measurements from two *Chlorophthalmus* species collected from Andaman Sea during April 2016

		SL (cm)	OW (mg)	FL (mm)	FW (mm)	Area (mm^2)	Perimeter (mm)	Elipticity	Roundness	Circularity	Rectangularity	Form factor
C. corniger	Min	11.36	4.0	3.74	1.96	5.56	9.53	10.08	0.41	16.03	0.66	0.68
(n = 25)	-Max	-14.3	-10.0	-4.95	-2.69	-9.51	-12.64	-19.19	-0.58	-18.40	-0.79	-0.74
	Mean±SD	12.88 ±0.78	6.3 ±1.40	4.39 ±0.34	2.25 ±0.19	7.23 ±0.97	11.09 ±0.81	14.46 ±2.46	0.47 ±0.04	17.11 ±0.73	0.73 ±0.03	0.74 ±0.03
C. acutifrons	Min	14.69	9.0	5.60	2.74	10.27	14.05	22.74	0.39	16.75	0.60	0.64
(n = 28)	-Max	-22.17	-25.0	-7.77	-4.0	-19.89	-19.24	-44.81	-0.50	-19.58	-0.76	-0.75
	Mean ±SD	17.84 ±2.00	16.81 ±4.15	6.58 ±0.59	3.35 ±0.31	15.17 ±2.56	16.53 ±1.44	32.34 ±6.22	0.44 ±0.03	18.03 ±0.89	0.68 ±0.03	0.70 ±0.03

SL - Standard length; OW - Otolith weight; FL - Feret length; and FW Feret width

Otolith morphology

Otolith of *C. corniger* is more or less oval in shape whereas, in *C. acutifrons* is more oblong (Fig. 2). Dorsal and ventral margin is smooth compared to *C. corniger*. Sulcus acusticus is heterosulcoid, ostial, supra median for both the species. Ostium is poorly defined, funnel-like, and shorter than the cauda for both the species. Cauda is tubular, curved, slightly flexed posteriorly ending close to the posterior-dorsal margin. Anterior region is more oblong for *C. corniger* and round for *C. acutifrons*; rostrum and antirostrum absent or poorly defined; excisura is wide without a notch. The posterior region of otolith in *C. corniger* is more oblong, whereas it is round and upper lobe is high for *C. acutifrons*. Colliculum and collum are absent. Edges are more irregular in shape. Intra-species variability is high for *C. acutifrons.*

Otolith samples and morphometric measurements

Linear regression analysis indicated significant correlations between otolith morphometric variables and SL (Table 2, Fig. 3A) with r^2 values ranging from 0.78 to 0.94 for *C. corniger* and 0.33 – 0.91 for

Table 2 — Correlation between various otolith morphometric variables and shape indices with fish size of two species of *Chlorophthalmus* collected from the Andaman Sea, India during 2016 April (statistically significant relationships are marked in bold)

	Relationship between	r^2	Significance level
C. corniger	SL X Feret length	0.87	***P* < 0.05**
	SL X Feret width	0.7	***P* < 0.05**
	SL X Area	0.85	***P* < 0.05**
	SL X Perimeter	0.94	***P* < 0.05**
	SL X Weight	0.72	***P* < 0.05**
	SL X Roundness	0.02	*P* > 0.05
	SL X Rectangularity	0.01	*P* > 0.05
	SL X Ellipticity	0.78	***P* < 0.05**
	SL X Form factor	0.09	*P* > 0.05
	SL X Circularity	0.08	*P* > 0.05
C. acutifrons	SL X Feret length	0.9	***P* < 0.05**
	SL X Feret width	0.64	***P* < 0.05**
	SL X Area	0.81	***P* < 0.05**
	SL X Perimeter	0.88	***P* < 0.05**
	SL X Weight	0.7	***P* < 0.05**
	SL X Roundness	0.32	***P* < 0.05**
	SL X Rectangularity	0.09	*P* > 0.05
	SL X Ellipticity	0.91	***P* < 0.05**
	SL X Form factor	0.34	***P* < 0.05**
	SL X Circularity	0.33	***P* < 0.05**

SL - Standard length and r^2 - correlation coefficient

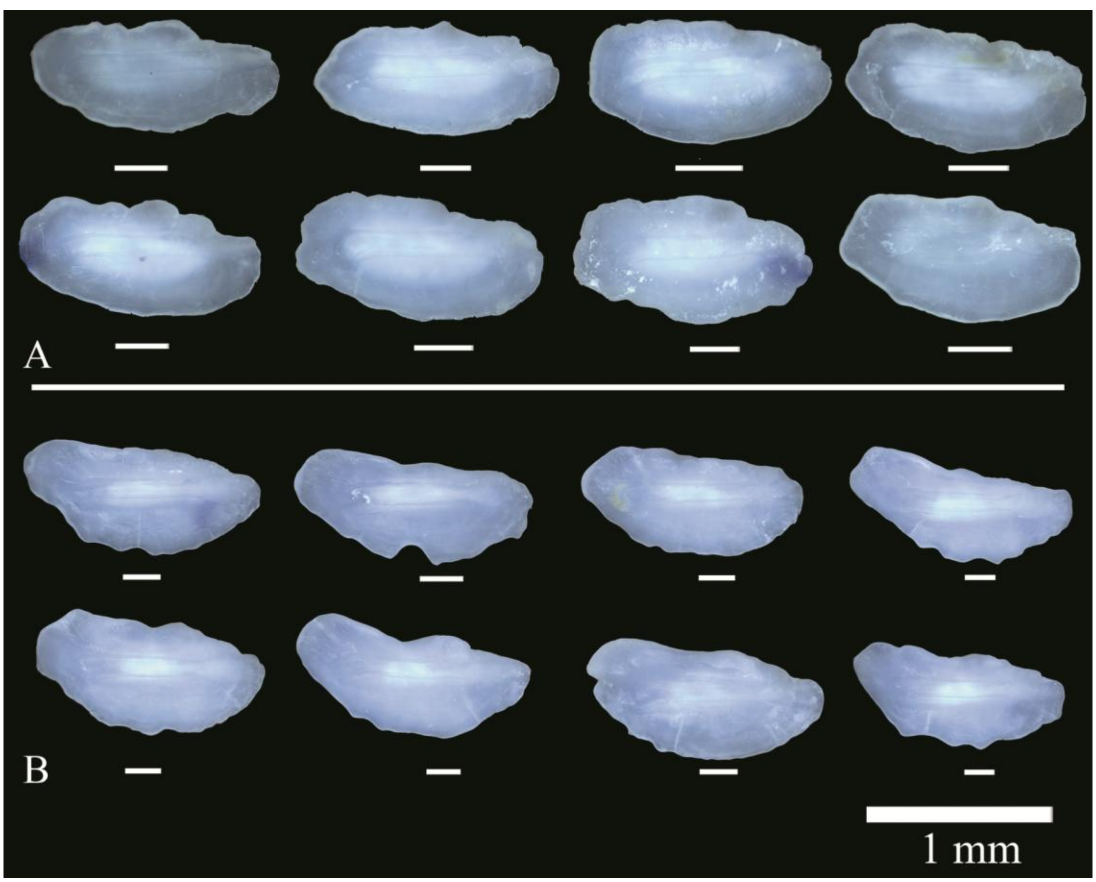

Fig. 2 — Representative otolith images of two *Chlorophthalmus* species (*C. corniger* (A), and *C. acutifrons* (B)) collected from Andaman Sea during April 2016 (scale = 1 mm)

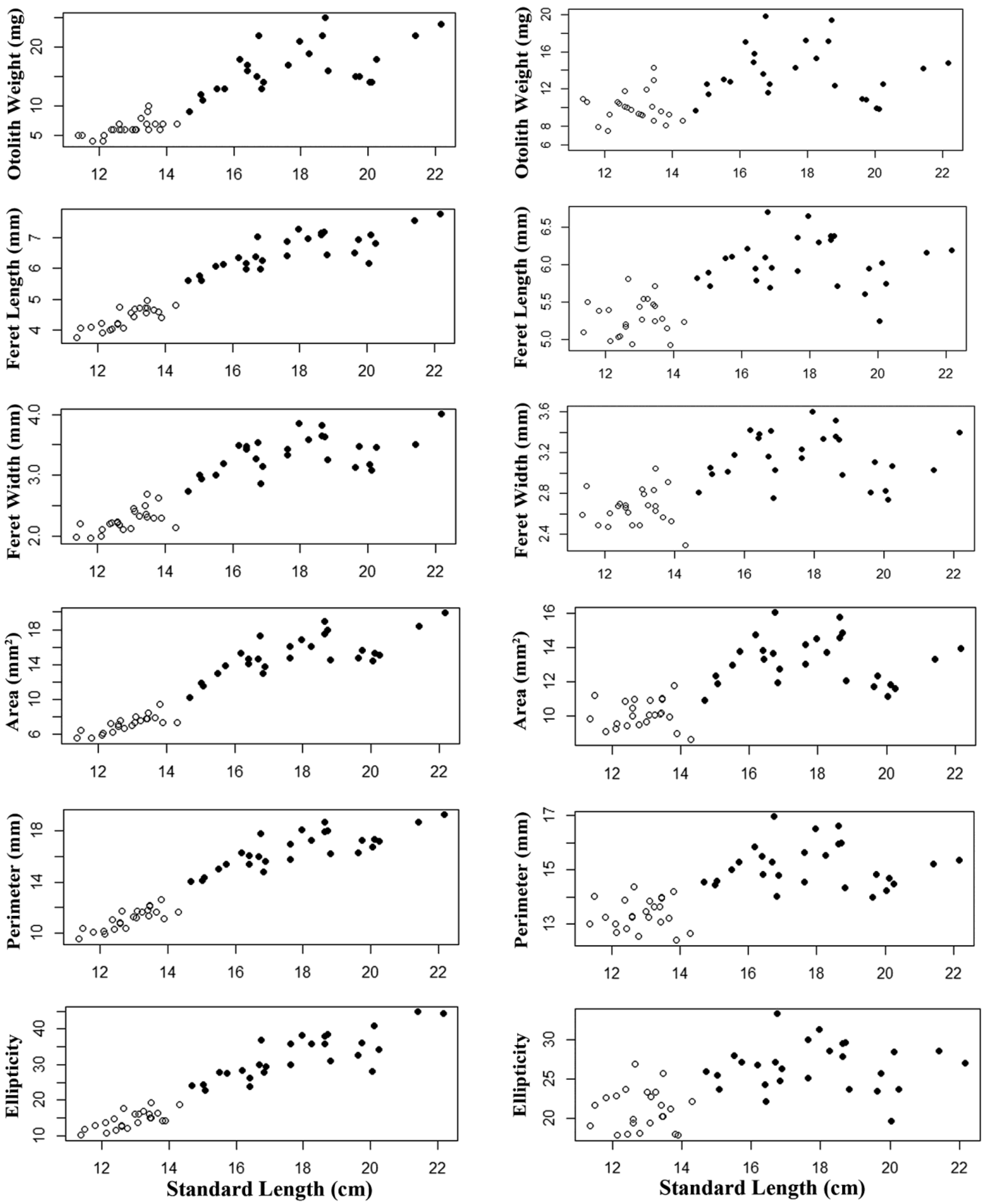

Fig. 3 — Scatter plots of original otolith measurements (A) and scaled up otolith measurements (B) with standard length for *C. corniger* (open circles) and *C. acutifrons* (filled circles) collected from Andaman Sea during April 2016

C. acutifrons ($p < 0.05$). Ellipticity is the only shape indices showed significant relations with SL for *C. corniger* whereas, other four shape indices showed significant relationship with size for *C. acutifrons* (roundness, ellipticity, form factor and circularity). All the morphometric variables showed significant relationship with fish size were allometrically corrected with SL using regression. The scaled-up otolith measurements did not correlate with SL ($r^2 < 0.01$, $p > 0.05$), indicating that the effect of fish size on otolith measurements was successfully removed from the data (Fig. 3B).

Multivariate analysis

The principal component analysis **(**PCA) was carried out to differentiate the species based on six different otolith morphometric variables and shape indices (scaled up) as well as to recognise major morphometric variables which in turn are responsible for the species differentiation. The first and second components described 84.78 and 11.80 % of the variation in otolith morphometry (Table 3). PC1 clearly differentiated the species based on ellipticity (r = 0.73) and otolith weight (r = 0.54) followed by area (r = 0.36) and perimeter (0.21; Table 4). Otoliths of *C. acutifrons* and *C. corniger* fall on the positive and negative values in the PC1 axis, respectively (Fig. 4). Otolith weight (r = 0.70) and ellipticity (r = -0.63) were responsible for the major differentiation in PC2. From PCA analysis, it was found that the two species of *Chlorophthalmus* are significantly differentiated based on the otolith morphometry. It was also found

Table 3 — Results of principal component analysis depicting of percentage of variance in the first five PC axes

PC axes	Eigen value	% Variance
1	25.6703	84.782
2	3.57445	11.805
3	0.72514	2.3949
4	0.289984	0.95773
5	0.0131792	0.043527

Table 4 — Correlation coefficient values between PC's components and otolith morphometric variables. In bold, higher absolute correlation values (r > 0.3)

	PC 1	PC 2	PC 3	PC 4	PC 5
Weight	**0.53584**	**0.69507**	**-0.4773**	0.04197	-0.0085
Feret length	0.09002	-0.0268	0.06016	0.05166	**0.69356**
Feret width	0.0529	0.05511	0.13242	0.1294	**0.65471**
Area	**0.35806**	0.21378	**0.71992**	0.16059	-0.1866
Perimeter	0.21351	0.02199	**0.32861**	**0.41727**	-0.0742
Ellipicity	**0.7267**	**-0.6282**	-0.1204	-0.2384	-0.0153
Roundness	-0.0006	0.01302	0.02105	-0.0017	-0.0585
Circularity	-0.0087	-0.2683	**-0.3311**	**0.84862**	-0.0806
Rectangularity	-0.0025	0.00303	0.00553	-0.0278	-0.1994
Form factor	0.00034	0.01058	0.01302	-0.0341	0.00493

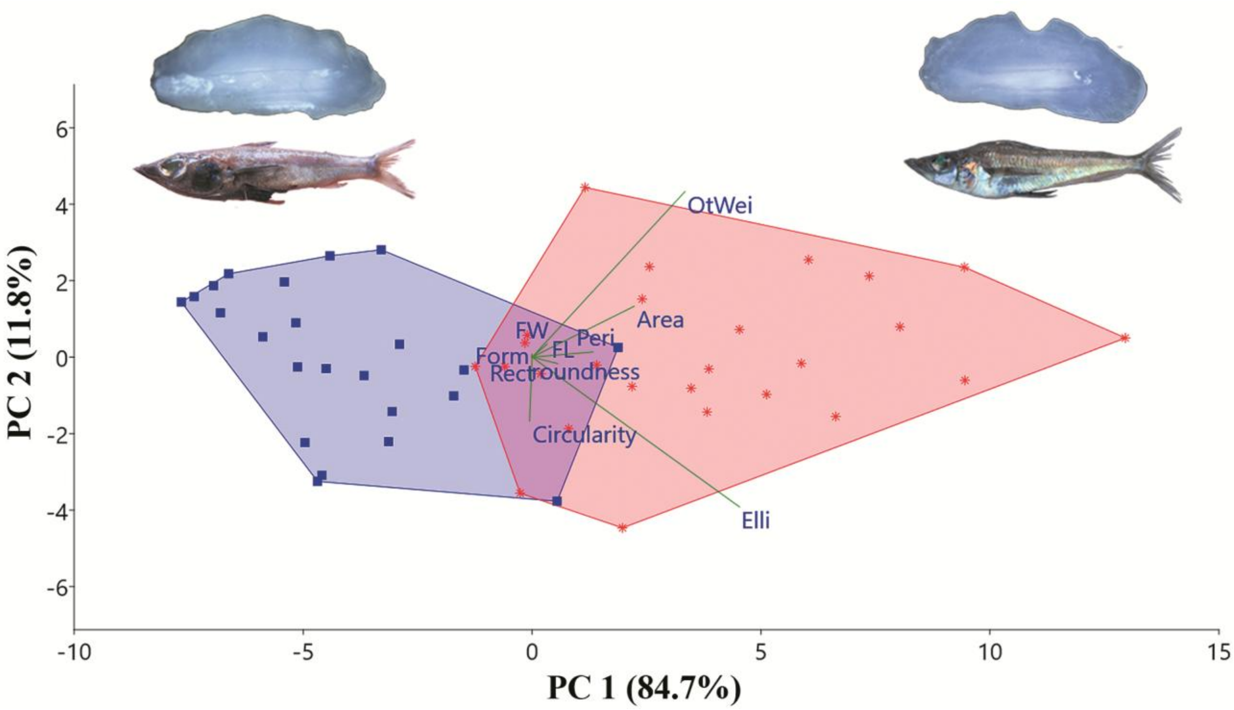

Fig. 4 — Principal component analysis showing the differentiation of two *Chlorophthalmus* species collected from Andaman Sea based on their otolith morphometry (blue squares - *C. corniger* and red star - *C. acutifrons*)

out that the shape index, ellipticity and the otolith measurement, otolith weight are the major factors responsible for the difference, followed by area and perimeter. Otolith of *C. acutifrons* is more elliptic, denser and is having more surface area compared to *C. corniger*.

The non-parametric permutation multivariate analysis One-Way PERMANOVA was found to clearly differentiate two *Chlorophthalmus* species (9999 Permutations, $F = 63.46$, $p = 0.0001$). Further, all pair-wise comparisons from the one-way PERMANOVA indicated that there is a significant difference in the species based on otolith morphometry.

Otolith morphometric analysis are widely used for the differentiation of stock/ populations/ species, taxonomic studies, food and feeding and palaeontological studies[9,33]. The regression equations derived from the relationship between fish size and otolith size help to estimate the size of fish for the food and feeding studies[19]. It is apparent from their small sizes and availability of the resources that many species of *Chlorophthalmus* is a potential food source for different deep-sea predators. The accurate identification of prey species is inevitable in understanding the food web dynamics of the species. The major objective of the present study was to check the suitability of otolith morphometric analysis to differentiate two species of *Chlorophthalmus* which are abundant in the deep-sea waters of Indian Exclusive Economic Zone[3,34].

In the present study, four otolith morphometric measurements (feret length, ferret width, area and perimeter), otolith weight and five shape indices (ellipticity, roundness, circularity, rectangularity and form factor) were studied for differentiating two *Chlorophthalmus* species[5]. Present study confirmed the suitability of otolith morphology to discern two *Chlorophthalmus* species collected from the Andaman Sea, India. PCA indicates that ellipticity, otolith weight and area are the major morphometric variables responsible for the variation. *C. acutifrons* possess more elliptic and heavier otoliths compared to its congener *C. corniger*. Relative area of the otolith is also large in *C. acutifrons*. The suitability of ellipticity and otolith weight to differentiate the species *Bembrops caudimacula* collected from Arabian Sea and Andaman Sea is reported earlier by Deepa *et al*.[9]. Otolith weight is widely used as a discriminating factor among the fishes and is very much sensitive to the variations in the growth as confirmed by the previous researches[9,11,35] with conflicting results[28]. Present study reiterates the suitability of otolith morphometric analysis to differentiate closely related species are in accordance with the previous researches[28,36]. The strong correlation between otolith measurements and fish size is reported by many authors[18,19] which helps to understand the interspecies variations in the otolith growth pattern[37,38]. The interspecies variation in the otolith shape is more closely related with the environmental characteristics of the habitat as well as the physiological constraints of the species than the phylogenetic variations[10,39,40]. *Chlorophthalmus* shows much variation in their bathymetric distribution. The known depth range of *C. acutifrons* and *C. corniger* is 184 – 285 m and 265 – 458 m, respectively[5]. The intra-species otolith variation is found to be high in *C. acutifrons* than in *C. corniger* (Table 1). Relative otolith size was found to be small in *C. corniger*. Studies confirmed that the low temperature decelerate the calcium carbonate incorporation in to the otoliths[29,41]. Environmental characteristics of the habitat where fish lives significantly influence the otolith size and shape along with the phylogenetic relationships between the species[16,37,42]. It is confirmed that the shallower depths experience large fluctuations of temperature when compared to deeper waters which have more stable oceanographic conditions[14]. Moreover, diet also found to influence the otolith morphology[20,41]. The availability of the prey varies in different depth regimes which could be reflected in the feeding habits of these two *Chlorophthalmus* species. Presently no information is available on the food and feeding habits of these two *Chlorophthalmus* species and their presence as prey in any deep-sea fishes. Fishes and crustaceans were the major prey item in the stomachs of its congener *C. agassizi*[24,43,44]. Diets of marine fishes reported to influence the protein component in the otolith which plays a major role in the biomineralization process ultimately reflecting in otolith 3D structure as observed[45].

Nonetheless, the present study demonstrated the usefulness of otolith morphology to identify the *Chlorophthalmus* species which are quite useful in understanding the food and feeding habits of their predators and show its significance in taxonomic as well as ecological insight on these deep-sea fishes. Further studies are essential with more samples with better understanding about various oceanographic characteristics of Andaman Sea to understand their influence on otolith morphology and morphometry.

This study supports the easiness of the procedure and accuracy to differentiate the two *Chlorophthalmus* species compared to expensive and more time demanded molecular analysis.

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Conflict of Interest

Authors declare that they no conflict of interest.

Author Contributions

RN & KVA involved in the conceptualisation, data collection and analysis, and manuscript writing. KO, MPR & HM: data collection and manuscript writing. KKB & SHM: manuscript writing. NS: manuscript writing, review and editing; and MS: supervision.

References

1 Russell B C, Chlorophthalmidae, In: *FAO species identification sheet for fishery purposes. Western Indian Ocean fishing area 51. Vol. 1. (Danish International Development Agency (DANIDA)*, edited by W Fischer & G Bianchi, (Rome, Food and Agricultural Organization of the United Nations), 1984. http://www.fao.org/3/ad468e/ad468eCO.pdf

2 Smith M M & Heemstra P C, *Smith's sea-fishes*, (J.L.B. Smith Institute of Ichthyology, Grahamston (South Africa)), 1986, pp. 261.

3 Venu S & Kurup B M, Distribution and abundance of Deep Sea fishes along the west coast of India, *Fish Technol*, 39 (2002) 20-26.

4 Bineesh K K, Molecular taxonomy of deep-sea fishes off the southern coast of India, Ph.D. Thesis, School of Industrial Fisheries Cochin University of Science and Technology (2015). http://dyuthi.cusat.ac.in/purl/5075

5 Kim Y U, kim Y S, Kang C B & Kim J K, New record of the genus *Chlorophthalmus* (Pisces: Chlorophthalmidae) from Korea, *Korean J Ichthyol*, 9 (1997) 163-168.

6 Kurup B M, Jiji T & Venu S, Distribution and biology of *Chlorophthalmus bicornis* Norman, beyond 250 m depth off the south west coast in the Indian EEZ, *J Mar Biol Assoc India*, 47 (2005) 57-62.

7 Zhuang L, Ye Z & Zhang C, Application of otolith shape analysis to species separation in *Sebastes spp.* from the Bohai Sea and the Yellow Sea, north-west Pacific, *Environ Biol Fishes*, 98 (2015) 547–558. http://dx.doi.org/10.1007/s10641-014-0286-z

8 Zischke M T, Litherland L, Tilyard B R, Stratford N J, Jones E L, *et al.*, Otolith morphology of four mackerel species (*Scomberomorus* spp.) in Australia: Species differentiation and prediction for fisheries monitoring and assessment, *Fish Res*, 176 (2016) 39–47. https://doi.org/10.1016/j.fishres.2015.12.003

9 Deepa K P, Kumar K V A, Oxona K, Nikki R, Bineesh K K, *et al.*, Population variations of Opal fish, *Bembrops caudimacula* Steindachner, 1876 from Arabian Sea and Andaman Sea: evidence from otolith morphometry, *Reg Stud Mar Sci*, 25 (2019). https://doi.org/10.1016/j.rsma.2018.100466.

10 Bani A, Poursaeid S & Tuset V M, Comparative morphology of the sagittal otolith in three species of south caspian gobies, *J Fish Biol*, 82 (2013) 1321–1332. https://doi.org/10.1111/jfb.12073

11 Tuset V M, Rosin P L & Lombarte A, Sagittal otolith shape used in the identification of fishes of the genus *Serranus*, *Fish Res*, 81 (2006) 316-325. https://doi.org/10.1016/j.fishres.2006.06.020

12 Zhang C, Ye Z, Panhwar S K & Shen W, Stock discrimination of the Japanese Spanish mackerel (*Scomberomorus niphonius*) based on the otolith shape analysis in the Yellow Sea and Bohai Sea, *J Appl Ichthyol*, 29 (2013) 368–373. http://dx.doi.org/10.1111/jai.12084.

13 Smale M J, Watson G & Hetch T, *Otolith Atlas of Southern African Marine Fishes*, (J. L. B. Smith Institute of Ichthyology, Grahamstown, (South Africa)), 1995, pp. 253.

14 Campana S E & Thorrold S R, Otoliths, increments, and elements: keys to acomprehensive understanding of fish populations, *Can J Fish Aquat Sci*, 58 (2001) 30–38. http://dx.doi.org/10.1139/f00-177.

15 Rooker J R, Secor D H, DeMetrio G, Kaufman A J, Rios A B, *et al.*, Evidence of trans-Atlantic movement and natal homing of blue-fin tuna from stable isotopes in otoliths, *Mar Ecol Prog Ser*, 368 (2008) 231–239. http://dx.doi.org/10.3354/meps07602.

16 Torres G J, Lombarte A & Morales-Nin B, Sagittal otolith size and shape variability to identify geographical in traspecific differences in three species of the genus *Merluccius*, *J Mar Biol Assoc UK*, 80 (2000) 333–342. http://dx.doi.org/10.1017/s0025315499001915.

17 Akhilesh K V, Bineesh K K, White W T, Shanis C P R, Hashim M, *et al.*, Catch composition, reproductive biology and diet of the bramble shark *Echinorhinus brucus* (Squaliformes: Echinorhinidae) from the south-eastern Arabian Sea, *J Fish Biol*, 83 (2013) 1112-1127. https://doi.org/10.1111/jfb.12201.

18 Battaglia P, Malara D, Romeo T & Andaloro F, Relationships between otolith size and fish size in some mesopelagic and bathypelagic species from the Mediterranean Sea (Strait of Messina, Italy), *Sci Mar*, 74 (2010) 605–612. https://doi.org/10.3989/scimar.2010.74n3605.

19 Kumar K V A, Nikki R, Oxona K, Hashim M & Sudhakar M, Relationships between fish and otolith size of nine deep-sea fishes from the Andaman and Nicobar waters, North Indian

Ocean, *J Appl Ichthyol*, 33 (2017) 1187-1195. https://doi.org/10.1111/jai.13467

20 Cardinale M, Doering-Arjes P, Kastowsky M & Mosegaard H, Effects of sex stock and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths, *Can J Fish Aquat Sci*, 61 (2004) 158–167. https://doi.org/10.1139/f03-151.

21 Lombarte A, Torres G J & Morales-Nin B, Specific Merluccius otolith growth patterns related to phylogenetics and environmental, *J Mar Biol Ass UK*, 83 (2003) 277–281. https://doi.org/10.1017/S0025315403007070h.

22 Reichenbacher B, Kamrani E, Esmaeili H R & Teimori A, The endangered cyprinodont *Aphaniusginaonis* (Holly, 1929) from southern Iran is a valid species: evidence from otolith morphology, *Environ Biol Fishes*, 86 (2009) 504–521. https://doi.org/10.1007/s10641-009-9549-5.

23 Bineesh K K, Akhilesh K V, Gomon M F, Abdussamed E M, Pillai N G K, *et al.*, Redescription of *Chlorophthalmus corniger*, a senior synonym of *Chlorophthalmus bicornis* (Family: Chlorophthalmidae), *J Fish Biol*, 84 (2014) 513–522. doi:10.1111/jfb.12305.

24 Hashim M, Distribution, diversity and biology of deep-sea fishes in the Indian EEZ, Ph.D. thesis, Cochin University of Science and Technology, Kerala, India, (2012) pp. 131. http://dyuthi.cusat.ac.in/purl/3056.

25 Sinha M K, Anrose A & Pratyush Das B C, Indian Deep sea Fisheries-Its Prospects, Issues and Challenges, *J Aquac Mar Biol*, 5 (2017) 00112. DOI: 10.15406/jamb.2017.05.00112.

26 Jo H S, Yeon I, Lim C, Hanchet S M, Lee D W, *et al.*, Fatty acid and stable isotope analyses to infer diet of Antarctic toothfish caught in the southern Ross Sea, *CCAMLR Science*, 20 (2013) 21-37. https://www.ccamlr.org/fr/system/files/science_journal_papers/Jo%20et%20al.pdf.

27 Alcock A W, *A descriptive catalogue on the Indian deep-sea fishes in the Indian Museum:* being a revised account of the fishes collected by the Royal Indian marine survey ship 'Investigator III', (Calcutta, Indian Museum), 1899, pp. 155-156.

28 Bostanci D, Polat N, Kurucu G, Yedier S, Kontas S, *et al.*, Using otolith shape and morphometry to identify four *Alburnus* species (*A. chalcoides, A. escherichii, A. mossulensis and A. tarichi*) in Turkish inland waters, *J Appl Ichthyol*, 31 (2015) 1013–1022. https://doi.org/10.1111/jai.12860.

29 Tuset V M, Olivar M P, Otero-Ferrer J L, López-Pérez C, Hulley P A, *et al.*, Morpho-functional diversity in *Diaphus spp*. (Pisces: Myctophidae) from the central Atlantic Ocean: Ecological and evolutionary implications, *Deep Sea Res I*, 138 (2018) 46-59. https://doi.org/10.1016/j.dsr.2018.07.005.

30 Rodgveller C J, Hutchinson C E, Harris J P, Vulstek S C & Guthrie C M, Otolith shape variability and associated body growth differences in giant grenadier, *Albatrossia pectoralis*, *PLoSONE*, 12 (2017) e0180020. https://doi.org/10.1371/journal.pone.0180020

31 Hammer Ø, Harper D A T & Ryan P D, PAST: Paleontological Statistics Software Package for Education and Data Analysis, *Palaeontol Electronica*, 4 (2001) pp. 9.

32 Treinen –Crespo C, Villegas-Hernández H, Guillén-Hernández S, Ruiz-Zárate M A & González-Salas C, Otolith shape analysis as a tool for population discrimination of the white grunt (*Haemulon plumieri*) stock in the northern coast of the Yucatan Peninsula, Mexico, *Rev Mar Cost*, 4 (2012) 157-168. http://hdl.handle.net/10256/9233.

33 Parisi-Baradad V, Manjabacas A, Lombarte A, Olivella R, Chic Ò,Piera J, *et al.*, Automated Taxon Identification of Teleost fishes using an otolith online database—AFORO, *Fish Res*, 105 (2010) 13-20. https://doi.org/10.1016/j.fishres.2010.02.005.

34 Sudarsan D, Sivaprakasam T E, Somavanshi V S, John M E, Nair K N V, *et al.*, An appraisal of the marine fishery resources of the Indian Exclusive Economic Zone, *Bull Fish Surv India*, 18 (1998) p. 81.

35 Ilkyaz A T, Metin G & Kinacigil H T, The use of otolith length and weight measurements in age estimations of three Gobiidae species (*Deltentosteus quadrimaculatus*, *Gobius niger* and *Lesueurigobius friesii*), *Turk J Zool*, 35 (2010) 819–827. doi:10.3906/zoo-1001-13.

36 Avigliano E, Jawad L A & Volpedo A V, Assessment of the morphometry of saccular otoliths as a tool to identify triple-fin species (Tripterygiidae), *J Mar Biol Assoc UK*, 96 (2016) 1167-1180. https://doi.org/10.1017/S0025315415001101.

37 Monteiro L R, Di Beneditto A P M, Guillermo L H & Rivera L A, Allometric changes and shape differentiation of sagittal otoliths in sciaenid fishes, *Fish Res*, 74 (2005) 288-299. https://doi.org/10.1016/j.fishres.2005.03.002.

38 Jawad L A, A comparative morphological investigation of otoliths of six parrot fish species (Scaridae) from the Solomon Islands, *J Fish Biol*, 93 (2018) 1046-1058. https://doi.org/10.1111/jfb.13787.

39 Parmentier E, Vandewalle P & Lagardère F, Morpho-anatomy of the otic region in carapid fishes: eco-morphological study of their otoliths, *J Fish Biol*, 58 (2001) 1046-1061. https://doi.org/10.1111/j.1095-8649.2001.tb00554.x.

40 Lombarte A, Palmer M, Matallanas J, Gómez-Zurita J & Morales-Nin B, Eco-morphological trends and phylogenetic inertia of otolith sagittae in Nototheniidae, *Environ Biol Fish*, 89 (2010) 607-618. https://doi.org/10.1007/s10641-010-9673-2.

41 Hussy K, Otolith shape in juvenile cod (*Gadus morhua*): ontogenetic and environmental effects, *J Exp Mar Biol Ecol*, 364 (2008) 35–41. https://doi.org/10.1016/j.jembe.2008.06.026.

42 Lombarte A & Cruz A, Otolith size trends in marine fish communities from different depth strata, *J Fish Biol*, 71 (2007) 53-76. https://doi.org/10.1111/j.1095-8649.2007.01465.x.

43 Anastasopoulou A & Kapiris K, Feeding ecology of the short nose green-eye *Chlorophthalmus agassizi* Bonaparte, 1840 (Pisces: Chlorophthalmidae) in the eastern Ionian Sea (eastern Mediterranean), *J Appl Ichthyol*, 24 (2008) 170-179. https://doi.org/10.1111/j.1439-0426.2007.01028.x.

44 Karuppasamy P K, Balachandran K, George S & Balu S, Food of some deep sea fishes collected from the eastern Arabian Sea, *J Mar Biol Assoc India*, 50 (2008) 134-138. http://eprints.cmfri.org.in/2152/.

45 Mille T, Mahé K, Cachera M, Villanueva M C, De Pontual H, *et al.*, Diet is correlated with otolith shape in marine fish, *Mar Ecol Prog Ser*, 555 (2016) 167-184. https://doi.org/10.3354/meps11784.