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SUPPLEMENTARY NOTE ON THE BLACK SNOEK, THYRSITOIDES MARLEYI FOWLER,1929 (PISCES: GEMPYLIDAE) FROM INDIAN WATERS

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ABSTRACT
A rare landed fish of the family Gempylidae were collected (n = 19), (8º22'38.43"N, 76º59'31.67" E) and the morphometric, meristic and molecular details were analysed from Indian waters. The DNA barcode (Ac-cession no.: MG886840), upon comparison with NCBI database, confirmed that the species was *Thyrsitoides marleyi,* which exhibited an identity of 99% to *T.marleyi* from Japanese waters (Accession: AP012505.1). Fin formula is D XVIII 17, A II 16, P I 14, V I 5, C 15. Among the morphometric characters in the percentage of standard length, all characters were genetically controlled (< 10%) and showed a high value of correlation coefficient (r > 0.96) indicates that most of the characters exhibited a direct proportional growth to each other. One of the specimens was deposited at the National Biodiversity Museum, (Details: EB 3162205, 887.0 mm, standard length (SL)), a designated national repository of CMFRI, Kochi.

INTRODUCTION

Taxonomy or systematics not only involves collecting, identifying, naming new species and developing sound classification but also the analysis of biological differences, biogeography, evolutionary biology and the relationship between host and parasites (Narendran 2006). The taxonomical study of a species reveals the extinction and biodiversity loss and thus can be prevented. Taxonomic knowledge of an organism is very useful in locating the abundance of fishes in a locality. Snake mackerels, Escolars or Snoeks, of the family Gempylidae comprised of 24 species fewer than 16 genera, one of the diverse group of fishes, distributed worldwide along the tropical and subtropical oceans (Kim *et al.*, 2012). The Gempilids were distributed widely from Indo-West Pacific Ocean, Korea, Japan, Taiwan, New Caledonia, New Hebrides, Malacca Straits, Andaman Sea, Western Australia, around Madagascar, La Reunion, East coast of South Africa and Red sea (Kim *et al.,* 2012). Gempilids reports from the Indian ocean were also very less. A study attributing to the Mean trophic index of fish fauna associated with trawl bycatch of Kerala, southwest coast of India (Bijukumar and Deepthi, 2009) has reported a fish under gempilidae family as *T. marleyi* specifying its habitat as benthopelagic, trophic index as 4.19 with very low resilience and the standard length reported ranged from 48.6 cm to 50.1 cm. The maximum size reported for the species was 200 cm (Fish base). Snake mackerels are oceanic predators which swim fast, usually found in deep waters during day time and migrate to the surface during the night. Studies of the taxonomical and biological aspects of the family gempilidae were very less. Usually, the species was noticed as bycatch along with ribbon fishes, seer fishes and tunas. Fish shows enormous diversity for their biological characteristics and behaviour. Morphological characters are external characters which are widely used in the identification of fishes. Morphological characters, such as body shape and meristic counts, have long been used to delineate stocks (Heincke, 1898), and continue to be used successfully (Villaluz and Maccrimmon, 1988; Haddon and Willis, 1995). Morphological characters such as morphometrics and meristics have been commonly used to identify stocks of fish (Teugels, 1982; Turan, 2004). A statistical analysis of morphometric characters gives a better idea of relationship within species and also to compare with the same species in different geographical areas. In fish, morphometric characters represent one of the major keys for determining their systematics, growth variability, ontogenetic trajectories (Kovac and Copp, 1999) and/or various population parameters. Both, the taxonomic classification of organisms, and understanding the diversity of biological life, were historically based on descriptions of morphological forms (Dean *et al.*, 2003). Morphological characters have been commonly used in fisheries biology to measure discreteness and relationships

among various taxonomic categories (Quilang *et al.*, 2007). Vizhinjam (8<2230 N 076<″ 5918.0µ E) is one of the major fish landing centres in Trivandrum coast having a high species richness (Baiju *et al.*, 2016). The physical structure of the rocky reef supports a high abundance of fishes and rich biodiversity (Baiju *et al.*, 2016). The monsoon fishery along the Vizhinjam coast during every year attributes to the landing of a variety of fish resources. The collection of rare fishes during this season by the researchers and fisheries students will be common. Our collection reported a rare fish from the family Gempilidae, led to the present study to reveal its morphometry, meristics and molecular details.

MATERIALS AND METHODS

The study is based on the samples collected from the hook and line fishers of Vizhinjam Coast, Thiruvananthapuram (8º22′38.43"N, 76º59′31.67" E). The photograph of the fish in fresh condition and collection site is shown in Figure 1 and 2 respectively. The samples were collected and brought to the laboratory without any physical damage and all the direct measurements were taken with a digital vernier calliper to the nearest 0.01 mm and weighed to the nearest 0.1 g by a digital analytical balance and get it identified by Nakamura (1980) Ajiad *et al*. (1982) and Kim *et al*. (2012). The examined specimens were preserved in 10% formalin. The morphometrics and meristics of the collected samples were studied properly; to get a detailed report of the fish from Indian waters followed the method of Hubbs and Lagler (2004). All the morphometric measurements calculated in percentage of Standard length were subjected to statistical analysis chiefly mean, standard deviation, range and correlation. Twenty-two morphometric measurements were documented and the various morphometric characters in the percentage of standard length have been studied. So in percentage standard length, the proportion to Standard Length (SL) vs Pre orbital length (PRL), Postorbital length (POL), Eye diameter(ED), Snout length (SNTL), Upper jaw length (UJL), Lower jaw length (LJL), Body depth (BD), Body width (BW), Head length (HD), Pre dorsal length (PDL), Pre pectoral length (PPL), Pre pelvic length (PVL), Pre anal length (PAL), Spiny dorsal height (SDH), Soft dorsal height (SODH), Pelvic fin height (PELH), Pectoral fin height(PECH), Anal fin height (ANH), Caudal depth (CD), Caudal peduncle length (CPL) and Interorbital length (IOL) were studied. Based on the difference in range, the different morphometric characters thus studied were then classified into genetically (<10%), intermediate (10 –15%) and environmentally (>15%) controlled characters (Surya *et al.,* 2016). The X-ray analyses of the samples were also done to clarify the skeletal structure of the fish. DNA barcoding, a species identification technique which involves the use of a marker region of approximately 650 base-pairs in the 5'-end of the mitochondrial cytochrome c oxidase subunit I gene (COI) was carried out to confirm the correctness of species identification of the specimens. Genomic DNA was isolated from the tissue stored in 90% ethanol using the phenol-chloroform method (Sambrook and Russell, 2001). Amplification of partial sequences of COI gene was carried out using the primer set LCO1490/ HCO2198 (Folmer *et al.*, 1994). PCR reactions were carried out in BIORAD T100 TM thermal cycler (Biorad, USA). The reactions were performed in 25μl containing 2.5 μl 10x assay buffer, 1.5 μl MgCl2 (1.5μM), 0.5μl of 10 μM of each primer, 0.5 μl of 10μM dNTPs, 1 U Taq DNA polymerase (Sigma Aldrich, USA) and 1μl of 50-100 ng template DNA. The PCR cycling profiles were as follows: An initial denaturation of 4 minutes at 94ºC, 30 cycles of denaturation for 30 seconds at 94ºC, 30 seconds of annealing at 42ºC, 45 seconds of extension at 72ºC, and a final extension of 7 minutes at 72ºC. The PCR products were checked on 1.5% agarose gels, bi-directionally sequenced and aligned in MEGA 7 (Kumar *et al*., 2016). Molecular identification of the specimens was conduct-ed by using the DNA sequences (650) of COI gene.

RESULTS AND DISCUSSION

The rare fish reported was *T.marleyi* a monotypic species of the family Gempilidae, landed along the Vizhinjam coast during the landing centre days of September 2017 and waited for few months to collect more samples but fishers together had intimated that the landings of such a fish was a rare occurrence and it occurred once or rarely twice in a year. The description of the fish is based on the samples collected from the hook and line fishers from a depth of over 80m. The genus *Thyrsitoides* comprised of only one species, *Thyrsitoides* marleyi Fowler,1929 worldwide. *T .jordanus*, a new species under the same genera were reported from the Gulf of Aqaba (Red sea) (Ajiad *et al*.,1982), but little is known about the distribution of the same species from anywhere in the world

Table 1: Comparison of morphometric and meristic measurements of *T.marleyi*

Morphometrics	Present study(India)	Kim (2012) (Korea)	Machidae (1985) (Japan)	Nakamura (1980)
Total length	887	976	-	794.8
Fork length	802	873	-	717.7
Standard length	785	818	471	688
Measurements (%SL)				
Body depth	11.25	10.7	1.7	11
Body width	8.4	5.6	4.7	5.4
Head length	25.47	26.6	25.7	24.8
Pre-dorsal length	25.6	24.3	23.9	23.1
Pre-pelvic length	31.21	29.6	30.1	29.5
Pre-pectoral length	24.56	26.5	-	25.2
Pre-anal length	75.79	78	78.1	76.6
Upper jaw length	11.71	11.3	-	11.3
Lower jaw length	13.75	12.3	-	-
Snout length	9.89	10.6	-	11.1
Interorbital length	4.01	4.1	-	3.9
Eye diameter	3.98	3.7	-	3.8
Caudal peduncle length	5.4	3.9	-	6.1
Caudal peduncle depth	2.92	3.1	-	3.1
Pectoral fin length	11.2	10.2	-	10.5
Pelvic fin length	5.29	6	-	6.4
Spiny dorsal length	47.77	-	-	-
Soft dorsal length	9.55	-	-	-
Anal fin length	6.5	7.7	-	-
Meristics				
Dorsal fins	XVIII, i + 11 + 6	XVIII, i + 11 + 6		XVIII, i + 11 + 6
XVIII, i + 17				
Pectoral fin rays	I,14	I, 13	I, 13	I, 14
Ventral fin rays	I, 5	II, 4	II, 4	I, 5
Anal fin rays	ii, 16	ii, 17	ii,17	ii, 16
Vertebrae	34	34	-	-
Branchiostegals	7	-	-	-
Caudal fin rays	15			

Table 2: Statistical estimates of various morphometric characters of *T.marleyi* in the percentage of Standard length

%SL	Mean	SD	SE	CV%	Range	Range difference	Correlation coefficient
PRL	13.97434	0.396695	0.125536	2.83874	16.45 - 17.76	1.31	0.974
POL	16.79208	0.395974	0.125308	2.358098	3.97 - 4.30	0.33	0.985
ED	4.115443	0.115021	0.036399	2.794864	3.9 - 4.3	0.4	0.971
SNTL	10.24677	0.351943	0.111374	3.434667	9.62 - 10.9	1.28	0.967
UJL	11.89987	0.142663	0.045146	1.19886	11.75 - 12.96	1.21	0.996
LJL	14.46392	0.39261	0.124244	2.714408	13.1 - 15	1.9	0.985
BD	15.75709	0.945526	0.299217	6.000636	14.2 - 17.1	2.9	0.858
BW	8.71942	0.313818	0.099309	3.599067	8.4 - 9.09	0.69	0.944
HL	25.60828	0.481623	0.152412	1.880732	25.3 - 26.9	1.6	0.986
PDL	25.90568	0.450371	0.142522	1.738503	25.6 - 27.1	1.5	0.987
PPL	29.51908	1.017102	0.321868	3.445574	27.18 - 31.26	4.08	0.962
PVL	31.54496	0.336986	0.106641	1.068273	31.2 - 32.32	11.2	0.999
PAL	76.75412	1.260414	0.398865	1.642145	75.85 - 79.72	3.87	0.999
SDH	48.48196	1.110639	0.351468	2.290831	47.25 - 51.1	3.85	0.997
SODH	10.0308	0.222894	0.070536	2.222099	9.5 - 10.43	0.93	0.988
PELH	6.712291	0.156107	0.049401	2.325689	6.2 - 7.3	1.1	0.991
PECH	10.77755	0.18143	0.057415	1.683411	10.5 - 11.01	0.51	0.993
ANH	7.23324	0.146197	0.046265	2.021181	7.1 - 7.9	0.8	0.984
CD	2.822932	0.136061	0.043057	4.819831	2.3 - 2.9	0.4	0.918
CPL	16.80672	0.248007	0.078483	1.475643	16.2 - 17.24	1.04	0.996
IOL	4.027023	0.113712	0.035985	2.823714	3.8 - 4.23	0.43	0.979

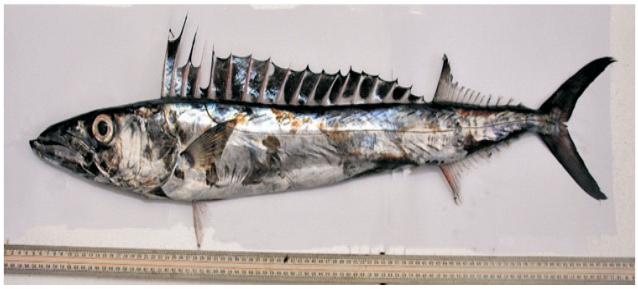

Figure.1: Photograph of *Thyrsitoides marleyi* Fowler, 1926

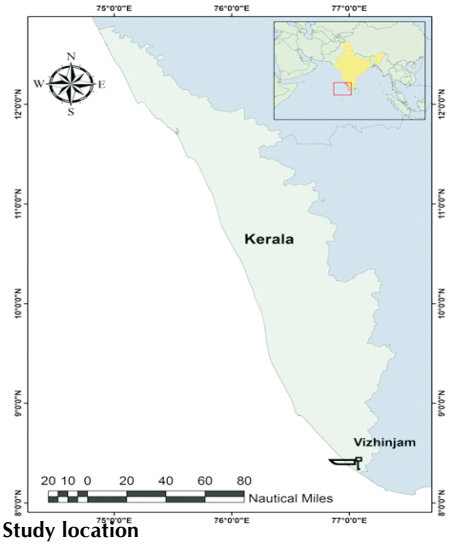

Figure. 2. Study location

rather than the above-said study. Initially, the genera *Thyrsitoides* were diagnosed by the following characters of the samples collected like bifurcated lateral line; separate two dorsal fins; well developed ventral fin, finlets behind dorsal and anal fins, almost similar second dorsal fin and anal fin counts; forked caudal fin, definite scale pattern on the different patches of the body; colour copper blue and brown dorsally and whitish to silver ventrally. The total length and weight of fishes varied from 56 - 85 cm and 0.85 - 1.925kg respectively. The body is elongated and slightly compressed with tiny cycloid scales at different patches of the body. Head is large and the dorsal profile of head sloping gently with an interorbital groove on the dorsal surface. Two pairs of nostrils present; pore-like on anterior and large slit-like on posterior. Mouth cleft of the fish is quite wide with lower jaw protrudes in front of the upper jaw. Both jaws bear sharp irregular canine-like teeth, of which 29 and 18 on upper and lower jaw respectively on each side. Tip of each jaw with a small cartilaginous process and 3 pairs of well-developed fangs like teeth on each side which are irregular in form. Operculum was smooth and without spines and scales. There is long gill arch without apparent gill rakers, but numerous sharp tiny spines along the gill arch.

Well distinguished spiny and soft dorsal fins present, spiny dorsal with black and white patches on the membrane and the soft with only a black patch on the anterior tip. Pectoral fin well developed with the upper ray reach up to the middle of 6th and 7th dorsal spine. Pelvic fin starts below the third dorsal spine, behind the pectoral fin and anal fin under the 2nd dorsal fin, followed by finlets. Finlets along the dorsal and ventral side are membraneous attachments with a longer finlet at the end on both sides. The lateral line originates from operculum and bifurcates between 4th and 5th dorsal spine, where the lower branch curves downward to pectoral fin and moves straight through the middle of the body to the caudal peduncle with a slight tilt between the 11th and 12th spine. The upper branch runs along with the dorsal profile of the body and ends near the soft dorsal fin. Caudal fin very well forked with a little long ventral lobe which is blackish. The body is blackish brown dorsally and silver ventrally with silver and brown crescent-shaped patches along the body in fresh condition.

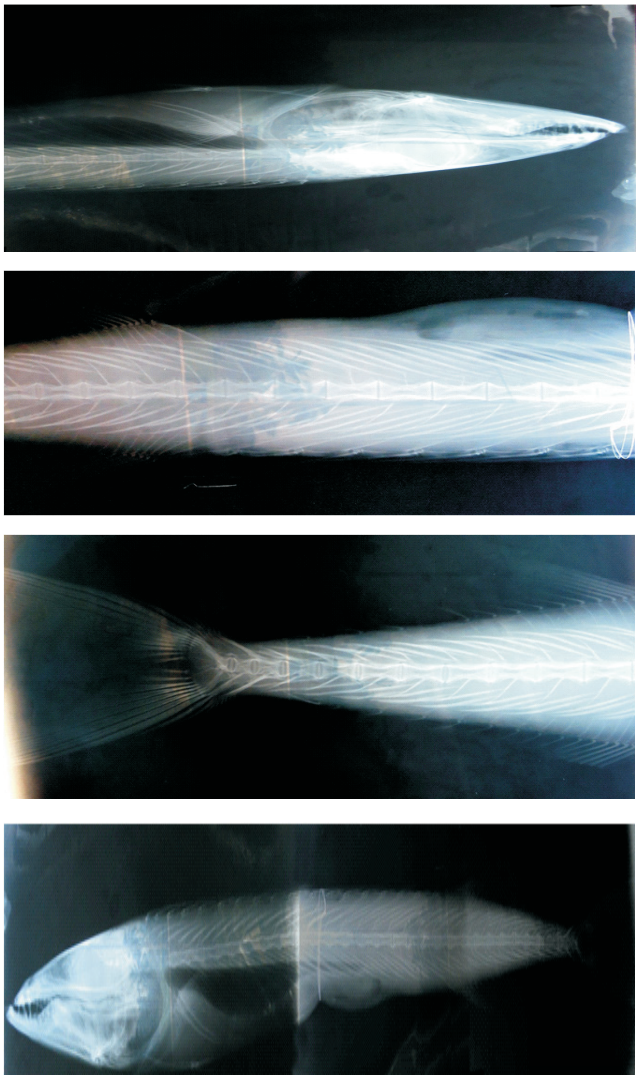

Figure.3: X-ray photograph of *T.marleyi* Fowler, 1926 showing the vertebrae and internal ribbings

The X-ray photograph of the fish showed that two rows of intramuscular bones stretching from the dorsal and ventral side of the vertebral column on both sides and it is depicted in Fig.3. A total of 32 ribbings from the dorsal and ventral sides of the body were counted from the photograph and the vertebral count was 34.

The morphometric and meristic counts of one of the specimens examined and its comparison to earlier workers from different countries was given in Table 1. The descriptive statistics of morphometric characters of *T.marleyi* in the percentage of standard length were given in Table 2. The morphometric characters studied in the percentage of standard length showed a high value of correlation coefficient ($r > 0.96$) to each other indicates most of the characters exhibited a direct proportional growth to each other. The better understanding of morphometric characters aids to identify unidentified taxa, investigate the mutated forms of groups and species and identify and classify biotypic associations (Straüss, 1985, Winans, 1985, Taylor *et al*., 1986). The morphometric characters of fishes are very keen to environmental distortion and quickly adapt themselves due to its phenotypic plasticity by modifying their physiology and behaviour. Many researchers opined about the high pliancy in the morphometric of fishes in response to the variations in the ecological parameters (Allendorf and Phelps, 1988, Swain *et al*., 1991, Stearns 1983, and Sajina *et al*., 2013). These adaptive capacities in fish ultimately changed its morphometric within and between populations than any other vertebrates and are more responsive to environmentally induced morphological variation (Wimberger 1992 and Allendorf *et al.,* 1987). Among the morphometric characters studied in the percentage of standard length, all characters were genetically controlled ($< 10\%$) and showed a high value of correlation coefficient ($r > 0.96$) indicates that most of the characters exhibited a direct proportional growth to each other. The absence of environmentally and intermediate controlled factors in the analysis revealed that the ecosystem disturbances affect least to its morphometric chiefly owing to its abundance in the deep sea.

The study also enlightens seven meristic characters of the fish *i.e*., a number of dorsal fin rays, pectoral fin rays, anal fin rays, ventral fin rays, caudal-fin rays, vertebrae and branchiostegal spines. The fin formula is D XVIII 17, A II 16, P I 14, V I 5, C 15. Meristic characters always with a distinct number and count, but varies to a certain range. Similar variations in the meristic characters were reported in case of *Megalaspsis cordyla* (Sajina *et al.,* 2013). There is absolutely no change in the meristic counts of *T.marleyi*. The DNA barcode from this study (Ac-cession no.: MG886840), upon comparison with NCBI database, confirmed that the species was T*hyrsitoides marleyi*, which exhibited an identity of 99% to *T.marleyi* from Japanese waters (Accession: AP012505.1). The DNA sequence (Ac-cession no.: MG886840) of the COI gene obtained from the present specimens were compared with those of *Thyrsitoides marleyi* (Accession No.: AP012505.1, Japan) deposited at the National Center for Biological Information (NCBI) database. One specimen examined for the present study was submitted to the National Marine Biodiversity Museum, a national repository of CMFRI, Kochi (Details: EB 3162205, 887.0 mm, standard length(SL), Hook and Line, National Marine Biodiversity Museum, Kochi, Kerala, India).

The present study is appurtenant to the marine finfish biodiversity along the Indian coast, which in turn articulates the importance of rare landings of the Indian fishery. DNA barcoding which is a validated tool for species identification (Henriques *et al*., 2015) was also employed as a supplemental identification method in addition to morphological characters in this study. The result indicated that COI sequences of present specimens were 99% identical to *T. marleyi* from Japanese waters (Accession: AP012505.1). The above details of the specified fish were not reported anywhere from India except a mention of the same as bycatch or sporadic occurrence. The site in which the samples collected were typically a reef area, lying close to the wadge bank zone documented the landings of a lot of deep-sea resources and other rare fishes (Abdussamad *et al*., 2016, Raheem *et al*., 2018, Raheem *et al*., 2019, Ambarish *et al*., 2017, Kishore et al., 2019, Gopakumar *et al*., 1991). The

close resemblance of *T. jordanus* described by Ajiad *et al*., 1982 with *T. marleyi* revealed the occurrence of a new species under the genera but the World Register of Marine Species (WORMS) considered it as a junior synonym to *T.marleyi* (Froese, R. and D. Pauly, 2020). The rare landing of *T.marleyi* along different coastal countries was reported by Nakamura, Izumi (1980) Kim *et al*. (2012) and Gon and Ofer (1987). Detailed studies rather than the sporadic occurrence of the species has not taken up properly by any of the researchers. An approach to estimate the basic details of *T.marleyi* from Indian waters forms a reference for further detailed studies. All the samples collected were in immature stage and the stomachs were emptied or in fully digested condition.

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