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SHORT PAPER

Narrow Barred Spanish Mackerel *(Scomberomorus commerson)* Confirmed with Leucism from East Coast of India

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Abstract

A single abnormally pigmented leucistic fish was collected from commercial fish catch from off Bay of Bengal, east coast of India. Using morphometric and DNA barcoding analysis, the fish species was identified as narrow barred Spanish mackerel (*Scomberomorus commerson*). The standard length of the fish was 130 cm and weight was 1600 g. Mitochondrial COI gene sequence search of the leucistic fish specimen showed more similarity towards the species *Scomberomorus commerson* in the scombroid fish group. This is the first report confirm the species narrow barred Spanish mackerel with leucism from the region and is probably caused by random genetic alterations.

Introduction

Body colour and colour patterns are important phenotypic characters in most of the vertebrates, which help for mate selection, species recognition, crypsis and warning or threatening of predators in a living environment (Zou, Zhang, Shi, Lin, Ouyang, & Zhang, 2015). Changes in the colour pattern often lead to misidentification of an organism in a closely related species. Colour pattern of an animal is contributed by two mechanisms namely, pigmentation and structural colourations. Pigments are the major substances for colouration of an animal, which are present in different parts of an animal in specialized cells called chromatophores (Ligon & McCartney, 2016). Loss of colour due to absence or lack of pigmentation is generally referred to as albinism in biology. Albinism is a genetically inherited condition produced by an autosomal recessive gene in the homozygous state in which the pigmentation protein melanin is either absent or non functional (Westerman & Birge, 1978; Reum, Paulsen, Pietsch, & Parker-Stetter, 2008; Kadir, Rasid, Wong, & Kwong, 2015). There are two kinds of albinism widely reported in fishes, complete albinism that refers to total absence of integumentary and retinal pigmentation and another is partial albinism or leucism, which is characterised by complete or partial loss of integumentary pigments, but with retinal pigments present (Goto, Sato-Matsumura, Sawamura, Yolota, Nakamura, & Shimiz, 2004). Albinism and leucism have been observed in different varieties of animals including fishes and they are not exclusive to any ecological or taxonomical group. It has been reported in more than 20 species of teleost and 36 species of cartilaginous fishes from freshwater and marine environment worldwide (Veena, Thomas, Raje, & Durgekar, 2011; Leal, Horst-Schulz, Lehmann-Albornoz, Machado, & Ott, 2013; Wakida-Kusunoki & Amador-del-Ángel, 2013).

The fish *Scomberomorus commerson* (narrow barred Spanish mackerel) is a commercially important high value epipelagic fish species, distributed in coastal tropical waters of the Indo-Pacific region (Dineshbabu, Muthaiah, Sasikumar, Rohit, & Bhat, 2012). The fish is mainly exploited by gill nets, trawls, hook and line, boat seines, shore seines and purse seines (Devaraj, Kasim, Muthiah, & Pillai, 1999). Normally, the freshly caught fish appears bluish grey colour in dorsal region and shading to silvery towards ventral side of the body

(Souissi, Golani, Mejri, Zaouali, & Capape, 2006). However, a single fish caught from a shoal exhibited partial loss of colour, and it was suspected to be a leucistic fish. Occurrence of leucism in narrow barred Spanish mackerel has been reported earlier from east coast of India. However, the species was not confirmed with proven methods. The occurrence of leucism may result in misidentification of fish species where the species mainly differentiated based on body colouration and which, need to be further confirmed with established methods for species confirmation. Therefore, the present study described the species confirmation of leucism fish observed from east coast of India using morphometric and molecular methods.

Materials and Methods

An adult leucistic narrow barred Spanish mackerel (Figure 1.) was caught in hook and line gear operated at a depth of 32 m and 11 km distance from shore in the Bay of Bengal, east coast of India (18° 04.004 N 83° 39.979 E) (Figure 2.). The leucistic fish was caught along with normal coloured individuals in a shoal during regular fishing. The fishermen communicated the information and the specimen was brought to the research laboratory of Visakhapatnam Regional Centre of ICAR-CMFRI, where it was examined and was measured using established methods (Collette, 2001). Further to confirm the species by molecular genetic analysis the tissue sample was collected for DNA barcode analysis by cytochrome c oxidase subunit 1 (COI).

The genomic DNA was isolated from the muscle tissues using NucleoSpin Tissue DNA extraction kit (Takara, Bio, Inc. Japan) following the manufacturer's protocol. The COI gene was amplified using universal primers (forward: 5' TCAACCAACCACAAAGACATTGGCAC-3'; reverse: 5'-

Figure 1. View of leucism (partial albino) fish specimen of *Scomberomorus commerson* possessing normal eye pigmentation collected from Bay of Bengal, east coast of India.

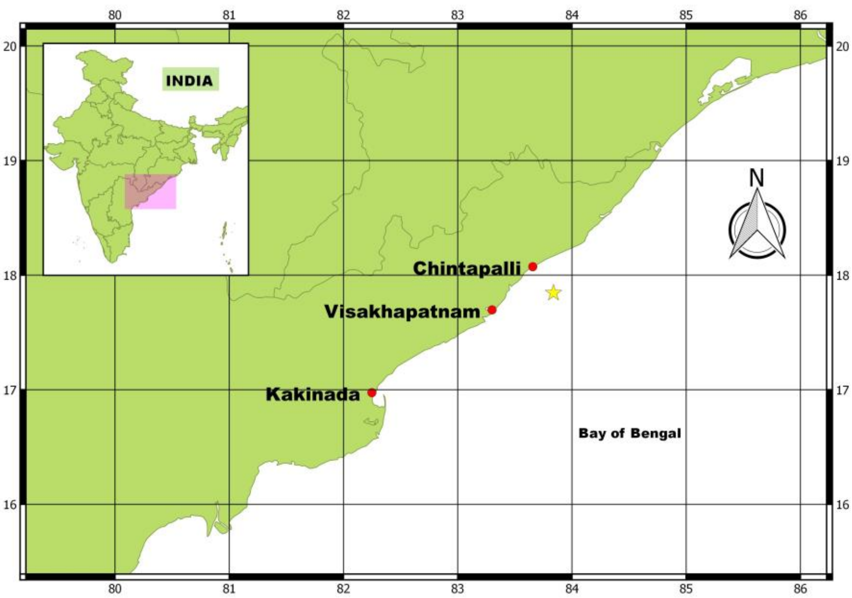

Figure 2. Collection site (Bay of Bengal, east coast of India) of the leucism fish *Scomberomorus commerson* indicated with star mark.

TAGACTTCTGGGTGGCCAAAGAATCA -3’) (Bioserve Biotechnologies Pvt. Ltd, India). Polymerase Chain Reaction (PCR) was performed using genomic DNA with the universal primers at 94 ^{o}C for 4 min; 30 cycles of 94 ^{o}C for 1 min, 56 ^{o}C for 1 min and 72 ^{o}C for 2 min and then final extension of single cycle at 72 ^{o}C for 10 min using thermo cycler (BIOER Little Genius, Bioer Technology company, China). The gene product was amplified using EmaraldAmp MAX PCR master mix (Takara, Bio, Inc, Japan). PCR product was visualized on 1.5% agarose gel, and subsequently purified by PCR purification kit (Qiagen, Germany) and then used for DNA sequencing. Nucleotide sequencing was carried out by ABI BigDYE terminator method (Applied Biosystems Inc., USA). The obtained sequence was analyzed for both forward and reverse sequences using Gene Runner software and pair-wise alignment using CLUSTALW tool. The sequence was subjected to nucleotide search in Barcode of Life Data Systems (BLOD) v3 data base to know the sequence match. Further, to establish the relationship between the suspected specimen with some of the related species, *COI* gene sequences of *S. commerson* (DQ885054.1, JF494453.1, KJ920128.1, KM677209.1, KJ134890.1, KF434774.1, HM007790.1) and other related species like *S. guttatus* (KJ134891.1, EF607535.1, EU871700.1, EU541328.11) and *Acanthocybium solandri* (EF653604.1, DQ080324.1, DQ497815.1, DQ197922.1) were retrieved from the NCBI GenBank public nucleic acid sequence repository. The genetic distance among different species was calculated by Kimura 2-parameter model using MEGA version 7. The phylogenetic relationship was inferred by using the Maximum Likelihood method with 100 bootstrap replicates in RAxML version 7.7.1 (Stamatakis, Hoover, & Rouge, 2008) and Neighbour-Joining method based on the Kimura 2-parameter model with 1000 bootstrap replicates in MEGA version 7.

Results and Discussion

The observed leucistic narrow barred Spanish mackerel was validated using traditional morphometric measurements and molecular genetic analysis for species confirmation. The morphometric parameters measured and their values are given in Table.1. The total length and weight of the fish was within the size range of earlier observation of narrow barred Spanish mackerel from India and elsewhere in the world (Yohannan, Jeyaprakash, Srinath, Thiagarajan, Livingston, Kasim, & Luther, 1992; Rao & Lakshmi, 1993; Kumar, Mahesh, Rao, Ghosh, & Maheswarudu, 2013). In east coast of India, the fish is observed with maximum size of 153 cm in length and 28 kg in weight by Kumar *et al*. (2013) and similarly, Shojaei, Taghavi, Seyfabadi, Bathe and Dehghani (2007) had reported the fish with 132 cm in length and 18.4 kg in weight from Iranian waters. The measured morphometric parameters of the fish were within the established range of percentage to the total length of the fish and these parameters agree with observations by Golani, Orsi-Relini, Massuti, and Quignard (2002); and Souissi *et al*. (2006). From the observed morphological characters of the fish specimen, it was concluded that the fish is narrow barred Spanish mackerel. However, the morphologically normal specimen showed abnormal body colour including devoid of integumentary colour, but with presence of retinal colour, suggesting that the particular fish could be affected by partial albinism or leucism. Normally the species exhibits dorsally bluish grey to ventrally silvery grey colour with transverse wavy bars; cheeks and lower jaw silvery white; first dorsal fin with bright blue to blackish blue; pectoral fin with light grey to blackish blue; caudal, anal, dorsal fin and finlets of anal fin greyish white to dark grey in colour (Souissi *et al*., 2006). The leucistic fish specimen was further examined, and it exhibited partial albino phenotypic characteristics of loss of pigments in the body. The entire body was dull whitish to pink in colour and the dorsal part of the body pigmented with grey and yellowish colours. The yellow pigmentation was high in the dorsal side, especially at the anterior region. The fins and finlets were pinkish in colour with yellow colour at the base. The eyes of the fish showed the normal retinal pigmentation. The morphological characters of the fish except the body colour suggested that the specimen was the narrow barred Spanish mackerel. This is the second report of leucism in this species from Indian waters as well as world. Previously, leucism in narrow barred Spanish mackerel was reported by Sethi, Rajapackiam, Poovannan, and Rathinam (2012) in India, and the size of the reported fish was 129 cm in length and 13.50 kg weight; however, the fish had more of yellowish pigmentation in dorsal portion of the body and also the fish specimen was confirmed based on morphometric characters alone.

The observed morphological characters hypothesised that the specimen is narrow barrel Spanish mackerel, belonging to scombroid fish group, however this hypothesis needs to be ascertained further because some of the species under this group have resemblances with same morphological characters at different size group (Jones & Silas, 1961). The narrow barred Spanish mackerel has more resemblance with *Scomberomorus guttatus* (Indo pacific king mackerel) at smaller size and with *Acanthocybium solandri* (Wahoo) at bigger size (Jones & Silas, 1961) with respect to morphological characters; however, all these fishes could be differentiated based on body colour pattern. The studied fish showed a different colour pattern because of leucism and therefore, it was little confusing to categorically describe the fish species based on its morphology alone. Therefore, confirmation using *COI*

Table 1. Morphometric characteristics of leucism fish, *Scomberomorus commerson*

Morphometric measurements	cm	% of TL
Total length (TL)	130.2	100.0
Standard length	113.4	87.1
Fork length	117.2	90.0
Pre-dorsal length	26.3	20.2
Pre-pectoral length	25.1	19.3
Pre-pelvic length	28.6	22.0
Pre-anal length	65.6	50.4
Body depth	19.3	14.8
Head length (HL)	24.9	19.1
		% of HL
Eye diameter	3.6	18.8

gene in mitochondrial region was taken to consideration for further confirmation of the fish species as is widely followed for most of the unknown fish species identification (Serra-Pereira, Moura, Griffiths, Gordoai, & Figueiredo, 2011; Ball, Jones, Lynghammar, Noble, & Griffiths, 2013). A 653 base pairs nucleotide sequence in the mitochondrial *COI* gene of the fish was deduced, and species identification was done based on sequence reads from the fish. The result of the analysis is in agreement with the hypothesis concluded from the morphological approach. The gene sequence searches in the NCBI Genbank and BOLD databases returned 100%, >99%, >96% & >95% matches with 7, 30, 48 and 15 individual *COI* gene sequence of *S. commerson* available in the data base, respectively. However, to further confirm the hypothesis, *COI* gene sequences from individuals of *S. commerson, S. guttatus* and *A. solandri* were downloaded from NCBI GenBank and aligned using CLUSTALW2 EMBL-EBI multiple sequence alignment programme. Genetic distance among the different fish species was calculated based on the variations in the *COI* gene sequences and given in Table 2. Phylogenetic relationship was established using Maximum Likelihood (Figure 3.) and Neighbour-Joining methods (Figure 4.) and phylogenetic tree was constructed accordingly. Result of genetic distance and phylogenetic tree analysis firmly supported the species identification based on morphometric and *COI* gene sequence based observations. All the sequences analysed were grouped in to three different categories according to species of origin such as *S. commerson*, *S. guttatus* and *A. solandri*. As expected the albino *CO1* gene sequence was grouped with the *S. commerson* and exhibited close relationship with gene sequence of different individuals of this species submitted from different parts of the world.

The mitochondrial *COI* gene sequence based identification confirmed that the fish specimen found with leucism is the narrow barrel Spanish mackerel. It has been reported in different studies that various factors like contaminated environmental parameters, light intensity, types of feeding during larval stages, endocrine hormones are responsible for body colour patterns and genetic factors including random genetic alteration, genetic alteration due to small population size are considered to cause colour abnormality in fishes (Tokac, Akyol, Aydin, & Ulas, 2013; Kadir *et al*., 2015). However, with respect to the location of the specimen caught, factors like heavy metals contamination in water and light intensity could be potentially excluded from the probable factors to cause colour change, because the open ocean is not generally contaminated and the species is a pelagic fish, thus light may not be an issue. The other factor namely feeding during larval stages may also be possibly excluded because, the fish generally moves in schools during larval stages for feeding, and feeds on a substance as a group, so individual fish getting affected by feeding may not be possible. The effect of small population size is also excluded, since the fish species is not impacted by overfishing in the wild and also good catches has been observed in most of the fish landings in this particular region (CMFRI, 2016). The other possible potential factors for the cause of abnormal colour in the individual fish are endocrine hormones and random genetic alterations. However, it has been observed in many of the lower vertebrates including fishes that the implication of endocrine hormones in chromatic changes is mostly temporary and fish returns to normal colour when the hormone level get stabilized (Ligon & McCartney, 2016). Therefore, the hypothesis of endocrine hormone being the causative agent for the colur changes in the fish is also not valid. Thus, leucism in this fish is probably by the result of genetic random alterations since the contaminated environmental factors, feeding behaviour and endocrine hormones seem to be improbable when considering the location of fish catch, behaviour of the fish in the water and nature of colour change. It has been stated in different studies that the lack of colouration in albino or leucism fish may result in increased chance for predation or render them less attractive for reproduction (Castillo, Melendez, & Garayzar, 2006). However, partial albinism may not be a handicap in the life of the studied specimen, since the

Table 2. Genetic divergence of *COI* gene among different group of fishes from scombridae family

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.*S.commerson* (Leucism): KT251199.1 (India)		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
2.*S.commerson*: DQ885054.1 (Canada)	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
3.*S. commerson*: JF494453.1 (Canada)	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
4.*S. commerson*: KJ920128.1 (India)	0.00	0.00	0.01		0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
5.*S. commerson*: KM677209 (India)	0.01	0.01	0.01	0.00		0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
6.*S.commerson*: KJ134890.1(Iran)	0.00	0.00	0.00	0.01	0.01		0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
7.*S. commerson* : KF434774.1 (India)	0.00	0.00	0.00	0.00	0.01	0.00		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
8.*S. commerson*: HM007790.1 (South Africa)	0.00	0.00	0.00	0.00	0.01	0.00	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
9.*A. solandri*: EF653604.1 (USA)	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76		0.00	0.00	0.01	0.02	0.02	0.02	0.02
10.*A. solandri*: DQ080324.1 (Canary Islands)	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.00		0.00	0.01	0.02	0.02	0.02	0.02
11.*A.solandri:* DQ497815.1(Taiwan)	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.01	0.01		0.01	0.02	0.02	0.02	0.02
12.*A.solandri:* 4DQ197922.1(Spain)	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.09	0.09	0.10		0.02	0.02	0.02	0.02
13.*S. guttatus:* KJ134891.1 (Iran)	0.73	0.73	0.73	0.73	0.74	0.73	0.73	0.73	0.76	0.76	0.76	0.74		0.01	0.01	0.01
14.*S. guttatus*: EF607535.1 (SouthChina)	0.73	0.73	0.73	0.74	0.74	0.73	0.73	0.73	0.76	0.76	0.76	0.74	0.02		0.01	0.01
15.*S. guttatus*: EU871700.1 (China)	0.73	0.73	0.73	0.74	0.74	0.73	0.73	0.73	0.76	0.76	0.76	0.74	0.02	0.00		0.01
16.*S. guttatus*: EU541328.11 (China)	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.75	0.75	0.75	0.74	0.04	0.02	0.02	

Genetic divergence value are shown in the below diagonal and the standard error values are shown above diagonal in the table

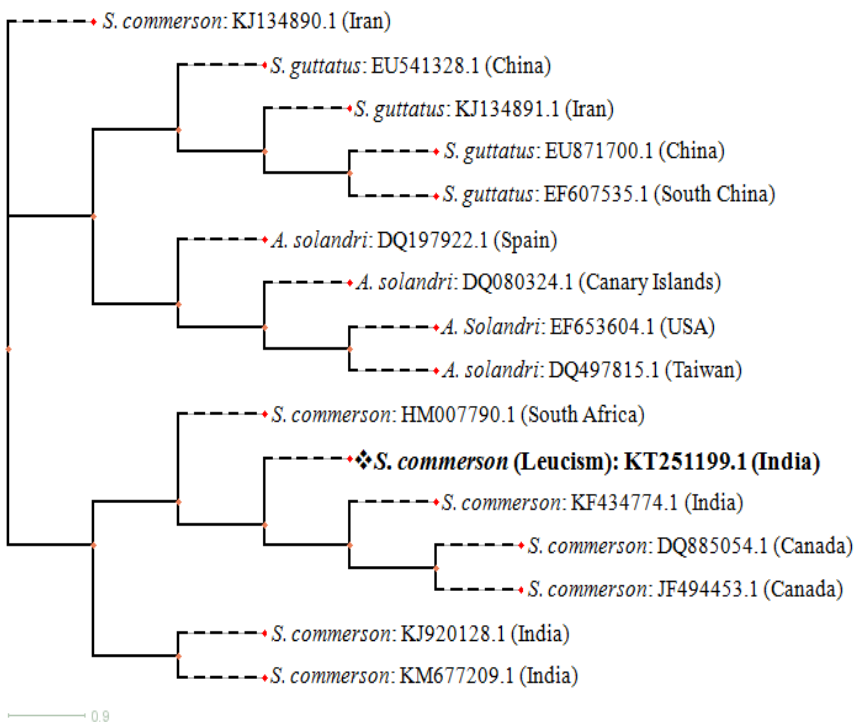

Figure 3. Phylogenetic tree obtained using *COI* gene sequence of fishes from three different closely related species from scombroid group by Maximum Likelihood method.

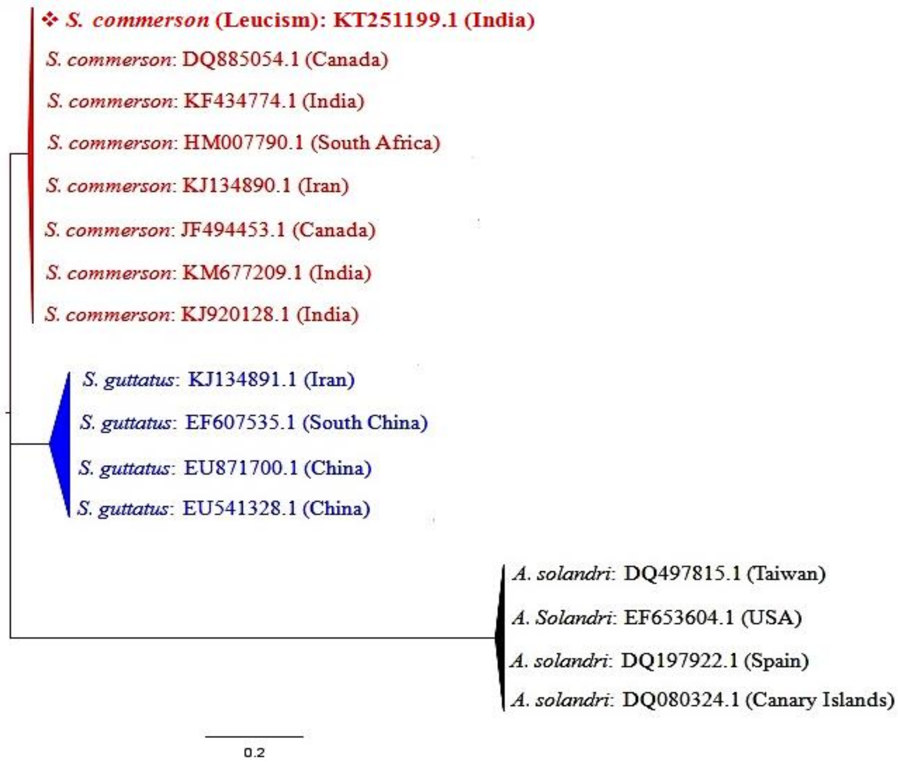

Figure 4. Phylogenetic tree obtained using *COI* gene sequence of fishes from three different closely related species from scombroid group by Neighbour Joining method.

fish could still potentially grow up to 16 kg in size like other normal fishes, which suggests that colour change is not disadvantageous for the survival of the marine fish.

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