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Short Communication

An account on the morphological identification and molecular confirmation of *Acanthopagrus berda* (Forsskal, 1775) from Malabar Coast, Kerala, India

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Family Sparidae is represented by ten species in Indian waters of which genus *Acanthopagrus* encompass three species namely, *Acanthopagrus berda*, *A. latus* and *A. bifasciatus*. Present study reveals regular catch of *A. berda* in different parts of Malabar coast, Kerala from Korapuzha, Kadalundi and Moorad estuaries and through conventional taxonomic as well as modern molecular approaches, the availability of *A. berda* at Malabar coast has been confirmed. Scientific reports regarding the geographic distribution of *A. berda* along the Indian coast are sparsely available. Previously, rare occurrence of *A. berda* has been reported from South east coast (Vishakhapatnam, Andhra Pradesh) of India.

[Keywords: *Acanthopagrus berda*, DNA barcoding, India, Kerala, Meristic, Morphometry]

Introduction

Seabreams commonly known as porgies belongs to the family Sparidae and are widely distributed in tropical and temperate seas. Most of the Sparidae fishes are commercially important and they play an major role in artisanal fisheries, industrial fisheries, sport-fishing as well as in aquaculture^{1,2}. Since the sparid fishes have excellent taste, they fetch high prices in local/domestic and international markets².

At present, more than 139 Sparidae species belonging to 36 genera are recognized³. The complexity in species identification in family Sparidae has resulted in addition of many more species with new descriptions after thorough investigation⁴. The major species complexes are found in the genus *Acanthopagrus*, which has been divided into six morphological forms⁵. Recently the

complexities in identification between the two species *A. berda* and *A. latus* have been resolved^{4,6}.

Peters (1855) proposed genus *Acanthopagrus* as a subgenus of *Chrysophrys*. There are currently 20 recognized species in *Acanthopagrus* genus⁴. *Acanthopagrus* species are high valued fishes that are caught commercially using hook and line, stake nets, fish traps, fish pots and trawl nets. They are also targeted in the recreational fishery, which is becoming increasingly popular⁷. Smith & Smith⁸ synonymized *Chrysophrys vagus* Peters, 1852 as *Acanthopagrus berda*.

The picnic seabream or river bream, Acanthopagrus berda (Forsskål, 1775) is globally considered as one of the prominent sparid fish in commercial fisheries and aquaculture⁹ because of its high recreational and economic value, market acceptance, good quality meat, disease resistance, tolerance to sudden variations in temperature and salinity, quick adaptation to captive conditions and fast growth rate. A. berda is an estuarydependent sea bream with a very wide distributional range throughout the tropical Indo-West Pacific region¹⁰, occurring from South Africa to India and extending to Japan, the East Indies and Northern Australia⁶. In India, a rare occurrence of *A. berda* from South east coast (Vishakapatnam, Andhra Pradesh) has been reported¹¹. Till date, only sparse scientific reports are available on the distribution of this species in Indian waters.

Due to similarity in the morphological features, species characterization is more complicated in closely related genera like *Acanthopagrus* and *Sparidentex*¹². A number of *Acanthopagrus* species such as *A. akazakii*¹³, *A. taiwanensis*¹⁴, *A. omanensis*⁶ and *A. pacificus*¹² have been described as new species (initially described as *A. berda*) collected from different waters.

Species identification is one of the important prerequisites for any biological research programme especially for biodiversity and conservation studies¹⁵. The current research was an attempt to confirm the identity of *A. berda* in Malabar coast through both conventional taxonomic and modern molecular approaches so that further studies on biology, reproduction and standardization of breeding programme of the species can be carried out with this species.

Materials and Methods

Korapuzha is a short river of 40 km, with a drainage area of 624 km², flowing through the Kozhikode district of Kerala state in India. Thirty specimens of A. berda were caught from Korapuzha estuary (11°39.858 N and 75°74.294 E) (Fig. 1) using cast net operated at 10 m depth during November to December 2015 and were brought to Calicut Regional Station of ICAR-Central Marine Fisheries Research Institute (CMFRI). The morphometric characters of the specimen were measured using a scale of 1 mm accuracy. The preservation of collected specimens were done using 4 % formaldehyde and representative samples were deposited in the Natural History Museum of Western Ghat Regional Centre (WGRC) of Zoological Survey India Calicut (Accession of at no ZSI/WGRC/IR.V.3005).

For species identification based on traditional methods, counts and measurements were taken following Hubbs & Lagler¹⁶ and the identification was carried out according to FAO Fish Identification sheets¹⁷. For molecular confirmation



Fig. 1 — Location map showing the sampling area

of the species, fish muscle tissue (approximately 0.1 g) was preserved in absolute ethanol at 4 °C for DNA analysis. Molecular analysis was carried out at the Marine Biotechnology Division of ICAR-CMFRL Cochin. The standard phenol/chloroform extraction protocol was used for carrying out the DNA extraction. Using a universal primer, 650 bp region of the Cytochrome C oxidase 1 was amplified employing a Biorad T100 Thermocycler (Biorad, USA) following standard protocol¹⁸. The PCR products were then sequenced with the primers using the BigDye Terminator Sequencing Ready Reaction v3.0 kit (Applied Biosystems, USA). The partial sequence of Acanthopagrus berda CO1 was deposited in NCBI. GenBank with the accession no: KY615237. The revised sequences were compared to reference sequences in the GenBank database for species identification using the BLAST algorithm (http://www.ncbi. nlm.nih.gov/ genbank/).

Results and Discussion

Morphometric measurements and meristic counts of 30 *A. berda* samples collected from Korapuzha, Calicut, India (Fig. 2) are given in Table 1. The prominent characters observed in the specimens were as follows: Body fairly deep and compressed with a pointed snout; 4-6 large compressed teeth in front followed by 3-5 rows of molar teeth in both upper and lower jaws. Gill raker count of first gill arch includes 6-8 on upper limb; 1 in the middle; and 7-9 on the lower limb. Single dorsal fin with 11 spines (broad and narrow spines arranged alternately with 4th, 5th and 6th spines longest) followed by 11 to 12 soft rays.



Fig. 2 — *Acanthopagrus berda* (Standard length: 28 cm) specimen collected from Korapuzha estuary

Table 1 — Morphometric and meristic counts of Acanthopagrus
<i>berda</i> collected from Korapuzha estuary, Calicut, Kerala ($n = 30$)

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Characters	Counts
Dorsal-fin rays	XI, 11- 12
Anal-fin rays	III, 9-10
Pectoral-fin rays	15
Pelvic-fin rays	I,5
Pored lateral-line scales	43-45
Scale rows between fifth dorsal-fin spine	3 1/2
base and lateral-line	
Scale rows between ninth dorsal-fin spine	3 1/2-4
base and lateral-line	
Scale rows above/below lateral-line	11-15
Scale rows on cheek	4-5
Scale rows on opercle	4-5
Gill-rakers (upper +angle+lower)	6-8+1+7-9
Standard length (mm)	184 (111-280)
As % of Standard length	Average (Range)
Body depth (highest)	49 (36-59)
Body depth at first anal-fin spine origin	42 (30-62)
Head length	30 (23-36)
Body width at pectoral-fin base	31 (24-39)
Snout length	10 (6-14)
Orbit diameter	8 (6-11)
Bony inter-orbital width	11 (6-15)
Inter-orbital width with membrane	11 (8-15)
Upper jaw length	9 (6-11)
Caudal peduncle depth	14 (8-24)
Caudal peduncle length	17 (11-26)
Pre-dorsal length	32 (23-42)
Pre-anal length	66 (51-88)
Pre-pelvic length	37 (30-54)
Dorsal fin base length	56 (45-65)
Anal fin base length	18 (13-24)
Caudal fin length	21 (14-45)
Caudal fin height Pelvic spine length	27 (11-44)
	16 (13-31)
First pelvic ray length	22 (16-30)
Pectoral fin length	37 (25-39) 7 (4-30)
First dorsal fin spine length Second dorsal fin spine length	10 (7-19)
Third dorsal fin spine length	13 (8-17)
Fourth dorsal fin spine length	15 (10-17)
Fifth dorsal fin spine length	14 (11-17)
Sixth dorsal fin spine length	14 (11-18)
Seventh dorsal fin spine length	13 (9-16)
Eighth dorsal fin spine length	12 (7-16)
Ninth dorsal fin spine length	11 (9-15)
Tenth dorsal fin spine length	11 (8-13)
Eleventh dorsal fin spine length	10 (8-14)
First dorsal fin ray length	11 (5-15)
First anal fin spine length	6 (4-19)
Second anal fin spine length	18 (8-27)
Third anal fin spine length	13 (9-16)
First anal fin ray length	12 (8-16)
2^{nd} anal fin spine/ 3^{rd} anal fin spine	1.44 (1.0-1.89)
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Pelvic fin with a single strong spine followed by five soft rays along with long pelvic axillary process. Anal fin consist of three spines (first anal spine shorter than eye diameter, second anal spine longer than the third) followed by 9 to 10 soft rays. Slightly forked round lobed caudal fin. Large scales, 43-45 scales in lateral line; $3^{1/2}$ scale rows between 5^{th} dorsal spine base and lateral line and $3^{1/2}$ to 4 scale rows between 9^{th} dorsal spine base and lateral line. Dark silver or grey colour body with silvery reflections; darker shade on the upper part of the body, base of scales and along the opercle edge. Soft dorsal, anal and pelvic fins black; dorsal spines often silvery with dark edges; pectoral fin with yellow shade at tinge and caudal fin with dark grey shading.

The morphometric and meristic characters match with the characters given in FAO fish identification sheets¹⁷. *A. berda* reported from the Red Sea (Yemen) were characterized by fairly deep body with silverygrey colour; anal fin rays rounded posteriorly; $3^{1/2}$ scale rows between the middle dorsal fin spine base and the lateral line^{8,19}. One of the unique character observed in the holotype of *A. berda* collected from the Indo-Pacific were the presence of a strong concavity above the posterior end of the maxilla, on the ventral edge of the first two infra-orbitals. Iwatsuki & Heemstra⁶ also reported that in *A. berda*, specimens over 13 cm SL, the strongly curved concavity above the posterior end of the maxilla were particularly obvious.

In sparids, the identification process mainly depends on the pattern of dentition which is more complicated. The important diagnostic characters of A. berda are 1) presence of four to six large compressed teeth in front of jaws followed by three to five molar teeth rows in both upper and lower jaws (Fig. 3a); 2) strong concavity above the posterior end of the maxilla (Fig. 3b); 3) 3.5 scale rows between the fifth dorsal fin spine base and the lateral line (Fig. 3c): and 4) uniformly blackish pelvic, anal and caudal fin with 2nd anal fin spine longer and stronger than the 3rd (Fig. 3d). All these characters are observed in the specimens collected from Korapuzha estuary confirming that the species is A. berda.

DNA barcoding is an efficient method used for fish species identification employing an array of species-specific molecular tags derived from the 5' region of the mitochondrial cytochrome c oxidase I (COI) gene²⁰. This technique has been effectively applied in fish identification²¹. The present study on DNA



Fig. 3 — (a) Mouth of *Acanthopagrus berda* showing 4 to 6 large more or less compressed teeth in front followed by 3 to 5 rows of molar-like teeth in both jaws; (b) strong concavity in the ventral edge of the first two infra-orbitals above posterior part of upper jaw; (c) 3.5 scale rows between the fifth spinous dorsal fin spine base and lateral line; and (d) anal fin with second spine longer than the third one

barcoding employing cytochrome oxidase gene (Accession no KY615237) and its comparison with existing published data revealed that the specimens collected from Calicut area was *Acanthopagrus berda* (Fig. 4).

Three species from the genera *Acanthopagrus viz. A. berda, A. latus and A. bifasciatus* have been reported so far from Indian waters¹¹. Behera *et al.*¹¹ reported the rare occurrence of the species at the Vishakhapatnam coast, Andhra Pradesh. But our observations reveal that there is a regular catch of this species using cast net or hook and line from Korapuzha, Kadalundi and Moorad estuaries of the Malabar Coast, Kerala.

Conclusion

A. berda is considered as one of the promising commercially important species for Indian mariculture. The confirmation of species is a basic requirement for their management in wild and captive conditions. The current study confirms the species identity in Kerala coast which could be a benchmark for further studies on this species from Indian waters.



0.06 0.04 0.02 0.00

Fig. 4 — Phylogenetic tree showing the similarity of Acanthopagrus berda DNA sequence with other published sequences

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Conflict of Interest

There is no conflict of interest.

Author Contributions

Specimen collection, identification, data analysis and manuscript writing: MTS and PPS; Manuscript editing, drafting and coordination of the work: PKA, KV and IJ; and Molecular biology analysis – SS.

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