Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), vol 13, 2025
http://dx.doi.org/

Morphological and molecular evaluation of *Etroplus suratensis* (Pices: Cichlidae) from three geographical locations of Peninsular India

Evaluación morfológica y molecular de Etroplus suratensis (Pices: Cichlidae) de tres localizaciones geográficas de la India peninsular

Nevin Raju[1], K.S. Sunish[2] and Kurian Mathew Abraham[3]

1, 2 Post Graduate and Research Department of Zoology, Maharaja's College, Ernakulam, Kerala, India.

3 Department of Aquatic Biology and Fisheries, University of Kerala, Kariavattom, Thiruvananthapuram 695 581, Kerala India

2. Corresponding Author: K S Sunish (ORCID - 0000-0002-1362-2939)

Tel.: +91-9847985555; E-mail address: sunishkss@gmail.com

ABSTRACT

Interspecific variations within species is common by having subspecies, races or strains and moreover, population differences are also evident in morphological, molecular, physiological and other characteristics, which will be added up by geographical variations. *Etroplus suratensis* (Pisces: Perciformes: Cichlidae) is an important brackish water food fish known for its high commercial value and recognised by government as the State fish of Kerala and distributed and cultured throughout the peninsular India. Identification sometimes fails to discriminate species while following the conventional morphometric measures due to phenotypic plasticity and presence of morphotypes. An attempt was made to differentiate the morphological and molecular characteristics of *E. suratensis* from three different states, Andhra Pradesh (Visakhapatanam), Karnataka (Mangalore) and Kerala (Cochin) of south India. Comparison of total of 8 morphometric measurements and phylogenetic evaluation using mitochondrial cytochrome oxidase subunit I (CoI) as molecular genetic marker was used assess geographical variation of the species. Morphologically, species from Visakhapatanam showed significant difference from other two localities whereas mean genetic divergence value between *E. suratensis* collected from Visakhapatnam and Mangalore with a native sample of *E. suratensis* were found to be significantly low (0.002%), which shows that *E. suratensis* distributed in Visakhapatnam and Mangalore though they are colour variants, are genetically similar.

Keywords: Molecular taxonomy; Morphometry; Molecular markers; Genetic Diversity; Intraspecific variation, pearl spot.

RESUMEN

Las variaciones interespecíficas dentro de las especies son comunes al tener subespecies, razas o cepas y, además, las diferencias poblacionales también son evidentes en las características morfológicas, moleculares, fisiológicas y otras, que se sumarán a las variaciones geográficas. Etroplus suratensis (*Pisces: Perciformes: Cichlidae*) es un importante pez comestible de aguas salobres conocido por su alto valor comercial y reconocido por el gobierno como el pez estatal de Kerala y distribuido y cultivado por toda la India peninsular. La identificación a veces no logra discriminar las especies siguiendo las medidas morfométricas convencionales debido a la plasticidad fenotípica y a la presencia de morfotipos. Se intentó diferenciar las características morfológicas y moleculares de *E. suratensis* de tres estados diferentes, Andhra Pradesh (Visakhapatanam), Karnataka (Mangalore) y Kerala (Cochin) del sur de la India. Se utilizó la comparación de un total de 8 medidas morfométricas y la evaluación filogenética utilizando la subunidad I de la citocromo oxidasa mitocondrial (CoI) como marcador genético molecular para evaluar la variación geográfica de la especie. Morfológicamente, las especies de Visakhapatanam mostraron diferencias significativas con respecto a otras dos localidades, mientras que el valor medio de divergencia genética entre *E. suratensis* recolectada en Visakhapatnam y Mangalore con una muestra nativa de *E. suratensis* resultó ser significativamente bajo (0,002%), lo que demuestra que las *E. suratensis* distribuidas en Visakhapatnam y Mangalore, aunque son variantes de color, son genéticamente similares.

Palabras clave: taxonomía molecular, morfometría, marcadores moleculares, diversidad genética, variación intraespecífica, mancha perlada.

INTRODUCTION

There are three *Etroplus* (Pisces; Cichlidae) species native to the Indian subcontinent *Etroplus suratensis* (Pearlspot or Green chromide), *E. maculates* (Orange chromide) and *E. canarensis* (Canara pearl spot or Banded chromide). *E. suratensis* is a euryhaline species that inhabits mainly brackish water and river mouths with commercial importance and designated as the State fish of the state of Kerala. It possesses certain requisite qualities essential for aquaculture such as good body weight, growth rate, high adaptability for food, tasty flesh and good market price (Joseph 1980; Rattan 1994) and more over many different aquaculture protocols like cage and raceway cultures were developed for the species (Abraham and Jayaprakas, 2011; Parakkandi *et al*., 2021). *E. suratensis* can be seen in different morphotypes with minor colour and/or morphometric variations with geographical distribution (Sreenivasan *et al*., 2021), moreover the phylogeny of subfamily Etroplinae warrants revisit based on molecular and other methods of systematics as an ambiguity persists (Sparks, 2008). Eventhough the fishes have evolutionary and economic importance, reports on its morphological heterogeneity and population differentiation were meagre. Suneetha (2007) studied the intra-specific phenotypic and genotypic variations of *E. suragensis* in Sri Lanka and Alex *et al*. (2013) reported the variations of the species from two different tropical lacustrine ecosystems of Kerala.

Morphometric studies play pivotal role in understanding the taxonomy of fish species in an environment and these morphometric features of the fish are unique to the species whereas the variations in its feature may be probably related to the habit and habitat (Avise, 1994). Conventional morphometric measures sometimes fail to discriminate species. Molecular genetic markers are considered to be powerful tools to detect genetic uniqueness of individuals, populations or species (Cavalcanti *et al*., 1999; Gunawickrama, 2007; Alex *et al*., 2013). DNA barcoding is a highly efficient method in the analysis of genetic divergences among species as well as for intraspecies-level identifications (Hebert *et al.* 2004a). One of the major properties of a DNA barcode is the possibility to easily associate all life-history stages and genders, to identify organisms from part/pieces, or to discriminate a matrix containing a mixture of biological species. Also, DNA barcoding is suitable for two different purposes: (1) the molecular identification of already described species (Askari and Shabani, 2013) and (2) the discovery of undescribed species (Gunawickrama, 2007).

An attempt has been made to resolve the taxonomic ambiguities among the various colourmorphs of *E. suratensis* from three different geographical locations of Peninsular India using morphological studies and the mitochondrial COI genes, as it is the widely accepted markers in the study of molecular phylogenetics (Hebert *et al.,* 2004; Wheeler, 2004). The cytochrome *c* oxidase I gene (COI) does have two important advantages. First, the universal primers for this gene are very robust, enabling recovery of its 5'end from representatives of most, if not all, animal phyla (Folmer *et al*., 1994; Yagi *et al*., 2001). Second, COI appears to possess a greater range of phylogenetic signal than any other mitochondrial gene (Zhang and Hewitt, 1997). Random Apmplified Polymorphic DNA (RAPD) markers have been employed to assess the population difference (Ali *et al*., 2004) and has been attempted for Tilapia and other cichlid species (Bardakei and Skibinski, 1994). Alex *et al*. (2013) employed RAPD technique along with morphometric evaluation to assess the variations of *E. suratensis* from two tropical lacustrine ecosystems of Kerala.

MATERIALS AND METHODS

Study Sites and Sample Collection

Fresh fish samples were collected from the commercial local fish landing centres at the time of landing and no live fish were sacrificed so that the study does not come under the purview of animal welfare laws, guidelines and policies. Specimens of *Etroplus suratensis* with similar maturity stages and/or size groups were collected from three different geographical locations i.e., three states of south India, Andhra Pradesh (Visakhapatnam), Karnataka (Mangalore) and Kerala (Kochi) (Figure 1). A total of 30 fishes each were collected and preserved in 10% formalin for morphometric analysis and in addition five more samples were preserved in alcohol for molecular assessment

Morphometric Analysis & Statistics

Morphometric studies of the samples were done by the standard methods of (Jayaram 1981). Apart from body colouration, a total of 15 morphometric characters were recorded including meristics. The

following morphometric measurements were taken (in millimeters, mm): head length, body depth, head depth, snout length, dorsal fin base, caudal fin base, and pectoral fin base. Meristic counts included dorsal fin elements, anal fin elements, pectoral fin elements, ventral fin elements, caudal fin elements, and scales on the lateral line. Data were converted to percentages of head length and standard length and expressed in its mean and standard deviation for comparison using analysis of variance (One Way ANOVA). Analyses were performed using R software (R Core team, 2021).

Figure.1 Sample collection location of *Etroplus suratensis*

Molecular Analysis

Muscle tissue samples along the lateral line were collected from each sample and stored in 95% ethyl alcohol at -20°C for DNA extraction and molecular analysis. Total DNA was extracted from muscle tissue using phenol/chloroform extraction protocol and ethanol precipitation method of (Sambrook *et al.,* 1989). The quality of the extracted DNA was evaluated electrophoretically in 0.8 percent agarose gel (Hoefer-HE33, Mini Horizontal Submarine Unit, Pharmacia Biotech,USA) and the quantity was determined using an Eppendorf biophotometer.

Universal primer amplification of Cytochrome C Oxidase 1 of marine fishes (Ward *et al*., 2005) were used to amplify a 650 bp region of the COI gene with the primers COI – F (5'- CCTGCAGGAGGAGGAGAGCC -3') and COI - R (5'- AGTATAAGCGTCTGGGTAGTC -3'). PCR amplifications were carried out in 25 µL reactions containing 1x assay buffer (100mM Tris, 500mM KCl, 0.1% gelatin, pH 9.0) with 1.5mM $MgCl_2$ (Genei, Bangalore, India), 10 p moles/µL of primer mix, 10 mMdNTPs (Genei, Bangalore, India), 1.5 U *Taq* DNA polymerase and 50 ng of template DNA. The PCR reaction was performed in PTC 200 gradient thermal cycler (M.J. Research, Inc., Watertown, Massachusetts, USA) programmed for an initial denaturation at 94°C for 4 min followed by 33 cycles of denaturation) at 94°C for 30 s, annealing at 53°C for 45 s, extension at 72°C for 1 min and a final extension at 72°C for 7 min. On successful completion of PCR, the amplifications were checked in 1.5% ethidium bromide-stained agarose gel. About 3 µL PCR product along with the marker (100bp DNA ladder, Genei, Bangalore, India) was run on the agarose gel, which was visualized under a UV transilluminator and documented using Image Master VDS (Pharmacia Biotech, USA). The remaining PCR product was cleaned using GeNeiTMQuick PCR purification kit (Genei, Bangalore, India). Sequencing was carried out on an ABI 3730 automated sequencer using Dye terminator mix v3.1 kit (Applied Biosystems).

Sequence data analysis

The COI sequence of *E. suratensis* was aligned using BIOEDIT sequence alignment editor version 7.0.5.2 (Hall, 1999) and was used to estimate genetic divergence values and for constructing phylogenetic tree (Neighbor Joining 'NJ') using MEGA 5.1 (Molecular Evolutionary Genetics Analysis) (Tamura *et al*., 2011) 'Indels' (insertions/deletions) or 'gaps' during aligning the sequences were treated as "fifth character". Kimura-2-parameter method was identified as the mutational model in the present study to accommodate the higher rate of transitions that are generally observed in teleosts.

RESULTS

Morphometric analysis

Morphometric characters were analysed and the parameters like body length, body depth, snout length, head depth, dorsal fin base length and body colourations showed variations in the samples from Visakhapatnam, and Mangalore compared to Kochi sample (Table 1). Visakhapatnam sample was smaller than the Mangalore and Kochi samples, with distinct body coloration (Figure 2). Meanwhile, the meristic characters of the three samples come in the similar range without a distinct change in any parameters.

Table 1. Analysis of variance (One Way ANOVA) of percentage morphological variations of *Etroplus suratensis* collected from three different locations of peninsular India

	Parameters	Visakhapatnam Sample	Mangalore Sample	Kochi Sample	F value
1.	Head length (% of SL)	28.69 ± 0.95	30.56 ± 0.78	30.87 ± 1.99	3.054
2.	Body depth (% of SL)	59.27 ± 0.90	69.48 ± 0.36	76.00 ± 1.32	4.545*
3.	Head Depth (% of SL)	37.71 ± 0.49	38.60 ± 0.55	41.00 ± 1.87	3.653*
4.	Snout length (% of HL)	45.62 ± 0.90	46.44 ± 0.46	49.09 ± 2.74	3.871*
5.	Dorsal fin length (% of SL)	25.67 ± 0.08	27.60 ± 0.20	28.27 ± 1.83	2.145
6.	Dorsal fin base (% of SL)	71.67 ± 0.77	74.77 ± 0.28	76.00 ± 1.2	2.981
7.	Caudal fin length (% of SL)	28.43 ± 1.6	34.55 ± 0.43	35.90 ± 0.49	4.351*
8.	Pectoral fin base (% of SL)	8.76 ± 0.58	10.11 ± 0.52	10.21 ± 1.83	2.654
9.	Body colouration	Black	Black	Green	

* $P < 0.05$

Molecular Analysis

COI sequences of samples collected from Visakhapatnam and Mangalore locations were compared with a native sample of *E. suratensis*. A total of 650 base pairs of sequences were aligned. Between the different *E. suratensis* species, 564 sites (87 %) were constant; 86 bases (13 %) exhibited variation. Among the substitutions 34 were transitions and 16 were transversions; the average pairwise ratio of transitions (Si) vs. transversions (Sv) was 2.13. The transitions were CT (17), AG (17) changes and transversions were AT (4), AC (4), GT (4), GC (4) changes. The observed frequency of nucleotide in the first codon position in *E. suratensis* is G (18.5%), A (23.3%), T (29.7%), C (28.5%). The mean genetic divergence value based on COI

gene sequences between four types of *E. suratensis* species with sequences of native *E. suratensis, E. canarensis, E. maculates* and *Oreochromis niloticus,* obtained from NCBI were 0.2%, 18.3%, 20% and 23.3% respectively (Table 2) (Figure 3).

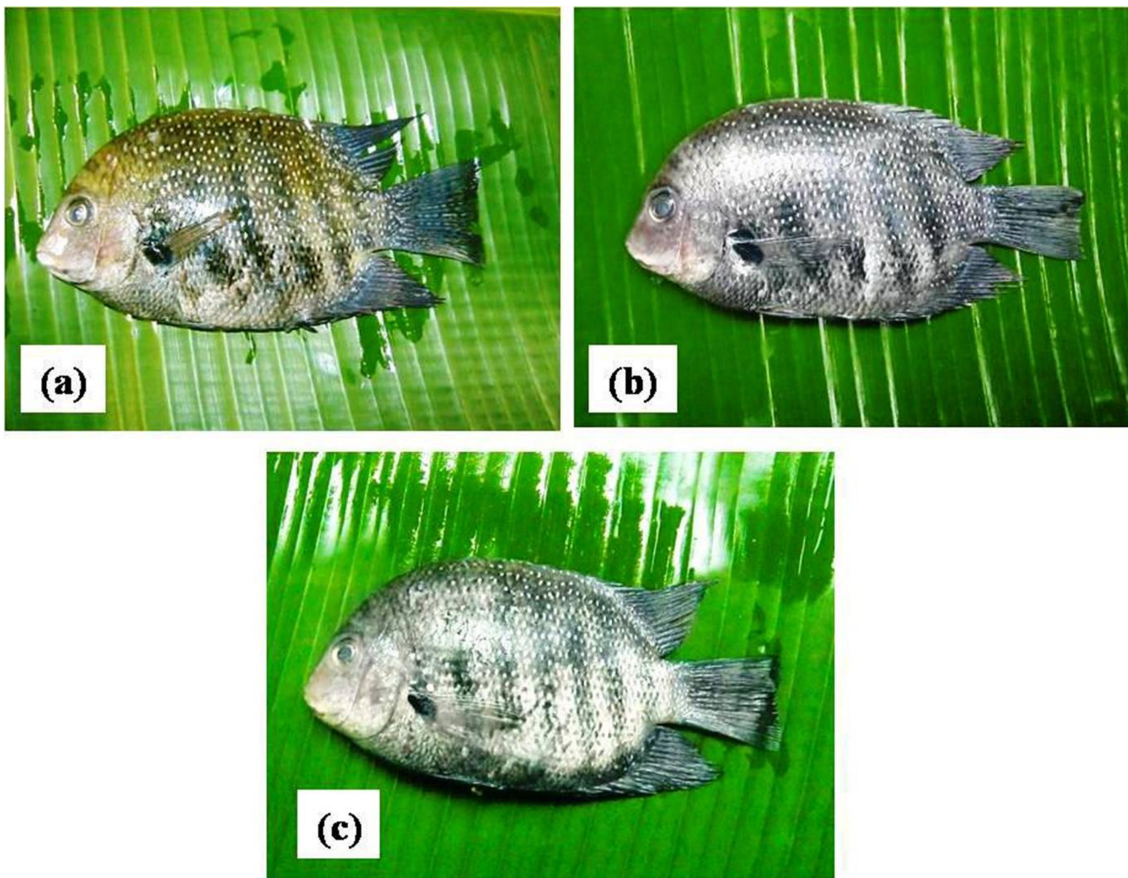

Figure 2a. *Etroplus suratensis* (Kochi Sample); Figure 2b *Etroplus suratensis* (Visakhapatnam Sample); Figure 2c *Etroplus suratensis* (Mangalore Sample)

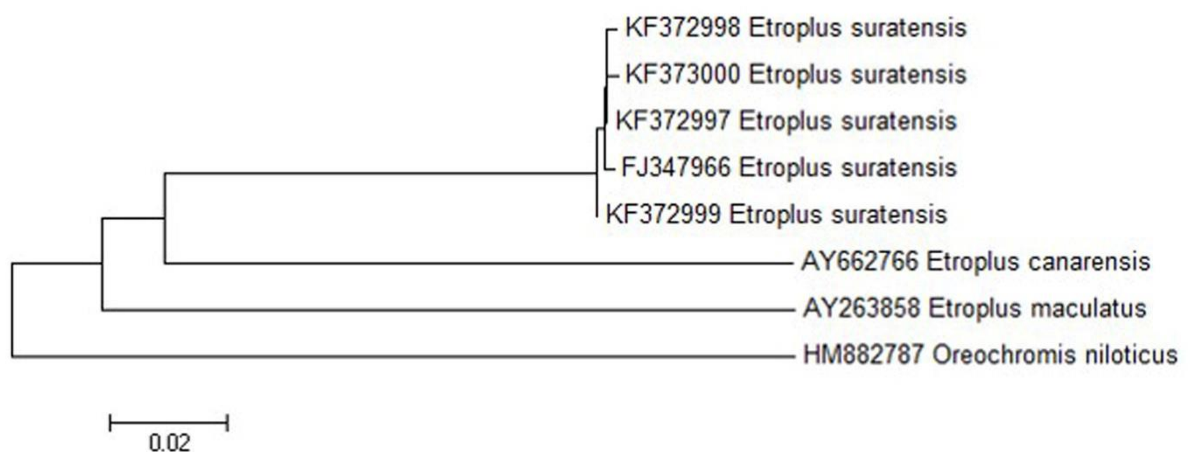

Figure 3. Neighbour joining tree from COI gene sequence data of *Etroplus suratensis* samples with sequences of native *E. suratensis*, *Oreochromis niloticus, E.*maculatus and *E.canarensis* downloaded from NCBI.

Table 2. Pair-wise genetic divergences of four types of *Etroplus suratensis* with sequences of native *E. suratensis, Oreochromis niloticus, E. maculatus and E. canarensis* downloaded from NCBI

	ES	ES1	ES2	ES3	ES4	EM	EC	ON
FJ347966 *Etroplus suratensis* (ES)								
KF372997 *Etroplus suratensis* (ES1)	0.002							
KF372998 *Etroplus suratensis* (ES2)	0.003	0.002						
KF372999 *Etroplus suratensis* (ES3)	0.003	0.002	0.003					
KF373000 *Etroplus suratensis* (ES4)	0.003	0.002	0.003	0.003				
AY263858 *Etroplus maculatus* (EM)	0.204	0.202	0.204	0.199	0.205			
AY662766 *Etroplus canarensis* (EC)	0.183	0.181	0.183	0.178	0.184	0.236		
HM882787 *Oreochromis niloticus* (ON)	0.231	0.233	0.233	0.236	0.237	0.265	0.263	0.00

The mean genetic divergence value between *E. suratensis* collected from Visakhapatnam, Mangalore and Kochi were found to be significantly low (0-0.002), which mean that *E. suratensis* distributed in Visakhapatnam, Mangalore and Kochi, though they were colour variants, genetically similar and is a single species of *E. suratensis*. Haplotype variation among samples collected from Visakhapatnam, Mangalore and Kochi were also found to be significantly low. Representative haplotypes from each locality were considered for analysis.

Accession Numbers

The COI sequences of *E. suratensis* haplotypes produced in the present study were submitted in the GenBank (NCBI) with accession no KF372997, KF372998, KF372999, KF373000 (Fig. 4).

10 20 30 40 50 60 70

....|....|....|....|....|....|....|....|....|....|....|....|....|....|

E1
EtroplussuratensisGGTGCTTGAGCTGGAATAGTAGGCACTGCTTTAAGCCTACTTATCCGAGCAGAACTAAGCCAAC
CAGGCT

E2
EtroplussuratensisGGTGCTTGAGCTGGAATAGTAGGCACTGCTTTAAGCCTACTTATCCGAGCAGAACTAAGCCAAC
CAGGCT

E3
EtroplussuratensisGGTGCTTGAGCTGGAATAGTAGGCACTGCTTTAAGCCTACTTATCCGAGCAGAACTAAGCCAAC
CAGGCT

E4
EtroplussuratensisGGTGCTTGAGCTGGAATAGTAGGCACTGCTTTAAGCCTACTTATCCGAGCAGAACTAAGCCAAC
CAGGCT

80 90 100 110 120 130 140

....|....|....|....|....|....|....|....|....|....|....|....|....|....|

E1
EtroplussuratensisCTCTCCTTGGAGACGACCAGATTTACAATGTTATCGTAACTGCGCACGCCTTCGTTATAATTTTCT
TCAT

E2
EtroplussuratensisCTCTCCTTGGAGACGACCAGATTTACAATGTTATCGTAACTGCGCACGCCTTCGTTATAATTTTCT
TCAT

E3
EtroplussuratensisCTCTCCTTGGAGACGACCAGATCTACAATGTTATCGTAACTGCGCACGCCTTCGTTATAATTTTCT
TCAT

E4
EtroplussuratensisCTCTCCTTGGAGACGACCAGATTTACAATGTTATCGTAACTGCGCACGCCTTCGTTATAATTTTCT
TCAT

150 160 170 180 190 200 210

....|....|....|....|....|....|....|....|....|....|....|....|....|....|

E1
EtroplussuratensisGGTTATGCCAATCATAATTGGCGGCTTCGGAAACTGACTAGTTCCCTTAATAATTGGTGCCCCCG
ATATA

E2
EtroplussuratensisGGTTATGCCAATCATAATTGGCGGCTTCGGAAACTGACTAGTTCCCTTAATAATTGGTGCCCCCG
ATATA

E3
EtroplussuratensisGGTTATGCCAATCATAATTGGCGGCTTCGGAAACTGACTAGTTCCCTTAATAATTGGTGCCCCCG
ATATA

E4
EtroplussuratensisGGTTATGCCAATCATAATTGGCGGCTTCGGAAACTGACTAGTTCCCTTAATAATTGGTGCCCCCG
ATATA

220 230 240 250 260 270 280

....|....|....|....|....|....|....|....|....|....|....|....|....|....|

E1
EtroplussuratensisGCCTTCCCCCGAATAAACAACATGAGCTTCTGACTCCTTCCTCCCTCATTCTTGCTCCTTTTAGCAT
CCT

E2
EtroplussuratensisGCCTTCCCCCGAATAAACAACATGAGCTTCTGACTCCTTCCTCCCTCATTCTTGCTCCTTTTAGCAT
CCT

E3
EtroplussuratensisGCCTTCCCCCGAATAAACAACATGAGCTTCTGACTCCTTCCTCCCTCATTCTTGCTCCTTTTAGCAT
CCT

E4
EtroplussuratensisGCCTTCCCCCGAATAAACAACATGAGCTTCTGACTCCTTCCTCCCTCATTCTTGCTCCTTTTAGCAT
CCT

290 300 310 320 330 340 350

....|....|....|....|....|....|....|....|....|....|....|....|....|....|

E1
EtroplussuratensisCTGGTGTAGAGGCAGGGGCAGGAACAGGGTGAACCGTATACCCTCCTCTAGCAGGCAACCTTG
CCCATGC

E2
EtroplussuratensisCTGGTGTAGAGGCAGGGGCAGGAACAGGGTGAACCGTATACCCTCCTCTAGCAGGCAACCTTG
CCCATGC

E3
EtroplussuratensisCTGGTGTAGAGGCAGGGGCAGGAACAGGGTGAACCGTATACCCTCCTCTAGCAGGCAACCTTG
CCCATGC

E4
EtroplussuratensisCTGGTGTAGAGGCAGGGGCAGGAACAGGGTGAACCGTATACCCTCCTCTAGCAGGCAACCTTG
CCCATGC

360 370 380 390 400 410 420

....|....|....|....|....|....|....|....|....|....|....|....|....|....|

E1
EtroplussuratensisAGGGGCCTCTGTTGATTTAACCATCTTTTCCCTACATCTAGCAGGTGTTTCATCTATTCTTGGGGC
AATC

E2
EtroplussuratensisAGGGGCCTCTGTTGATTTAACCATCTTTTCCCTACATCTAGCAGGTGTTTCATCTATTCTTGGGGC
AATC

E3
EtroplussuratensisAGGGGCCTCTGTTGATTTAACCATCTTTTCCCTACATCTAGCAGGTGTTTCATCTATTCTTGGGGC
AATC

E4
EtroplussuratensisAGGGGCCTCTGTTGATTTAACCATCTTTTCCCTACATCTAGCAGGTGTTTCATCTATTCTTGGGGC
AATC

430 440 450 460 470 480 490

....|....|....|....|....|....|....|....|....|....|....|....|....|....|

E1
EtroplussuratensisAACTTTATCACCACAATCATTAATATGAAACCTCCCGCTATTTCACAATATCAAACCCCCTTATTTG
TCT

E2
EtroplussuratensisAACTTTATCACCACAATCATTAATATGAAACCTCCCGCTATTTCACAATATCAAACCCCCTTATTTG
TCT

E3
EtroplussuratensisAACTTTATCACCACAATCATTAATATGAAACCTCCCGCTATTTCACAATATCAAACCCCCTTATTTG
TCT

E4
EtroplussuratensisAACTTTATCACCACAATCATTAATATGAAACCTCCCGCTATTTCACAATATCAAACCCCCTTATTTG
TCT

500 510 520 530 540 550 560

....|....|....|....|....|....|....|....|....|....|....|....|....|....|

E1
EtroplussuratensisGAGCTGTTCTTATTACGGCCGTCCTTCTTCTTCTCTCTCTCCCCGTACTAGCAGCCGGCATCACAA
TGCT

E2
EtroplussuratensisGAGCTGTTCTTATTACGGCCGTCCTCCTTCTTCTCTCTCTCCCCGTACTAGCAGCCGGCATCACAA
TGCT

E3
EtroplussuratensisGAGCTGTTCTTATTACGGCCGTCCTTCTTCTTCTCTCTCTCCCCGTACTAGCAGCCGGCATCACAA
TGCT

E4
EtroplussuratensisGAGCTGTTCTTATTACGGCCGTCCTTCTTCTTCTCTCTCTCCCCGTACTAGCAGCCGGCATCACAA
TGCT

570 580 590 600 610 620 630

....|....|....|....|....|....|....|....|....|....|....|....|....|....|

E1
EtroplussuratensisTCTAACAGATCGAAATTTAAATACGACCTTTTTCGATCCCGCAGGGGGAGGAGACCCCATCTTAT
ACCAG

E2
EtroplussuratensisTCTAACAGATCGAAATTTAAATACGACCTTTTTCGATCCCGCAGGGGGAGGAGACCCCATCTTAT
ACCAG

E3
EtroplussuratensisTCTAACAGATCGAAATTTAAATACGACCTTTTTCGATCCCGCAGGGGGAGGAGACCCCATCTTAT
ACCAG

E4
EtroplussuratensisTCTAACAGATCGAAATTTAAATACGACCTTTTTCGATCCCGCAGGGGGAGGAGACCCCATCTTAT
GCCAG

640 650 660

....|....|....|....|....|....|....|..

E1 EtroplussuratensisCACCTCTTCTGATTCTTTGGCCACACAGAAAGTCTAA

E2 EtroplussuratensisCACCTCTTCTGATTCTTTGGCCACACAGAAAGTCTAA

E3 EtroplussuratensisCACCTCTTCTGATTCTTTGGCCACACAGAAAGTCTAA

E4 EtroplussuratensisCACCTCTTCTGATTCTTTGGCCACACAGAAAGTCTAA

Figure 4. Pairwise nucleotide alignment of *Etroplus suratensis* (ES1) ; *Etroplus suratensis* (ES2) ; *Etroplus suratensis* (ES3) & *Etroplus suratensis* (ES4)

DISCUSSION

Morphometrics and meristics are widely used to taxonomical identification, sexual dimorphism evaluations and to distinguish stocks or populations (Jayasankar *et al*., 2004). The morphometric investigation of *Etroplus suratensis* species of three geographical locations agree with those in taxonomic compilations (Jayaram, 1999) and evaluations of morphological variations revealed that the samples from Visakhapatnam and Mangalore were found to be similar to that of the Kochi variety. Of the fifteen morphological measures studied, most of them were in the similar range despite differences in the body length, body depth, snout length, head depth and dorsal fin base length along with distinct colour variations in the body. *E. suratensis* of Kochi origin exhibited a greenish body colouration while that of the Visakhapatnam and Mangalore specimens exhibited a blackish or smoky ash colouration with spots. The results of the morphological evaluation corroborates with the observations of Suneetha (2007) and Alex *et al*. (2013) in the same species. Both the study registered morphological heterogeneity between different ecosystems. Environmental factors induce morphological variability among spatially separated fish populations (Carvalho, 1993), and phenotypic plasticity in fish morphology has been documented for various species, including cichlids (Wimberger 1991; Wimberger, 1992). As the morphometric measurements could lead to misidentification of the species, molecular taxonomy was chosen as the best tool for the species identification.

Molecular markers including nuclear and mitochondrial DNA markers are recently been widely used to study the taxonomy and phylogeny of various species. DNA barcoding employs standardized genomic fragments to facilitate species identification and discovery (Hebert, *et al*., 2003; Savolainen *et al*., 2005). Studies on various groups of animals have shown that a 650-bp fragment of the mitochondrial gene, cytochrome *c* oxidase I (COI, *cox1*) is generally effective as a barcode sequence, delivering more than 95% species-level resolution (Hebert *et al*., 2004b; Meyer and Paulay, 2005; Hajibabaei *et al*., 2006; Smith, *et al*., 2006). COI possess greater phylogenetic signals than any other genes. In this study, mitochondrial DNA molecular marker COI was used to resolve the taxonomic ambiguity of *E. suratensis* collected from Visakhapatnam, Mangalore and Kochi sample. *E. suratensis* collected from Visakhapatnam and Mangalore had colour variation compared to the native sample of Kochi. The present study evidenced that freshwater fish species can be effectively identified through the use of DNA barcoding, especially the complex and

small-sized species and that the present COI sequences can be used for subsequent applications in ecology and systematics.

Mitochondrial DNA is known to evolve much faster than the nuclear genome. Consequently, most of the mitochondrial protein-coding genes have been used to examine the phylogenetic relationships in relatively lower categorized levels such as families, genera or species. Due to the high rate of substitution occurring in the third codon positions of protein-coding genes, the DNA sequences of protein-coding genes have frequently been used for species identification. It has been proposed that the mitochondrial gene sequence of the cytochrome c oxidase subunit 1 (COI) gene could serve as the basis for a global identification system for animals (Sparks, 2008). The suggestion was that each species would be delineated by a particular sequence or a tight cluster of very similar sequences. The total length of cytochrome oxidase I in vertebrates is about 1545 base pairs (bp), and a region of about 650 bp long commencing near the start of the COI reading frame was nominated as the 'barcode' region. The precise length of the barcode region will vary slightly depending on the primer sequences used for amplification. The primer pairs for amplifying a partial sequence of COI used in the present study was developed by (Ward et al. 2005) and this could successfully amplify an appropriately 650 base pair segments of mitochondrial COI gene in *E. suratensis* of all three locations.

The low divergence value among *Etroplus* collected from three locations gave hint that the variation can only be intraspecific. The number of haplotypes reported was also less. This may be due to the high proportion of the identical haplotype in the samples or the limited number of individuals (5 each) collected for the study. The observed genetic divergence values (0.0 - 0.002) and transition *vs*. transversions ratios (2.16) are comparable to the mean 2.3% in sharks (Martin, 1995) and in many perciformes (Garcia *et al*., 2000). Transitions outnumbered transversions in the present study which came following the previous studies of mitochondrial DNA in fish, which is typically observed (Page and Holmes, 1998).

Phylogenesis from molecular data is often computed by pair-wise genetic distance-based (numerical) methods like Neighbour Joining (NJ) tree. NJ tree making method is a widely used distance-clustering algorithm that allows unequal rates of divergence among lineages. The phylogenetic tree based on the pairwise genetic distance indicated that *Etroplus* reported from Visakhapatnam, Mangalore and Cochin are genetically similar. Though *E. canarensis* shares similar genera with *E. suratensis,* the genetic distance is found to be considerably high (≈19%). In future, sequences developed through this study may be utilised for studying the taxonomy and phylogeny of *E. suratensis* species. The present study has constructed a DNA barcode of native local and unknown samples of *E. suratensis* using the COI genes and compared these mitochondrial COI gene sequences to examine the taxonomic status and their phylogenetic relationship with local *E. suratensis* samples.

CONCLUSION

The fish identified from Visakhapatnam, Mangalore and Kochi were morphometrically similar with minor variations and by DNA barcoding method using COI gene sequence were one and same species of *E. suratensis,* though they are colour variants, were genetically similar. The present study evidenced that freshwater/brackish water fish species can be effectively identified through the use of DNA barcoding, especially the complex and small-sized species and that the present COI sequences can be used for subsequent applications in ecology and systematics.

ACKNOWLEDGEMENT

This work was technically supported by Peninsular and Marine Fish Genetic Resources (PMFGR) Unit, Central Marine Fisheries Research Institute (CMFRI-ICAR), Kochi. The first author thanks Dr. Divya P.R., Senior Scientist, Peninsular and Marine Fish Genetic Resources (PMFGR) Unit, CMFRI, Kochi for all the expertise rendered.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: No animal testing was performed during this study.

Data availability: The manuscript has no associated data.

Authors' contributions: NR wrote the manuscript with input from SKS and KMA. NR and SKS conducted the field study. NR and SKS contributed to the taxonomic identification. KMA analysed the data statistically and edited/finalized the document.

REFERENCES

Abraham, K.M. and Jayaprakas, V., 2011. Evaluation of nucleic acid content as growth index in the culture of Etroplus suratensis (Bloch) fed Nutripro-Aqua. Journal of Aquaculture in the Tropics, 26(3-4): 131-141.

Alex, D.M., Remya M. and Biju Kumar A., 2013. Morphometric and genetic variations of Etroplus suratensis (Bloch)(Actinopterygii: Perciformes: Cichlidae) from two tropical Lacustrine Ecosystems, Kerala, India. Journal of Aquatic Biology and Fisheries, 1(1&2): 140-150.

Ali, B.A., Huang, T.H., Qin Da, N. and Wang, X.M., 2004. A review of random amplified polymorphic DNA (RAPD) markers in fish research. Review of Fish Biology and Fisheries, 14: 443-453.

Askari, G. and Shabani A., 2013. Genetic diversity evaluation of Paraschistura bampurensis (Nikolskii, 1900) in Shapour and Berim rivers (Iran) using microsatellite markers. Journal of Cell Biology and Genetics, 3(2): 29–34.

Avise J.C., 1994. Molecular Markers, Natural History and Evolution. Chapman and Hall, New York, London, pp 511.

Bardakei, F. and Skibinski, D.O.F., 1994. Application of the RAPD technique in Tilapia fish species and subspecies identification. Journal of Hereditary, 73:117-123.

Carvalho, G.R., 1993. Evolutionary aspects of fish distribution: genetic variability and adaptation. Journal of Fish Biology, 43(sA): 53–73.

Cavalcanti, M.J., Monteiro L.R. and Lopes, P.R., 1999. Landmark-based morphometric analysis in selected species of serranid fishes (Perciformes: Teleostei). Zoological Studies, 38(3): 287–294.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3(5): 294–299.

García, G., Wlasiuk, G. and Lessa, E.P., 2000. High levels of mitochondrial cytochrome b divergence in annual killifishes of the genus Cynolebias (Cyprinodontiformes, Rivulidae). Zoological Journal of the Linnean Society, 129(1): 93–110.

Gunawickrama K.B.S. 2007. Morphological heterogeneity and population differentiation in the green chromid Etroplus suratensis (Pisces: Cichlidae) in Sri Lanka. Ruhuna Journal of Science, 2(1): 70-81.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. and Hebert, P.D., 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America, 103(4): 968–971.

Hall, T.A., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Nucleic acids symposium series (Vol. 41, No. 41, pp. 95-98). [London]: Information Retrieval Ltd. c1979–c2000.

Hebert, P.D., Cywinska A. and Ball S.L., 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences, 270 (1512): 313–321.

Hebert, P.D., Penton, E.H., Burns, J.M., Janzen, D.H. and Hallwachs, W., 2004a. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings of the National Academy of Sciences of the United States of America, 101(41): 14812–14817.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S. and Francis, C.M., 2004b. Identification of birds through DNA barcodes. PLOS Biology, 2(10): e312.

Jayaram, K.C, 1999. The Freshwater Fishes of the Indian Region. Narendra Publishing Company, Delhi.

Jayaram, K.C., 1981. Freshwater Fishes of India, Pakistan, Bangladesh, Burma and Sri Lanka. Arbindo Press, Calcutta. pp: 356.

Jayasankar, P., Thomas, P.C., Paulton, M.P. and Mathew, J., 2004. Morphometric and genetic analysis of Indian Mackerel (Rastrelliger kanaguta) from Peninsular India. Asian Fisheries Society, 17: 201-215.

Joseph M.M., 1980. Brackish water finfish and shell fish resources of Mangalore India. In Proceedings seminar on some aspects of Inland aquaculture in Karnataka. pp. 17–26.

Martin, A.P., 1995. Mitochondrial DNA sequence evolution in sharks: rates, patterns, and phylogenetic inferences. Molecular Biology and Evolution, 12(6): 1114–1123.

Meyer, C.P. and Paulay, G., 2005. DNA barcoding: error rates based on comprehensive sampling. PLOS Biology, 3(12): e422.

Mohanta, S.K., S.K. Swain, S.P. Das, A. Bit, G. Das, S. Pradhan, J.K. Sundaray, P. Jayasankar and P. Das, 2016. Complete mitochondrial genome sequence of Etroplus suratensis revealed by next generation sequencing. Mitochondrial DNA Part BRes., 1(1): 746-747.

Page, R.D.M. and Holmes, E.C., 1998. Molecular evolution: A phylogenetic approach. Blackwell Science Oxford. UK Google Scholar.QH390. p: 34.

Parakkandi, J., Das B.K, Saha A., Leela, R.V. and Gunasekharan J., 2021. Cage culture of Etroplus suratensis (Bloch, 1790) in a tropical reservoir in India and associated impacts on water and sediment quality, Journal of World Aquacultuer Society, 1-13 (DOI: 10.1111/jwas.12828).

R Core Team, 2021. R: A language and environment for statistical computing. F Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rattan P., 1994. Ecobiology of Pearl Spot, Etroplus suratensis (Bloch) in Goa Waters. Ph.D Thesis, National Institute of Oceanography, Goa. 1994, 266.

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. Molecular cloning: A laboratory manual (No. Ed. 2). Cold Spring Harbor Laboratory press.

Savolainen, V., Cowan R.S., Vogler, A.P., Roderick, G.K. and Lane, R., 2005. Towards writing the encyclopedia of life: An introduction to DNA barcoding. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462): 1805–1811.

Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W., Hebert, P.D., 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). Proceedings of the National Academy of Sciences of the United States of America, 103(10): 3657–3662.

Sparks, J.S., 2008. Phylogeny of the Cichlid Subfamily Etroplinae and taxonomic revision of the genus Paretroplus (Teleostei: Cichidae). Bulletin of the American Museum of Natural History, 314: 1-151 (DOI: doi.org/10.1206/314.1).

Sreenivasan, N., Mahesh, N. and Raghavan, R., 2021. Freshwater fishes of Cauvery Wildlife Sanctuary, Western Ghats of Karnataka, India. Journal of Threatened Taxa, 13(1): 17470-17476. DOI: https://doi.org/10.11609/jott.6778.13.1.17470-17476.

Suneetha, G.K.B., 2007. Morphological heterogene and population differenctiation in the green chromide Etroplus suratensis (Pisces: Cichlidae) in Sri Lanka. Ruhuna Journal of Science, 2: 70-81.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28(10): 2731–2739.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Hebert, P.D., 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462): 1847-1857.

Wheeler, Q.D., 2004. Taxonomic triage and the poverty of phylogeny. Philosophical Transactions of the Royal Society B: Biological Sciences, 359 (1444): 571–583.

Wimberger, P.H., 1992. Plasticity of fish body shape. The effects of diet, development, family and age in two species of Geophagus (Pisces: Cichlidae). Biological Journal of the Linnean Society, 45(3): 197–218.

Wimberger, P.H.,1991. Plasticity of jaw and skull morphology in the neotropical cichlids Geophagus brasiliensis and G. steindachneri. Evolution, 1545–1563.

Yagi, T., Katoh, T., Chichvarkhin, A., Shinkawa, T. and Omoto, K., 2001. Molecular phylogeny of butterflies Parnassius glacialis and P. stubbendorfii at various localities in East Asia. Genes and Genetic Systems, 76(4): 229–234.

Zhang, D.X. and Hewitt, G.M., 1997. Assessment of the universality and utility of a set of conserved mitochondrial COI primers in insects. Insect Molecular Biology, 6(2): 143–150.

Received: 06th May 2022; Accepted: 19th January 2023; First distribution: 25th April 2023