

1.1 INTRODUCTION

The grey mullet is first mentioned in historical records from the Mediterranean region, where it was recognised as a valuable source of nutrition by the ancient Greeks and Romans (Thompson 1947). In his work "The History of Animals" written in the latter half of the 4th century BC, Aristotle provided a detailed account of the life cycle of various species of grey mullets. For *Mugil cephalus*, he used the term "kefalos," which means "head," "kestrefs," for *Liza Ramada*, "myxinos," for *L. aurata*, and "chelon," emphasising the large lips, for *Chelon labrosus* (Thompson 1947; Koutrakis 1999). Subsequently, Linnaeus documented *M. cephalus* in his publication 'Systema Naturae' in 1758.

The Mugilidae, classified by Jarocki in 1822, is a family of grey mullets that falls under the class Actinopterygii and the order Mugiliformes (Fricke *et al.* 2024). The initial endeavour to establish the systematics of the Mugilidae family was undertaken by Cuvier & Valenciennes (1836). Since then, there has been ongoing discussion on the number of species and genera within the family, as well as their evolutionary relationships. Berg (1940) classified the Atherinidae, Mugilidae, and Sphyraenidae families into the Mugiliformes order, specifically at the Sub Perciformes level. Later, Greenwood *et al.* (1966) and Nelson (1984) examined the sub-order classification of these three families and categorised them under the Perciformes order. Nelson (1994) finally classified mullets in the distinct taxonomic order Mugiliformes. Schultz (1946) conducted a thorough review of the genera within the Mugilidae family. His focus was on the taxonomic characteristics of mouth parts, specifically the position (inferior or terminal) of the mouth, the relative thickness of the lips, the extent to which the lips are covered by papillae and crenulations, the structure of the upper attachment of the maxilla, the curvature and degree of exposure of the posterior angle of the maxilla, the shape and distribution of teeth, and the presence or absence of the symphyial knob. By observing the differences in the aforementioned characteristics, he was able to identify a total of 13 genera. Smith (1948) conducted a comprehensive review of the South African mullets, utilising the characteristics established by Schultz. He verified the taxonomic significance of the mouthparts, but noted that Schultz did not analyse samples from around the world. He supplemented Schultz's (1946)

descriptions with five additional taxa. In a subsequent analysis, Schultz (1953) re-evaluated his own research together with Smith's findings. After making necessary revisions and incorporating new information, Schultz confirmed the validity of 14 genera.

Thomson (1954) conducted a revision of the mullets of Australian waters and adjacent seas, based on characters of mouth parts, dentition, digestive system, morphometric and meristic. He listed 38 nominal genera (excluding fossils) world-wide, of which 13 genera were recognized as valid, and described 17 species belonging to nine genera in Australia and the South Pacific region. Thomson (1981) considered 64 species in 14 genera (of 282 nominal species) as valid, and presented a detailed description of distinctive characters and diagnostic features useful for recognition of mullet species. Thomson (1997) undertook a world-wide revision of the family Mugilidae and recognized as valid 14 of 40 described genera, and 62 of 280 nominal species. He observed that the nostrils may be variously placed in different species of mullets. In some species, the nostrils are nearer to each other than the posterior is to the eye or the anterior to the lip; in other species their position may be different. Crosetti & Blaber (2016) proposed that the head is an informative organ from the taxonomic point of view, normally employed in any identification key of mullets. Fricke *et al.* (2020) performed the revision of the systematics of Mugilidae and recognized 20 genera and 79 species. Whitfield & Durand (2023) reviewed the grey mullet global occurrence and recognized 77 valid species belonging to 26 genera under Mugilidae.

Marine species exhibit universal variability in DNA sequence, morphology, coloration, and biochemistry. A polymorphism refers to the presence of multiple distinct forms or variations that can be identified. Alternatively, in certain instances, variation can just be quantified as continuous variation, shown by disparities in bodily dimensions or the relative size of a fin. Since Oren's last thorough work on Mugilidae in 1981, there have been notable alterations in the taxonomy and systematics of this family. The area is currently undergoing a significant transformation in terms of the study of the evolutionary relationships and classification of mullets. Recent advancements in methodologies, including molecular taxonomy using mitochondrial and/or nuclear gene sequencing, as well

as the use of geometric morphometrics (a discipline that assesses shape differences in organisms based on anatomical landmarks), have improved the precise differentiation of taxa.

Phylogenetic classification offers the advantage of revealing the fundamental biological mechanisms that contribute to the variety of organisms. Scientists utilised phylogenetics to track the genetic lineage of several species, providing evidence for the process of speciation (Wiens *et al.* 2003). Previously, the classification of organisms was determined by identifying their physical characteristics. However, with the progress of technology, this approach has been replaced. Now a days, Research organisations are classifying fish using the DNA barcode method, which represents a paradigm shift in categorization. Furthermore, the process of classifying organisms based on their physical characteristics can be difficult and requires skilled taxonomists.

The morphology-based procedures mentioned in the study by Cywinska *et al.* (2007), Rindi *et al.* (2008), and Packer (2009) are typically time-consuming and may not always accurately identify species. Additionally, the considerable phenotypic plasticity of fishes can contribute to misidentification, as highlighted by Crosetti & Blaber (2016). Various molecular studies have revealed the presence of cryptic fish species that have developed significant genetic differences without corresponding differences in physical characteristics. As a result, these species cannot be detected using the traditional approach of classifying species based on their physical features. The identification of morphologically cryptic species presents a substantial obstacle for contemporary taxonomy (Heinrichs *et al.* 2011).

DNA barcoding, a nucleotide-based taxonomic classification system, was created by scientists as a result of aforementioned problems (Hebert *et al.* 2003, 2004). This method is considerably more effective than the usual morphological approach since it depends on changes in the nucleotide sequence that occur as a result of environmental adaptation. DNA barcoding is a comprehensive method that uses multiple dimensions to accurately identify fish species. Accurate identification of fish species is crucial in various applications that include the economic and social significance of the country. Under these circumstances,

immediate identification is imperative. DNA barcoding has been successfully used in various studies to rapidly assess biodiversity, biomonitoring, track the spread of pathogens and their vectors, aid in forensic investigations, combat illegal trade of endangered species, for feeding ecological studies, and to support conservation initiatives. DNA barcoding addresses numerous challenges associated with morphological taxonomy. Modern taxonomic analysis now relies heavily on molecular methods due to the decline in taxonomists and rise in identified species. Species determination only requires a minimal amount of tissue, even as little as a single cell. This allows for analysis of the material without any prior knowledge and can be applied at any stage of development (Hebert *et al.* 2003).

In 2003, researchers suggested using a specific 658 bp fragment of the mitochondrial cytochrome C oxidase subunit I gene (*cox1* or COI) as a universal marker for identifying species. This fragment, referred to as a "DNA barcode," might be used to tag any taxonomic group within the animal kingdom (Hebert *et al.* 2003). The quantity of partial COI gene sequences that are accessible in public data sources has peaked in accordance with this theory. Till 2016, approximately 4.7 million COI barcodes have been sequenced and stored in the Barcode of Life Data Systems database (BOLD, <http://www.boldsystems.org/>). Additionally, over 3000 papers have been published on the use of COI barcodes for identifying and discovering animal species (Pentinsaari *et al.* 2014).

Owing to advances in sequencing, COI is currently the most widely used method for building the phylogeny of the animal kingdom. It is worth mentioning that the majority of DNA barcoding research conducted so far consider this specific gene area as a simple tool for identification, aligning with the idea of a simply readable "barcode." Hence, the 5' region of the mitochondrial cytochrome c oxidase subunit I (COI) gene is suggested as the universal and standard marker for barcoding most animal species (Hebert *et al.* 2004; Ward *et al.* 2005; Hajibabaei *et al.* 2007; Ratnasingham & Hebert 2007; Hebert *et al.* 2016).

The use of these methods has provided recent information on the Mugilidae family, revealing the existence of cryptic species and resulting in disputes between genera and even subfamilies. Although there have been significant breakthroughs, there is still a considerable amount of work that needs to be done in order to define

and consolidate the taxonomy and systematics of Mugilidae. The purpose of this chapter is to examine the variety of mullets in the Gujarat coast by using morphological and molecular approaches, as well as studying their distribution.

1.2 METHODOLOGY

1.2.1 Study Area

Gujarat is located on the westernmost portion of India and possesses the longest coastline in the country, extending around 1600 km, which accounts for 24% of India's total coastline. The Gujarat coastline is characterised by some sixty rivers that flow annually or seasonally, as well as two significant gulfs - the Gulf of Cambay and the Gulf of Kachchh. This combination of rivers and gulfs creates a distinctive landscape along the coastline of Gujarat. A total of 04 major rivers, 25 minor rivers, and 5 desert rivers flow into coastal waters. The estuarine ecosystem, formed by the mouth of a river, is one of the most diverse habitats on Earth.

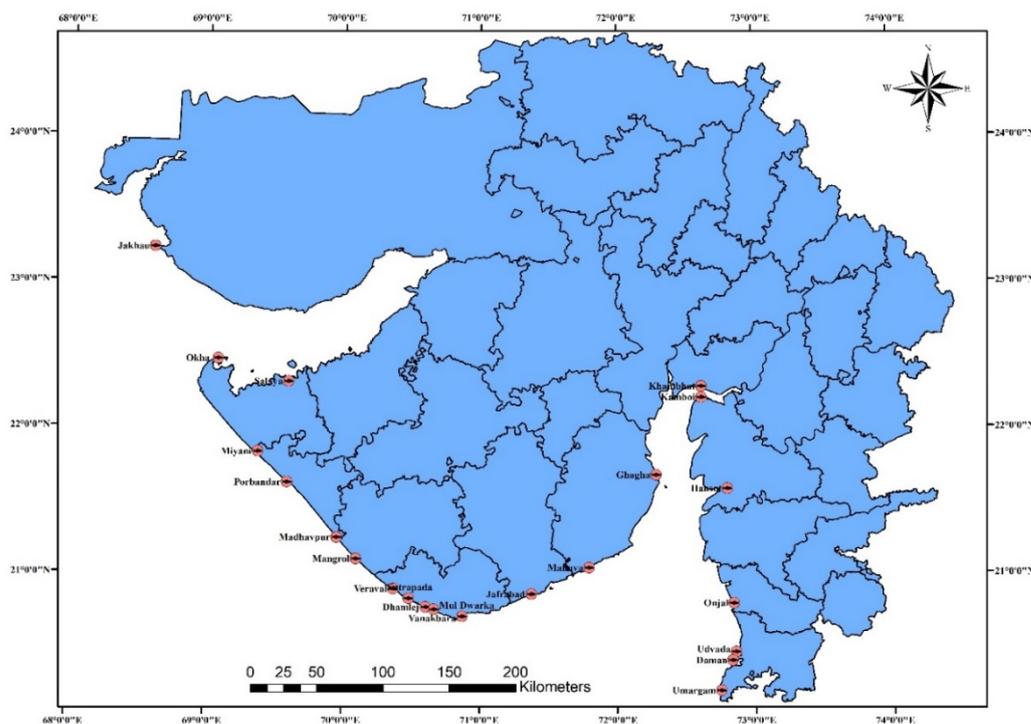


Figure 1.1: Map of collection sites of mullets from the major fish landing centres of Gujarat Coast, India

To study the species diversity of family Mugilidae Jarocki, 1822, samples were collected after the cooperation with nearby fishermen from major landing centres of the Gujarat coast, India (Fig. 1.1). On field, photographs of site and fish samples were clicked as well as their morphological characters have been noted down. Further, specimens were kept in Ice-box and transported to the Laboratory

at the Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara.

1.2.2 Morphological Identification

Morphological identification was carried out to identify the distinguishing features of each individual fish species, as each species possesses unique traits that distinguish it from others. The morphological identification involved measuring the number of dorsal fin spines and rays, caudal fin rays, anal fin rays, pelvic fin spines and rays, pectoral fin spine and rays, lateral line scale count, transverse lateral line scale count, and branchiostegal rays (Fig.1.2). The fish formula was created using these data. The confirmation of the notable fish examples was completed using standard available keys such as "Fishes of India - Francis Day"; FAO factsheets, www.fishbase.org, a verified and approved website of the Food and Agricultural Organisation; Day 1888; Thomson 1997.

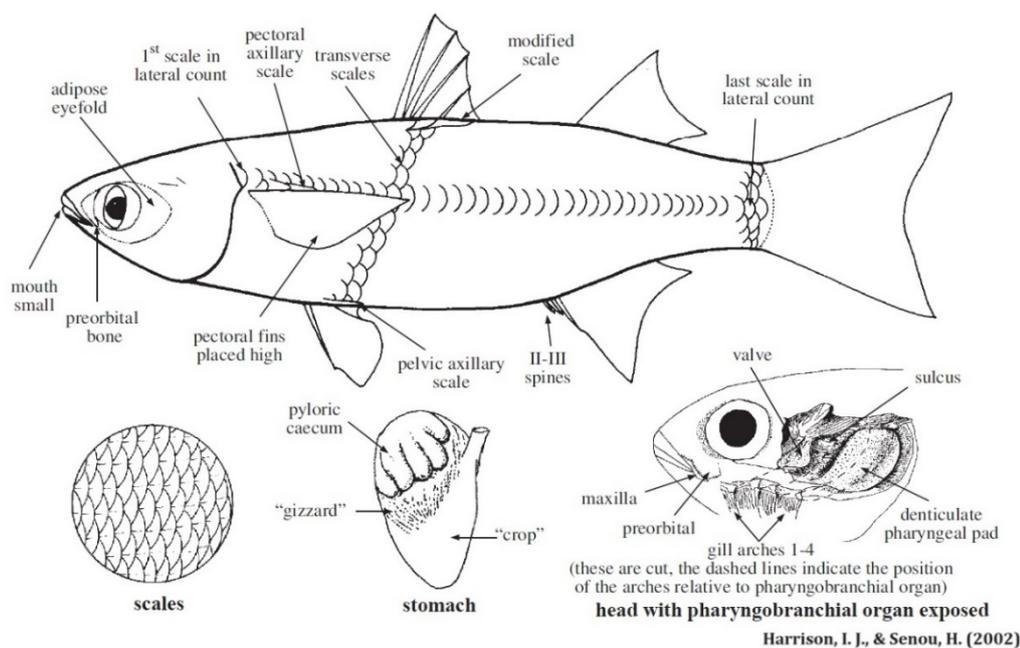


Figure 1.2: Representation of typical mullet's morphological features used for identification

1.2.2 Molecular Identification

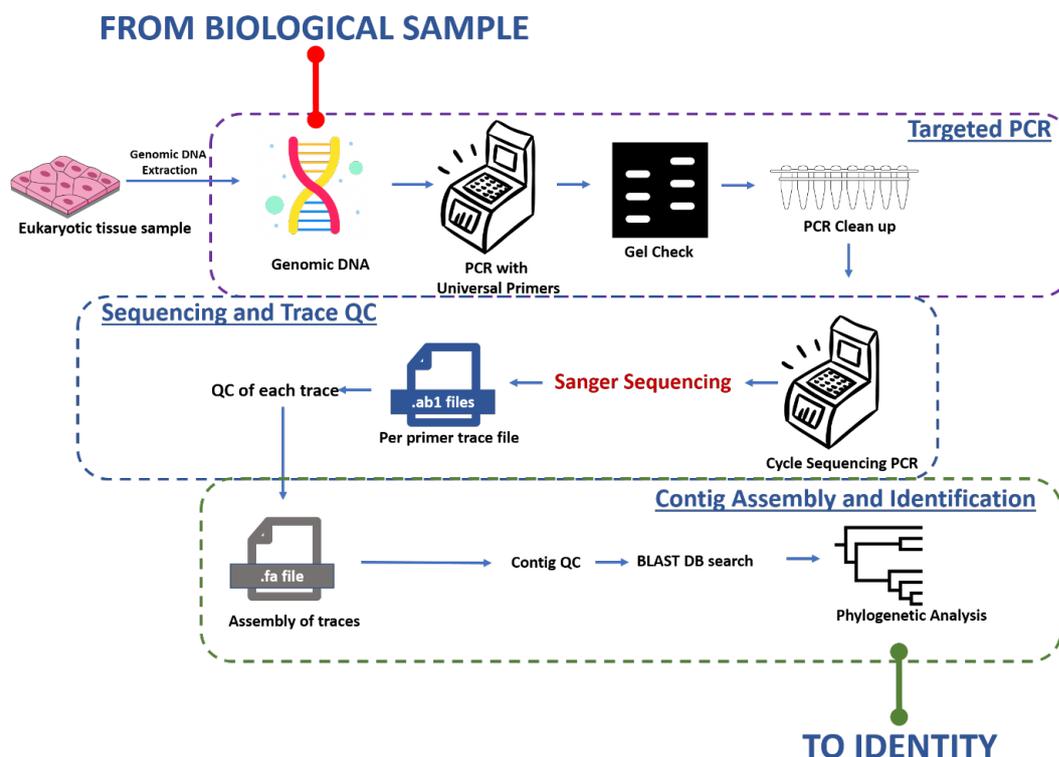


Figure 1.3: Illustration of molecular steps involved in identification of the mullets collected from Gujarat coast

1.2.2.1 Reagents

- Lysis Buffer: 8ml 10% SDS solution + 2ml 10X PBS,
- Proteinase K (10mg/ml)
- RNase (10mg/ml)
- Isopropyl alcohol
- 1.2 M NaCl
- Chilled Ethanol
- Nuclease free water (Milli Q)

1.2.2.2 Protocol

- The tissue from the frozen fishes was collected and stored at -20° C till further use.
- Tissues were chopped using sterile blade and taken into 2ml micro-centrifuge tubes.
- 600µl of Lysis buffer (10% SDS {8ml} + 10X PBS {2ml}) was added and kept in water bath at 60°C for 20 minutes.

- Add 400ul of chloroform
- Centrifuged at 10000 rpm for 15 minutes.
- Supernatant was taken and equal volume of Iso-propenol was added.
- Then tubes were kept in -20°C for 1 hour.
- Centrifuged at 10000 rpm for 5 minutes.
- Remove the supernatant
- Approx. 60-100 µl of 1.2 M NaCl was added to dissolve the pellet.
- 200µl of absolute Ethanol was added to it.
- Again, centrifuged at 10000 rpm for 7-10 minutes.
- Pellets were taken and were dissolved in Milli Q water.

1.2.2.3 Quantification of genomic DNA

After extraction of genomic DNA, quantification was carried out using Spectrophotometer. Purity and concentration of DNA were measured by taking ratio at 260/280 nm.

1.2.2.4 Gel Electrophoresis

The extracted DNA samples of 5 µl volume was mixed with 1 µl gel loading dye and were carefully loaded on the 0.8% Agarose gel. Electrophoresis was carried out at 150V. The gel images were recorded in JPEG or TIF formats using gel documentation system E-Gel Imager (Thermo Fisher Scientific). The gels were analysed by using the software Gel capture version 2.4.4 (Thermo Fisher Scientific).

1.2.2.5 Polymerase Chain Reaction (PCR)

PCR was carried out using 1X final concentration of ReadyMix™ Taq PCR Reaction Mix (Dream Taq) and, template DNA (50 ng/µl). The reaction was carried out in Thermal cycler (Applied Biosystems Veriti®). Detail of Final concentration of PCR reagents in reaction mixture (20 µl) is given below (Table 1.1).

Table 1.1: Final concentration of PCR reagents in reaction mixture (20 µl)

Requirements	Amount (µl)
PCR Ready Mix Buffer	10µl
Reverse Primer	1µl
Forward Primer	1µl
Template DNA	2µl
Milli Q Water	6µl

1.2.2.6 Cytochrome C Oxidase subunit I gene (COI) amplification using primer set Fish BCL and Fish BCH

COI gene in Arthropods was amplified in a volume of 20 µl containing 10 µl Taq PCR reaction mix, 10pmol forward primer (Fish BCL), 10 pmol reverse primer (Fish BCH) (Baldwin *et al.*, 2009), 50 ng template DNA and sterile ion-free water (to make up the final desired volume). Primer with its sequence detail is given below in Table 1.2. Amplification was carried out in Thermal cycler (Applied Biosystems Veriti®).

Reactions were amplified through 35 cycles with the following conditions.

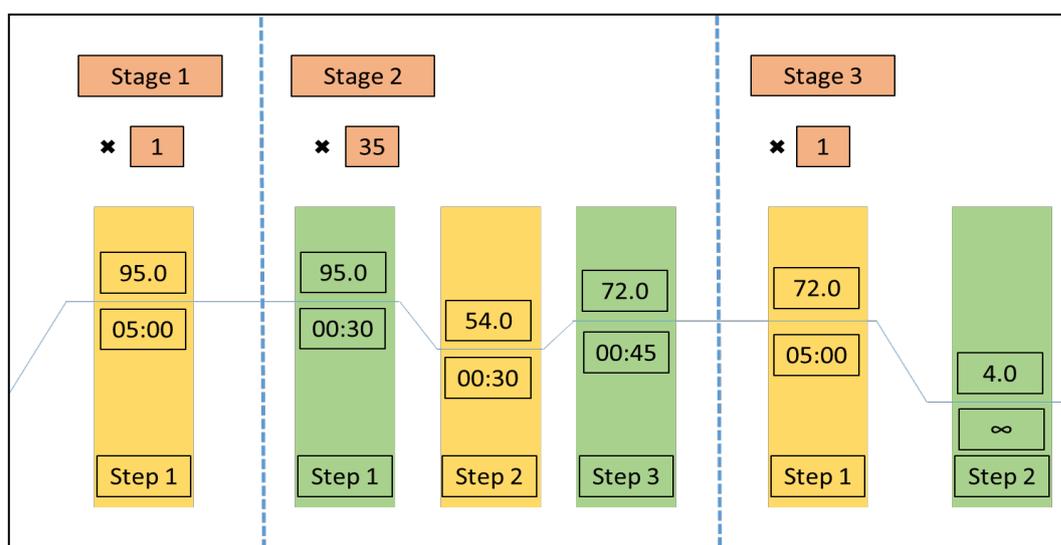


Figure 1.4: PCR conditions for Fish BCL and Fish BCH

Table 1.2: Primer list with sequence

Primer	Sequence	Reference
FISH-BCL F	5'-TCAACYAATCAYAAAGATATYGGCAC -3'	Folmer <i>et al.</i> 1994
FISH-BCH R	5'-ACTTCYGGGTGRCCRAARAATCA -3'	Folmer <i>et al.</i> 1994
LCO-1490 F	5'-GGTCAACAAATCATAAAGATATTGG-3'	Baldwin <i>et al.</i> 2009
HCO-2198 R	5'-TAAACTTCAGGGTGACCAAAAATCA-3'	Baldwin <i>et al.</i> 2009

The amplified DNA samples having approximately 5 µl volume were mixed with 1 µl gel loading dye and were carefully loaded on the 2% Agarose gel and analysed against 100 bp Marker.

1.2.2.7 Purification of amplified PCR products

Purification of COI gene amplified products were done using ExoSAP-IT® of Affymetrix using following reaction mixture given in Table 1.3. Clean up was carried out in Thermal cycler (Applied Biosystems Veriti®) using conditions given below (Fig. 1.5).

Table 1.3: PCR clean-up reaction mixture

Reaction Mixture	Amount
PCR Amplified Product	10µl
ExoSAP-IT®	4µl

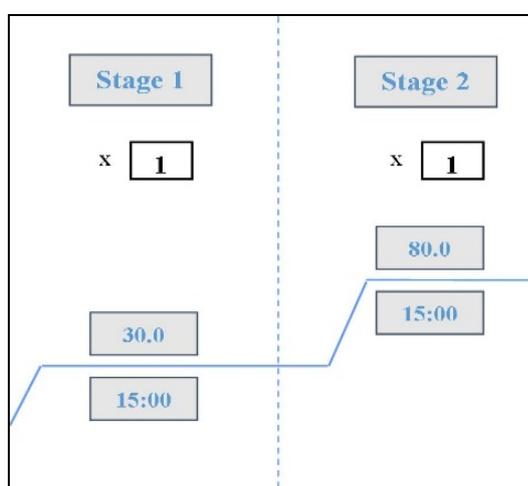


Figure 1.5: Condition for PCR clean-up (ExoSAP-IT®)

1.2.2.8 Cycle Sequencing

Sequencing was carried out using Big Dye® Terminator v 3.1 Cycle sequencing kit. The Big Dye Terminator v3.1 Cycle Sequencing Kit provides the required reagent components for the sequencing reaction in a ready reaction, pre-mixed format.

Cycle sequencing was performed in 10 µl volume. Reaction mixture was prepared as follows. Two reaction tubes were prepared for forward sequencing primers and reverse sequencing primers. In this experiment COI amplification primers (LCO 1490, HCO 2198) served as sequencing primers. Cycle sequencing reagents and concentration for 10µl mixture is given below (Table 1.4).

Table 1.4: Cycle Sequencing reagent and concentration

Reagent	Quantity
Terminator Ready Reaction mix v 3.1	4 µl
Bigdye Sequencing buffer	1 µl
Template	5 µl
Primer	10 pm
Deionized water	To make the volume up to 15 µl

Before going to amplification reaction mixture was mixed well in an individual tube and spin down briefly. Amplification was carried out in Thermal cycler (Applied Biosystems Veriti®). Reactions were amplified through 35 cycles with the following conditions (Ivanova *et al.* 2005) (Fig. 1.6).

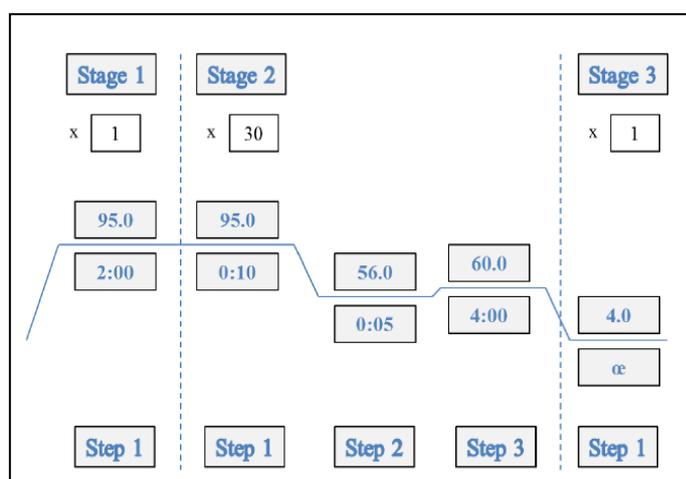


Figure 1.6: Cycle sequencing conditions

1.2.2.9 Purification of Cycle Sequencing Products

In order to achieve optimal results, complete removal of unincorporated dye terminators before performing capillary electrophoresis necessary. Excess dye terminators in sequencing products can obscure data in the early part of the sequence and interfere with base calling. Purification was done using BigDyeX Terminator® Purification Kit. Kit contains SAM™ Solution and Big Dye® X Terminator™ Solution. Following purification protocol was followed:

- Cycle sequencing reaction plate was spun at 100 g for 1 minute.
- Purification reaction premix was prepared by adding 10 µl of BigDyeX Terminator® to 45 µl of SAM™ solution. Total reaction was 55 µl for each well.
- After removing seal of the 96-well plate 55 µL of SAM™ Solution / X Terminator® Solution premix was added to each well.
- Plate was sealed using MicroAmp® Clear Adhesive Films and subjected to vortex for 30 minutes at 2000 rpm on IKA® Vortex 4 digital.
- After vertexing plate was centrifuged at 1000 rpm for 2 minutes.
- Plate kept at room temperature (25°C) before going to capillary electrophoresis.

1.2.2.10 Capillary electrophoresis

- Capillary electrophoresis of cycle sequenced products was performed on **3500** XL platform (Applied biosystems).
- Instrument software 3500 was used to give commands to instrument. Dye Set Z and the Sequencing Install Standard, BigDye® Terminator v3.1 Kit was used to create the BigDye® Direct spectral calibration information to apply to the data.
- BigDye® mobility and calibration files were used for optimal base calling with the BigDye® Cycle Sequencing Kit v 3.1.
- Plate containing cycle sequenced products was loaded on Position A/ Position B.
- Sample information was loaded on plate preparation mode.
- Capillaries were filled with POP-7™ polymer.

Selecting following parameters capillary electrophoresis was performed (Table 1.5).

Table 1.5 Capillary electrophoresis parameters

Polymer	Array	Run Module	Mobility file
POP-7™	50 cm	BDX_StdSeq50_POP7xl	KB_3500_POP7xl_BDTv3direct.mob
Polymer			

Run time for 24 samples was 2 hours and 17 minutes.

1.2.2.11 Sequence analysis and submission of barcodes to Barcode of Life Database Systems (BOLD) and National Centre of Biotechnology Information (NCBI)

- Sequence analysis was done using sequencing analysis version 5.4 (Applied Biosystems) and BioEdit, biological sequence alignment editor (Ibis Biosciences).
- Consensus sequences generated after aligning gene sequences from forward and reverse primers. These sequences were subjected to Sequence match analysis using Basic Local Alignment Search Tool (BLAST) on NCBI.
- Consensus sequences which showed significant match with the earlier identified data on NCBI were submitted to BOLDSYSTEMS according to the guidelines provided onto BOLD website (<http://www.boldsystems.org/>) and NCBI also.
- Phylogenetic tree was constructed using MEGA11 software (Hall 2013; Kumar *et al.* 2016).

1.3 RESULTS

In present study, A total of seven species has been recorded from the various fish landing centres of Gujarat coast. All the species [*Crenimugil seheli* (Forsskål, 1775), *Mugil cephalus* Linnaeus, 1758, *Planiliza parsia* (Hamilton, 1822), *Planiliza planiceps* (Valenciennes, 1836), *Planiliza tade* (Forsskål, 1775) and *Planiliza subviridis* (Valenciennes, 1836), *Rhinomugil corsula* (Hamilton, 1822)] were distributed throughout the coast of Gujarat.

1.3.1 Morphological description

Classification of mullets according to Froese, R. and D. Pauly, 2024 and Fricke, R., Eschmeyer, W. N. & Van der Laan, R., (eds), 2024.

Gigaclass: Actinopterygii

Superclass: Actinopteri

Class: Teleostei

Order: Mugiliformes

Family: Mugilidae Jarocki, 1822

Crenimugil Schultz, 1946

***Crenimugil seheli* (Fabricius 1775) (Fig. 1.7)**

Diagnosis: BR 6, D₁ iv, D₂ i/8, C 15-16, A iii/9, V i/5, P 13-14, L 38-42, tr 13-14, Ceca pyl 7. Body moderately robust; head bluntly pointed; scale-free to between posterior nostril and anterior rim of eye; Interorbital twice eye diameter, slightly convex; eye diameter longer than snout; adipose tissue rim around eyes; Well-spaced fine ciliate teeth are present in both lips, shorter and sharper in upper; Tongue low-domed, without median ridge; Mouth corner on vertical from posterior nostril tip of upper jaw below the line of gape, reaching vertical between posterior nostril and interior rim of eye; Pad over tendon to mouth corner hidden; Posterior nostrils reaching above level of upper rim of eye; Pectoral fin reaching between anterior eye rim and posterior nostril when laid forward; Anal fin slightly higher than second dorsal fin, both higher than first dorsal fin.

Colouration: The dorsal surface is bluish brown or green, while the abdomen and flanks are silvery. The upper row of scales has dusky dots that result in indistinct longitudinal stripes. The dorsal and upper lobe of the caudal fin have a dark-blue tip, while the anal, pelvic, and pectoral fins are yellow. The pectoral fins also have a dark blue patch on the dorsal side near the origin point (Harrison & Senou 2002).

IUCN category: Least Concern (LC) (Palmer-Newton 2020)

Common name: Blue spot mullet;

Vernacular name: Boi (Gujarati), Velisa, Sarya, Madavai (Tamil)

Habitat: Resides in shallow waters along the coast. Juvenile individuals go to estuaries and lagoons. Forms schools around coral reefs. they feed on algae, diatoms, detritus, and crustaceans (Crosetti & Blaber 2016).

Distribution: Extensively distributed in the Indo-Pacific region, spanning from the Red Sea and East Africa to the Marquesas Islands, north to Hawaii and Japan, and south to southern Queensland and New Caledonia (Crosetti & Blaber, 2016).



Figure 1.7: Specimen of *Crenimugil seheli* (Fabricius, 1775) collected from Diu

***Mugil* Linnaeus, 1758**

***Mugil cephalus* Linnaeus, 1758 (Fig. 1.8)**

Diagnosis: BR 6, D₁ iv, D₂ i/8, C 15, A iii/9, V i/5, P 16, L 42-44, tr 14. Body slender, cylindrical, compressed at posterior; head with no scales in front of anterior nostrils; scales cycloid type; interorbital region fattened; eye diameter larger than pre-orbital length; adipose eyelid covering entire eye; upper lip comparatively thin; anterior nostril situated below and nearer to the upper lip, posterior nostril

situated above the level of the upper rim of the eye; maxilla straight; pad at mouth corner; posterior tip not curved; inferior mouth; anal fin longer than the second dorsal fin; body depth comparatively less; caudal peduncle height comparatively small.

Colouration: The dorsal side of the fish has a light olive colour, while the ventral and lateral sides have a silvery appearance. The lateral side is marked with stripes. The pelvic fins are a pale yellowish colour, while the other fins are dusky.

IUCN category: Least concern (LC) (Camara *et al.* 2019).

Common name: Flathead grey mullet.

Vernacular name: Boi, Gandhiyo (in Gujarati).

Habitat: Saltwater and Brackish water. Sometimes enters freshwater body.

Distribution: Cosmopolitan in distribution from warm temperate to tropical seas (Crosetti & Blaber 2016).

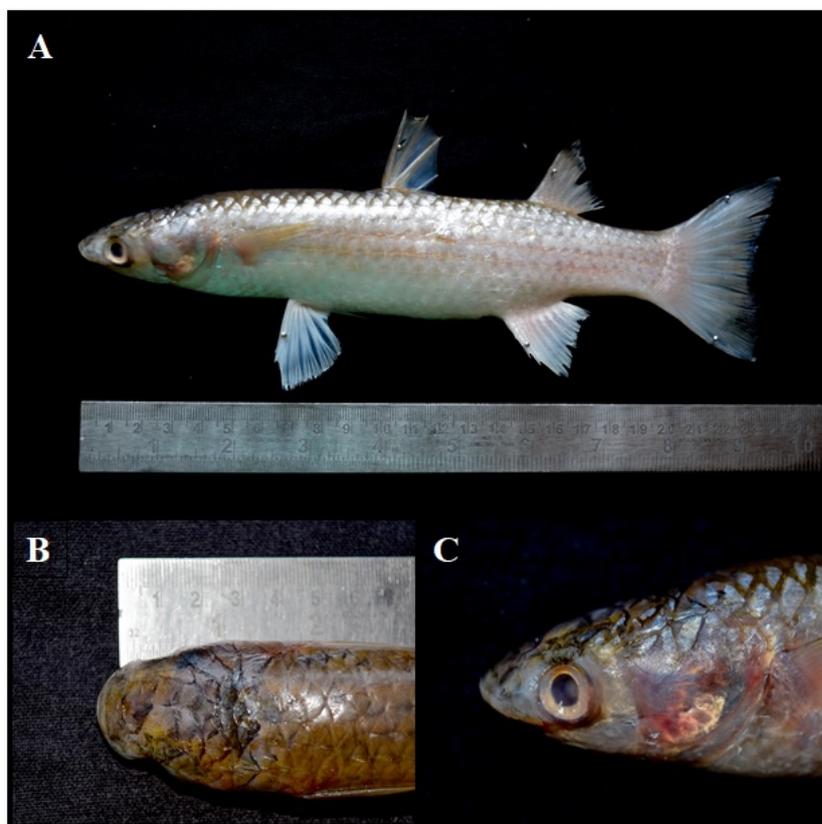


Figure 1.8: *Mugil cephalus* Linnaeus, 1758 collected from Jakhau, Gujarat (a) Specimen examined in the laboratory, (b) Lateral head view, (c) Dorsal head view

***Planiliza* Whitley, 1945**

***Planiliza parsia* (Hamilton 1822) (Fig. 1.9)**

Diagnosis: BR 6, D₁ iv, D₂ i/8, C 14-15, A iii/8-9, V i/5, P 14-15, L 34-35, tr 11-12, Ceca pyl 5. Scales pavement ctenoid, body moderately robust, head bluntly pointed, interorbital slightly less than twice eye diameter, slightly convex; eye diameter slightly greater than or equal to snout length; Adipose tissue covering 2/3 of posterior iris, rather less anteriorly; Mouth corner on vertical from posterior nostril, tip of upper jaw reaching vertical between posterior nostril and anterior rim of eye; Pad over tendon to mouth corner; Pelvic fin origin nearer vertical from pectoral fin origin than that from origin of first dorsal fin; First dorsal fin origin slightly nearer snout tip than caudal base; Caudal fin deeply forked.

Colouration: The coloration of the fish is greenish brown on the upper side and white to silvery on the underside. There is a golden mark on the upper operculum, and the base of the second dorsal, anal, and caudal fins is yellowish.

IUCN category: Data deficient (Not available)

Common name: Gold-spot mullet

Vernacular name: Boi (Marathi, Gujarati), Kanbo (Malayalam), Shirto (Marathi), Madavai (Tamil), Bonthalu (Telugu)

Habitat: Found in shallow coastal waters, estuaries, lagoons, and even extending into tidal rivers. They consume diatoms and other forms of organic materials as a source of food (Crosetti & Blaber 2016).

Distribution: Indian Ocean, found along the coasts of Pakistan, India, Sri Lanka and Andaman Islands (Crosetti & Blaber 2016).



Figure 1.9: *Planiliza parsia* (Hamilton, 1822) collected from Diu, Gujarat

***Planiliza planiceps* (Valenciennes, 1836) (Fig. 1.10)**

Diagnosis: BR 6, D1 iv, D2 i/9, C 17, A iii/9, V i/5, P 15, L 36-38, tr 12, Ceca pyl 5. Body subcylindrical, elongated; body depth comparatively much more; pointed head, depressed upper profile; ctenoid scales; interorbital flat and twice to eye diameter; adipose tissue obsolescent; mouth corner vertically parallel to posterior nostril and slightly curved downwards; terminal mouth; tip of the upper jaw reaching vertical between posterior nostril and anterior edge of eye; nostrils equidistance from each other and from eye and lip; dorsal and anal fins almost equal in size; body depth much longer; first dorsal fin origin nearer to snout tip than to caudal fin base.

Colouration: The dorsal side is pale yellow with a dark olive colour on the head. The ventral side is silvery and the fins are dusky. There are also dark lines present.

IUCN category: Least Concern (LC) (Palmer-Newton 2020)

Common name: Tade grey mullet

Vernacular name: Boi (in Gujarati).

Habitat: Saltwater and brackish water.

Distribution: Indian ocean to West Pacific, Red sea to Australia, Philippines, China, the Marianas and Guam (Thomson 1997).

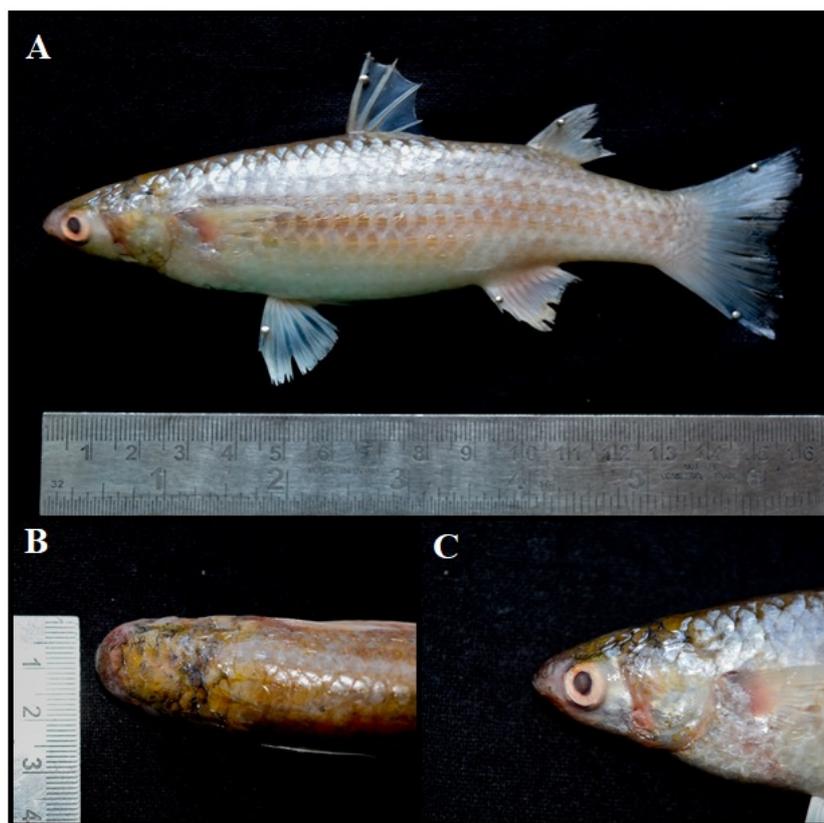


Figure 1.10: *Planiliza planiceps* (Valenciennes, 1836) collected from Khambhat, Gujarat (a) Specimen examined in the laboratory, (b) Lateral head view, (c) Dorsal head view

***Planiliza tade* (Forsskål, 1775) (Fig. 1.11)**

Diagnosis: BR 6, D1 iv, D2 i/8, C 14, A iii/9, V i/5, P 15, L 30-33, tr 10-11, Ceca pyl 5. Body slender, elongate; Head pointed, upper profile depressed, scale free to anterior nostril, more-or-less bulging laterally; scales pavement ctenoid; interorbital twice eye diameter, flat; Adipose tissue covering most of iris, eyes relatively small; Teeth on upper lip, straight, single row sparse Ciliiform teeth in lower lip; tongue highly keeled; Mouth corner at vertical from posterior nostril; Tip of upper jaw reaching vertical between posterior nostril and anterior rim of eye; Posterior end of preorbital curving up sharply, anterior end reaching half up upper lip, above line joining midpoints of posterior and anterior nostrils; Anterior nostrils entirely below vertical span of posterior nostrils, nostrils equidistance from each other and from eye and lip; Pectoral fin not reaching to eye in large specimen; Tips of anterior rays reaching well behind tips of posterior rays; Doral

and anal fin almost equal in height; Second dorsal and anal fins densely scaled; Caudal fin moderately forked.

Colouration: The coloration of the upper side of the body is a mixture of green and brown, while the underside is whitish. The sides of the body have 5 to 7 indistinct lines that run down the rows of scales.

IUCN Category: Data deficient (Hoese 2018)

Common name: Tade mullet

Vernacular name: Tiri (In Gujarati)

Habitat: Typically inhabiting marine environments, these creatures are commonly observed in groups in shallow coastal waters, estuaries, and lagoons. Young individuals can be located in rice fields and mangrove marshes. Spawning takes place in the open ocean, away from the shore. The species consumes floating algae, benthic invertebrates, and organic matter found in sand and mud (Crosetti & Blaber 2016).

Distribution: In Oceania it is reported from Fiji, Samoa, Tonga, New Guinea and Indonesia to Borneo and Guam. Tropical Australia also has an abundance of it. The species has a vast distribution, ranging from the Red Sea and South Africa to the North Indian Ocean, Bay of Bengal, and all Indo-Pacific waters, including the Philippines, Thailand, Myanmar, China, Taiwan, Hong Kong, and Korea (Crosetti & Blaber 2016).



Figure 1.11: *Planiliza tade* (Forsskål, 1775) collected from Sikka, Gujarat

***Planiliza subviridis* (Valenciennes, 1836) (Fig. 1.12)**

Diagnosis: BR 6, D1 iv, D2 i/8, C 14, A iii/(8)9, V i/5, P 15, L 27-33, tr 11-12, Ceca pyl 4-5. Body moderately robust; Head broad, scale free to anterior nostril, dorsally flattened and bluntly pointed, head length equal or slightly bigger than body depth; Snout shorter than eye diameter; Ctenoid scales; Interorbital slightly less than twice eye diameter, gently convex; Eye diameter longer than snout; Adipose tissue covering iris; Small fine teeth along edge of upper lip with several rows behind, ciliiform teeth in lower lip; Mouth corner at vertical just behind anterior nostrils; Tip of upper jaw reaching vertical from anterior rim of eye; Pad over tendon to mouth corner; Nostrils equidistance from lip and eye; Pectoral fin reaching posterior edge of pupil when laid forward; Pelvic fin origin nearer vertical from origin of pectoral fin than that from first dorsal fin origin; First dorsal fin origin nearer snout tip than caudal base; Tips of anterior rays reaching behind tips of posterior rays; Anal and first dorsal fins subequal, higher than second dorsal fin; Second dorsal and anal fins densely scaled; Caudal fin moderately forked.

Colouration: The coloration of this organism is dark greenish on the back, brownish on the top, and white on the underside. It also has 3-6 indistinct dark stripes running along the upper rows of scales.

IUCN Category: Least concern (LC) (Bru 2022)

Common name: Greenback mullet

Vernacular name: Gandhiyo, Boyea (Gujarati), Bata (Bengali)

Habitat: Resides in shallow coastal waters, estuaries, bays, and mangroves. Young individuals can also be found in rice fields and freshwater embayments. Adults consume microalgae, filamentous algae, diatoms, and benthic detrital material that is ingested along with sand and mud (Crosetti & Blaber 2016).

Distribution: This species has a wide distribution range that spans from the Red Sea, Persian Gulf, South Africa, North Indian Ocean, Bay of Bengal, China Sea, and Japan to the western Pacific. Reports indicate that this species can be found in several regions, including Oceania and the Indo-Pacific waters. These regions

include Fiji, Samoa, Tonga, New Caledonia, Indonesia, New Guinea, the Philippines, Thailand, Myanmar, China, Taiwan, Hong Kong, and Korea. The species is found in large numbers in the tropical regions of Australia, specifically in North Queensland, the northern Territory, and North-western Australia. It is also present in New South Wales and Queensland, but there have been no reports of its presence in Tasmania, Victoria, and South Australia (Crosetti & Blaber 2016).



Figure 1.12: Specimen of *Planiliza subviridis* (Valenciennes, 1836) collected from Sikka, Gujarat

***Rhinomugil* Gill, 1863**

***Rhinomugil corsula* (Hamilton 1822) (Fig. 1.13)**

Diagnosis: BR 6, D1 iv, D2 i/8, C 17, A iii/9, V i/5, P 13, L 48-45, tr 16. Body slender, elongated; blunt pointed head; ctenoid scales; V-shaped mouth, upper lip overhang; interorbital slightly longer than eye diameter, eye diameter and snout almost equal; mouth corner extended up to mid-eye; both nostrils below level of lower edge of eye, anterior nostril almost 1/3 distance from eye to snout tip, posterior nostril vertically parallel to anterior edge of eye; posterior nostril comparatively nearer to eye than anterior nostril to lip; prominent raised cutaneous rim around anterior nostril; pectoral fin reaching up to mid eye when extended anterior side; second dorsal arises comparatively far behind the origin of the anal fin, anal and second dorsal fins equal in size.

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Colouration: The anterior dorsal side has a light olive colour, while the abdomen is white and the caudal portion is grey. The pectoral fin has a pale golden tint, whereas the other fins are a dusky shade.

IUCN category: Least concern (LC) (Dahanukar 2010).

Common name: Corsula.

Vernacular name: Boi, Dokari (in Gujarati).

Habitat: Freshwater inhabitant mainly and brackish water.

Distribution: India, Bangladesh, Myanmar, Nepal (Rahman 1989).

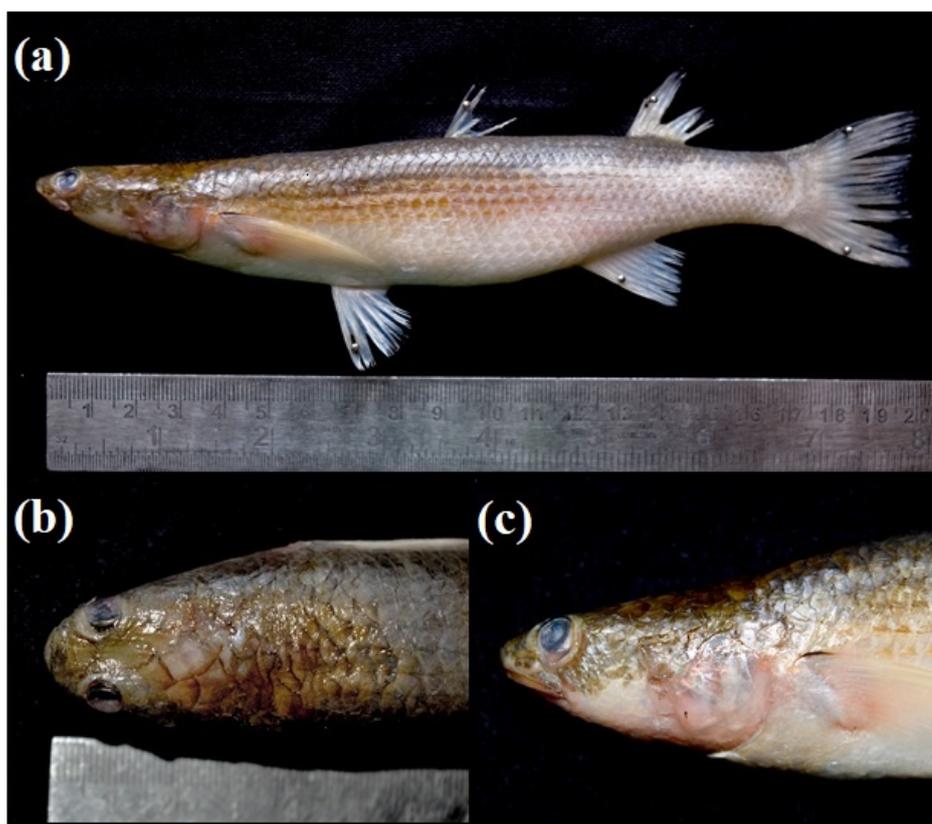


Figure 1.13: *Rhinomugil corsula* (Hamilton, 1822) collected from Narmada, Gujarat (a) Specimen examined in the laboratory, (b) Lateral head view, (c) Dorsal head view

1.3.2 Molecular Identification

A total of three species namely *Mugil cephalus* Linnaeus, 1758, *Planiliza tade* (Forsskål 1775) and *Planiliza subviridis* (Valenciennes 1836) were identified using molecular identification technique (submission IDs: 2818600 for *Planiliza subviridis*, 2818604 for *Planiliza tade*, 2818607 for *Mugil cephalus*). Among these, *P. subviridis* barcoded for the first time from Gujarat coast. The quality of the genomic DNA indicated an appreciable amplification process with an approximate 720 bp and 620 bp size for COI (Fig. 1.14). The sequences' similarity was checked using BLAST, and the identity ranged between 95 and 100%. To check the evolutionary divergence and construct phylogenetic tree, a total of 11 sequence of mullet species recorded from India were extracted from NCBI portal.

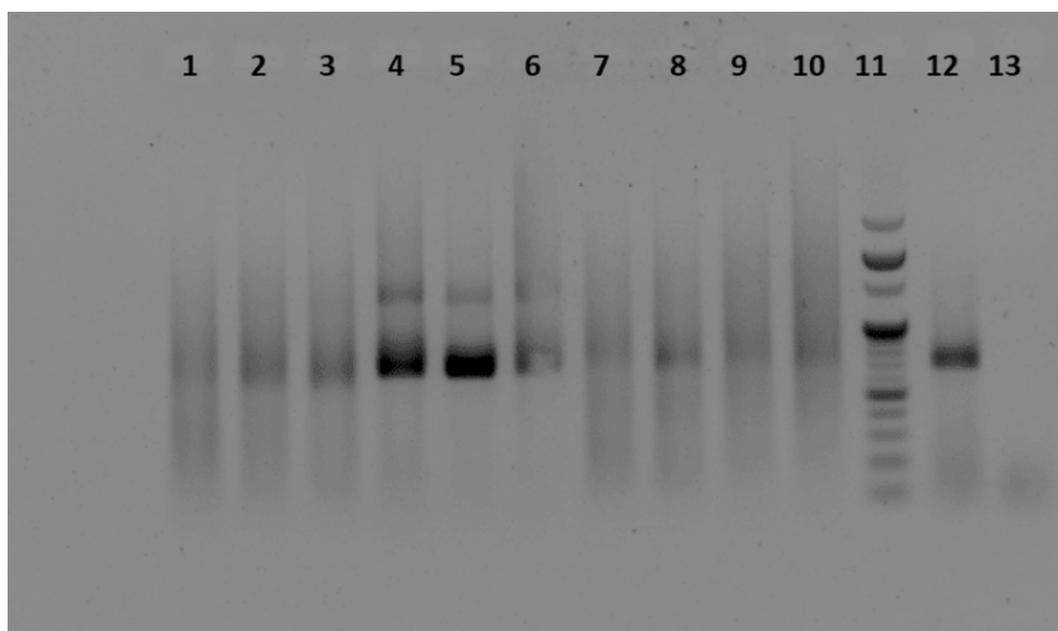


Figure 1.14: Electrophoresis Gel Image of PCR product of COI of Mullet

The nucleotide frequencies are A = 23.79%, T/U = 30.63%, C = 26.82%, and G = 18.77%. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -13089.190. The overall mean pairwise distance for mullets was calculated to be 2.97 ± 0.73 for COI. The number of base substitutions per site from averaging over all sequence pairs are shown. Standard error estimate(s) are shown in the second column and were obtained by a bootstrap procedure (500 replicates). Analyses were conducted using the Maximum Composite Likelihood model (Tamura et al., 2004).

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This analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 737 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.* 2021).

The pairwise distance for *Planiliza subviridis* and *Valamugil buchani* (3.16) was found to be maximum, and *Planiliza tade* and *Liza klunzingeri* (2.15) was found to be least for COI (Table 1.6).

Table 1.6: Pair Wise distance analysis COI sequence of Mullet

	MS	MiC	VB	OP	MP	CC	LK	PM	LP	OL	RC	MC	PT	PS
MS														
MiC	3.36													
VB	3.68	2.41												
OP	3.05	3.02	3.16											
MP	2.47	3.02	2.56	3.36										
CC	3.43	2.62	2.89	3.59	2.87									
LK	2.70	3.06	3.36	2.53	3.18	2.92								
PM	2.25	2.90	2.69	3.25	0.16	2.91	3.16							
LP	2.59	3.01	3.48	2.70	3.28	3.06	0.19	3.24						
OL	2.72	3.31	3.33	1.96	3.48	2.99	3.00	3.38	2.86					
RC	3.39	2.90	3.12	3.22	2.10	2.80	3.18	2.07	3.18	3.65				
MC	2.91	3.05	3.06	3.31	2.98	2.83	2.53	3.04	2.76	3.16	3.16			
PT	2.92	3.40	3.11	3.15	2.97	3.49	2.15	2.89	2.30	3.02	3.09	2.65		
PS	2.86	3.28	3.76	3.25	3.68	3.51	2.98	3.72	2.95	3.21	3.19	3.33	3.50	

[Abbreviations: MS – *Moolgarda seheli*, MiC – *Minimugil cascasia*, VB – *Valamugil buchani*, OP – *Osteomugil cf. perusii*, MP – *Moolgarda perusii*, CC – *Chelon carinatus*, LK – *Liza klunzingeri*, PM – *Planiliza macrolepis*, LP – *Liza parsia*, OL – *Oedalechilus labiosus*, RC – *Rhinomugil corsula*, MC – *Mugil cephalus*, PT – *Planiliza tade*, PS – *Planiliza subviridis*]

1.3.3 Phylogenetic analysis of mullets

The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The optimal tree is shown. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004) and are in the units of the number of base substitutions per site. This analysis involved 14 nucleotide sequences. Codon positions included were

1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 737 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.* 2021). Among the three species analysed, *Planiliza subviridis* found to be monophyletic to *Moolgarda seheli* and polyphyletic to other mullet species. While *Mugil cephalus* displayed paraphyly to *Planiliza tade* and polyphyletic to other mullet species. However, *P. tade* was closest to *L. parsia*. Hence, the phylogeny resolved for the sequence species is (*Oedalechilus labiosus* + *Osteomugil cf. perusii* + (*Planiliza subviridis* + *Moolgarda seheli* + (*Liza parsia* + *Liza klunzingeri* + (*Planiliza tade* + *Mugil cephalus* + (*Planiliza macrolepis* + *Moolgarda perusii* + (*Rhinomugil corsula* + (*Chelon carinatus* + (*Valamugil buchhanani* + *Minimugil cascasia*)))))))) (Fig. 1.15, 1.16).

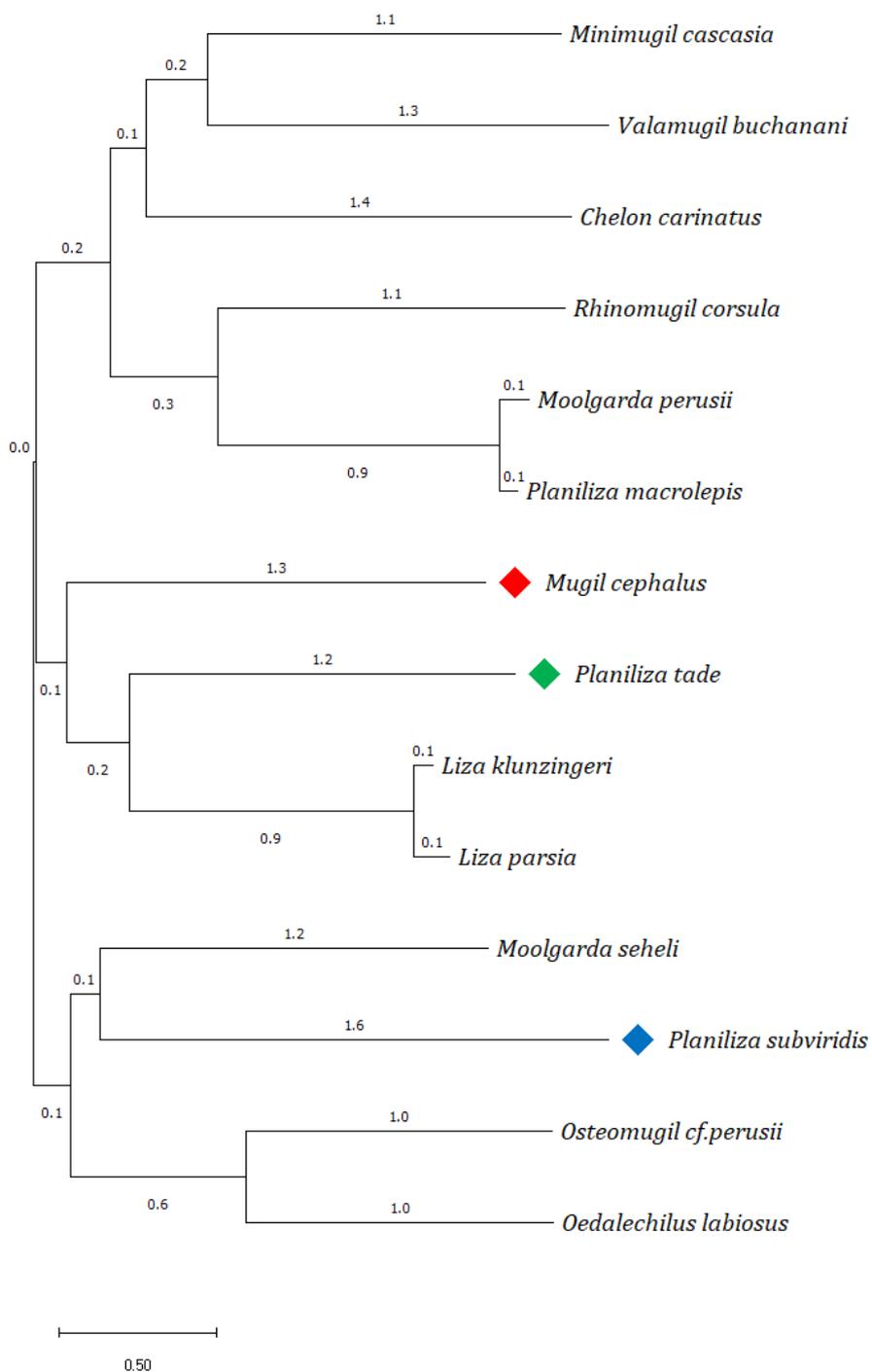


Figure 1.15: Phylogenetic tree of Mullet with its species

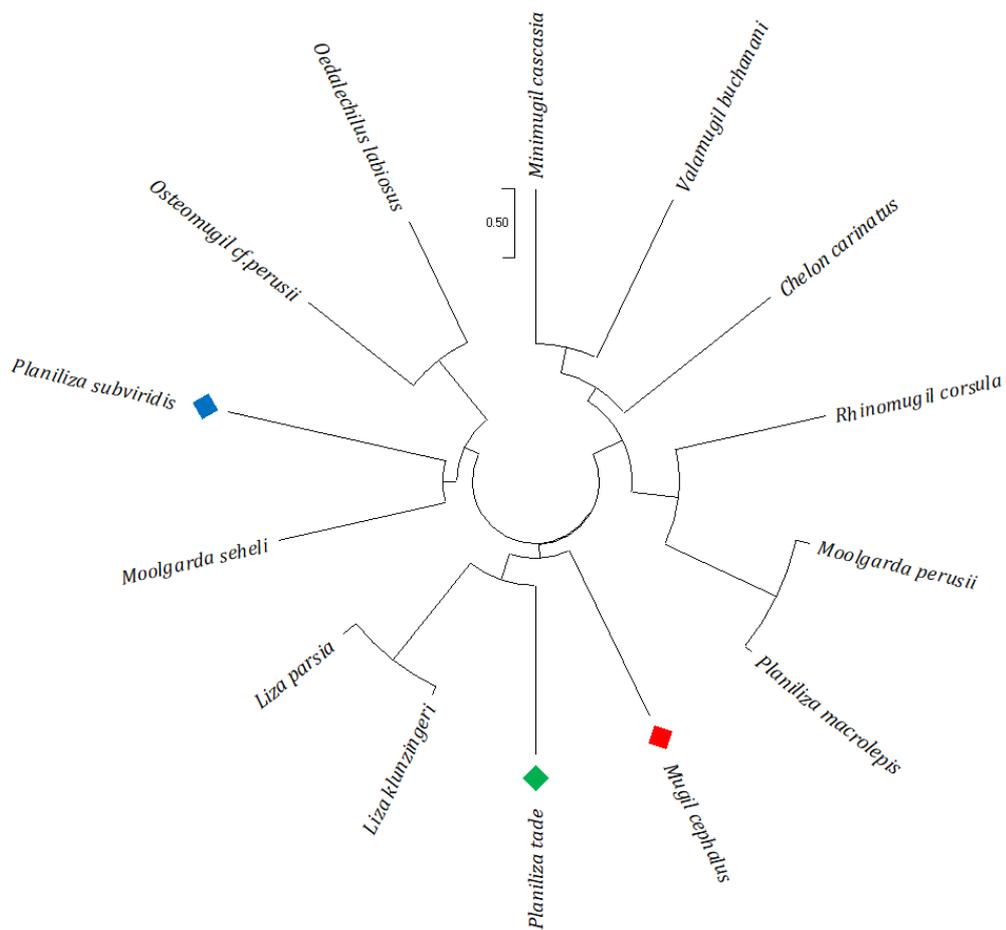


Figure 1.16: Overall Phylogeny Tree of Mullet

1.4 DISCUSSION

Since the 19th century, researchers in India have examined the diversity of the Mugilidae family. Hamilton (1822) conducted a comprehensive study on the diversity of fish species found in the river Ganga and its tributaries. He described a new species of the only representative member of the genus *Rhinomugil* Gill, 1863, found in the lower parts of the Ganga River in West Bengal. Cuvier & Valenciennes (1836) later documented two new species in their work "Histoire naturelle des poissons": *Mugil subviridis* Valenciennes, 1836 from the Malabar coast and Ganga River, and *Mugil labiosus* Valenciennes, 1836 from Mumbai. Francis Day had documented common species of Southern Indian mullets between 1865 and 1888; Whitehouse (1922) produced a more thorough report. In addition to them, several researchers have made significant contributions to the field of Mugilidae systematics in India. These include Bleeker 1853, Chaudhuri 1917, Herre 1939, Herre 1941, Jacob & Krishnamurthy 1948, Pillay 1954, John 1955, Sarojini 1957, Sarojini 1958, Misra 1959, Pillay 1962a, Pillay 1962b, Luther 1968, Blanc & Hureau 1971, Luther 1973, Luther 1974, Luther 1977, and Jayram 1981.

In the Mugilidae family, 24 species of mullets from 10 genera have been identified in Indian waters. *Planiliza* Whitley 1945 has the greatest number of species (10) among all the genera that have been reported, followed by *Osteomugil* Luther, 1982 (4 species), and *Crenimugil* Schultz 1946 (3 species). While there is only one species in each of the remaining genera: *Ellochelon* Whitley, 1930; *Minimugil* Durand, Chen, Shen, Fu & Borsa 2012; *Mugil* Linnaeus 1758; *Parachelon* Durand, Chen, Shen, Fu & Borsa 2012; *Paramugil* Ghasemzadeh, Ivantsoff & Aarn 2004; *Plicomugil* Schultz in Schultz, Herald, Lachner, Welander & Woods 1953; and *Rhinomugil* Gill 1863. The current study demonstrates that the species *Rhinomugil corsula* (Hamilton 1822) is found in the majority of states and union territories in India, but *Parachelon grandisquamis* (Valenciennes 1836) is only found along the West Coast of India. Tamil Nadu has the highest reported number of Mugilidae species (22 sp.), followed by Gujarat (18 sp.), Maharashtra (18 sp.), Karnataka (18 sp.), Kerala (18 sp.), West Bengal (18 sp.), Andaman & Nicobar (18 sp.), Goa (17 sp.), Pondicherry (17 sp.), Andhra Pradesh (17 sp.), Odisha (17 sp.), Lakshadweep (17 sp.), Pulicat lake (16 sp.), and Madhya Pradesh (9 sp.) (Fig. 1.17). A total of two

species have been documented from Uttarakhand, Delhi, Uttar Pradesh, Bihar, Assam, Tripura, and Meghalaya. The remaining states, namely Rajasthan, Punjab, Jharkhand, Chhattisgarh, Telangana, and Manipur, were found to have only one species according to Bhatt & Mankodi (2023).



Figure 1.17: Distribution of Mullet in India (Bhatt & Mankodi 2023)

In Gujarat, 18 species were identified using morphological characteristics out of the 24 species documented in India (Bhatt & Mankodi 2023). Several Indian scientists used DNA barcoding to conduct molecular identification (Bhatt & Mankodi, 2023). In Gujarat, a group of scientists successfully barcoded a total of five species from various taxa. The species that were barcoded are as follows: The species *Minimugil cascasia* (Hamilton 1822) was studied by Sarma *et al.* (2015). *Mugil cephalus* Linnaeus 1758 was studied by Khedkar *et al.* (2014). *Planiliza tade* (Fabricius 1775) was studied by Sidat *et al.* (2013). *Rhinomugil corsula* (Hamilton 1822) was studied by Khedkar *et al.* (2014). *Planiliza klunzingeri* (Day 1888) was studied by Shukla *et al.* (2012), Sidat *et al.* (2013), Khedkar *et al.* (2014), and Ayachit & Bagatharia (2015).