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Abstract: Fishes of the Mugilidae family are poorly known from a taxonomic perspective, largely because of their conservative morphology. In this paper, we aim to fill the knowledge gap and data deficiency regarding the genetic diversity of mullets occurring in southern peninsular India. A comprehensive mitochondrial phylogeny based on 238 *COI* sequences (78 newly generated) and three species delimitation methods (ASAP, RESL, and ABGD) confirmed the presence of 21 species of mullets within eight genera in southern peninsular India. These include one species each under the genera *Rhinomugil* Gill 1863, *Minimugil* Durand, Chen, Shen, Fu, and Borsa, 2012, *Plicomugil* Schultz 1953, and *Ellochelon* Whitley 1930; seven species of *Planiliza* Whitley 1945; four species of *Crenimugil* Schultz 1946; four species of *Osteomugil* Luther 1982; and two species of the "*Mugil cephalus* Linnaeus 1758" complex—though with uncertainty regarding the exact geographic origin of one of the two species of the "*Mugil cephalus*" complex and one species phylogenetically close to *Planiliza subviridis* (Valenciennes 1836). We provide an overview of the specific geographical regions in southern peninsular India, and "species groups", which require future research using an integrative approach, so as to unravel the true extent of mullet diversity and their distribution in the region.

Keywords: barcoding; cryptic species; cytochrome oxidase I; Indian Ocean; systematics

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1. Introduction

Mullets, members of the family Mugilidae, are distributed in offshore, coastal, and inland waters along temperate, subtropical, and tropical seas [1], where they are popular in capture fisheries and aquaculture, intricately connected to cultural values [2], and play an influential part in the estuarine and coastal processes [3]. Around 600,000 tons of wild-caught and 266,048 tons of farmed mullets were harvested in 2019 [4], contributing to food security and livelihoods across the tropics.

Though extensively studied since the time of Linnaeus [5], the taxonomy and systematics of Mugilidae have been in a constant state of confusion, with the resolution and delimitation of species (and even generic) boundaries hindered by their conservative morphology and paucity of useful taxonomic characters [6]. For example, only very slight phenotypic variations exist between species within the genera *Mugil* Linnaeus 1758 and *Planiliza* Whitley 1945, making delimitation based on traditional morphological characters and meristics challenging, and identification problematic, particularly for non-specialists [7,8]. This has resulted in mullets being underrepresented [9], or erroneously represented, in field guides,

checklists, and other compilations. Though the family Mugilidae currently comprises 78 valid species under 26 genera, more than 300 nominal names are known to exist [10].

Many of the advancements in the taxonomy and nomenclature of mullets began after the application of molecular genetic information, which challenged two hundred years of morphology-based taxonomy and indicated that the group comprises a large number of cryptic species [7,11–13] and evolutionarily distinct lineages [14]. Considerable levels of genetic divergence have also been observed between (30–5 mya), and within (~10 mya) species, suggestive of long and independent evolutionary histories [15].

India has a long history of utilizing mullets [16,17], with many estuaries and backwaters contributing to both small-scale fisheries [18] and traditional aquaculture. The earliest studies on mullets in India date back to the 19th century with Hamilton's [19] work in the Ganges, followed by those in southern peninsular India by Cuvier and Valenciennes [20], and Day [21], who recorded 26 species. From the early decades of the 1900s [22], and for the next one hundred years, many studies [23–32] either entirely (aquaculture, toxicology, biology, life history) or partially (biodiversity checklists, fishery, trade) focused on mullets. Limited taxonomic studies in the 20th century were carried out by John [33], who recorded 15 species from Kayamkulam Lake (Southern India), Sarojini [34], who reviewed the Indian genera of mullets, and Luther [35], who suggested the occurrence of 13 species of mullets in India and subsequently provided new characters for an improved understanding of their taxonomy [36]. Mullet species in India have also been investigated from the perspective of genetic divergence [37], species delimitation [38], and biogeography [39]. Recently, Bhatt and Mankodi [40] provided an annotated checklist on the diversity of mullets from the Indian subcontinent, largely based on secondary literature, accounting for 24 species within 10 genera. The occurrence of species such as *Parachelon grandisquamis* (*Valenciennes 1836*) and *Planiliza carinata* (*Valenciennes 1836*) in India, which are mentioned in their checklist, is, however, doubtful [41,42], and needs further confirmation.

The mitochondrial cytochrome c oxidase subunit I (*COI*) gene, utilized in this study, proved its relevance for species boundaries in the Mugilidae family as demonstrated by Durand et al. [9] by comparing the *COI* variability alone to a dataset involving three different markers. Even though interspecific hybridization cannot be ruled out when using a mitochondrial marker such as the *COI* gene [43], there have been no previous reports of natural interspecific hybridization in Mugilidae. Furthermore, even if hybridization remains possible, such a process cannot account for the frequency of cases where there is a discrepancy in species identification between morphology and mitochondrial DNA.

In this paper, we aim to determine the genetic diversity of mullets occurring in the marine, coastal, and inland waters of southern peninsular India, a region known for its mullet fisheries, but where only limited research, especially using topotypic specimens, has been conducted on their taxonomy. By characterizing the existing genetic diversity, we aim to identify major species groups and biogeographical regions, which requires comprehensive explorations and taxonomic studies, using an integrative approach [44]. Through this manuscript, we aim to initiate the development of a meticulously curated repository comprising reference DNA barcodes for Indian mullet species. Additionally, we seek to establish a foundational framework that will contribute to shaping the future debate on the taxonomy and systematics of this fish family within the Indian subcontinent.

2. Materials and Methods

Mullet specimens were purchased from fish landing centers, markets, and/or from local fishers operating in the coastal and estuarine regions along the southern Indian peninsula (southwest and southeast coasts) and the Laccadive archipelago. They were primarily identified (as far as possible) using the "Species Identification Sheets" of the Food and Agricultural Organization (FAO) [45] of the United Nations, and González-Castro and Ghasemzadeh [1]. No fish were killed or sacrificed specifically for the purpose of this study, and the sampling protocols were entirely based on dead fish available in markets/landing centers. Therefore, no specific permissions or ethical clearances were required. The fishing

locations are represented in Figure 1. Information on the localities where the mullets were captured, was collected from the fishers, and passive georeferencing of sampling locations was achieved through the utilization of fishing-related data, specifically incorporating details such as distance and direction from landing centers (as provided by fishers or vendors).

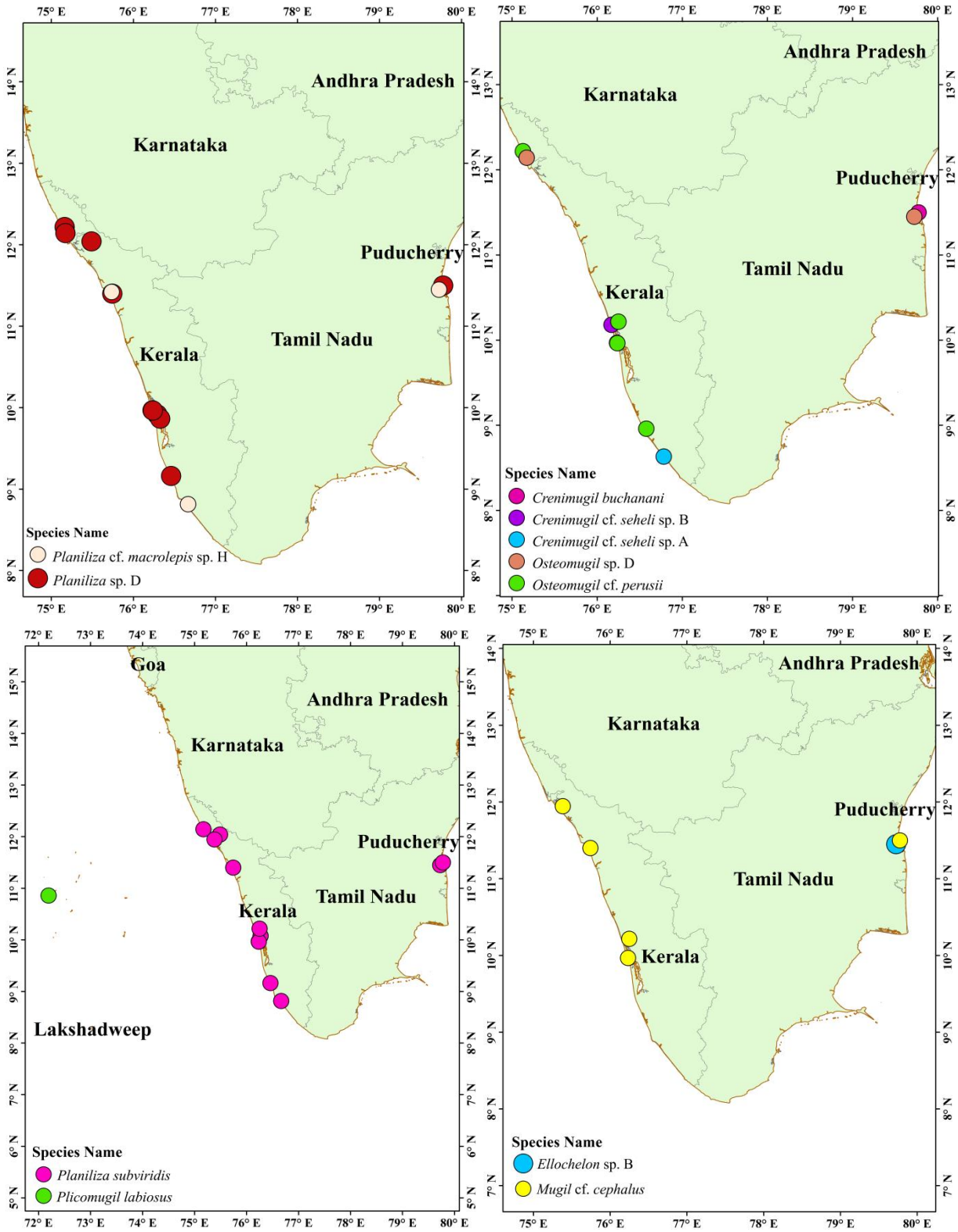

Figure 1. Maps showing locations of sample collection in southern peninsular India.

DNA extraction, PCR amplification (using primer pair FishF1 + FishF2/FishR1; Ward et al. [46]), and sequencing of the mitochondrial *COI* gene were carried out following Dahanukar et al. [47]. Sequences were aligned using MUSCLE [48], and a maximum likelihood (ML) analysis was performed in IQ-TREE [49], with the best partition scheme and ultrafast bootstrap support for 1000 iterations [50]. Three distinct phylogenetic trees were constructed as follows: (1) A comprehensive phylogenetic tree using a dataset comprising 238 *COI* sequences representing species of the family Mugilidae, and a 658 bp sequence of *Oryzias latipes* (Temminck & Schlegel 1846) as an outgroup. This included 78 new sequences generated as part of the current study (archived in BOLD under project MULIN), and 160 published sequences (including mullet *COI* sequences from the Indian subcontinent, topotypic sequences, and other reference sequences representing Molecular Operational Taxonomic Units (MOTUs) under eight genera concerning the Indian scenario of mullets, as denoted in References [7,9,11,12,39,41]). These sequences were retrieved from the GenBank (www.ncbi.nlm.nih.gov/genbank/ (accessed on 5 June 2023)) [51]. (2) A concise phylogenetic tree (alignment length 501 bp) using representative sequences (comprising a total of 76 sequences, including an outgroup) from each clade as delineated in the comprehensive phylogenetic tree. This tree was developed to provide a brief, yet informative overview of the genetic diversity of Indian mullets. (3) A phylogenetic tree with 78 newly generated sequences along with an outgroup. The newly generated sequences as part of our studies are presented in Table 1, and information about the complete dataset of mullet sequences used to construct the comprehensive phylogenetic tree, including topotypic and reference sequences obtained from GenBank and newly generated sequences, is provided in Supplementary Table S1. In order to avoid misinterpretations of molecular phylogenetic analyses resulting from misidentifications of sequences in genetic databases, the sequences we used, including both topotypic and other reference sequences, were sourced from research that employed a morphological as well as a molecular taxonomic approach (Supplementary Table S2).

Table 1. Details of species, locations, and barcode length, and GenBank accession number of newly generated sequences.

Species Name	Fishing Locations	Latitude (N)	Longitude (E)	*COI* Sequence Length (bp)	GenBank Accession
Crenimugil buchanani (Bleeker 1853)	Vellar, Tamil Nadu	11.498	79.776	645	MW137482
Crenimugil buchanani	Vellar, Tamil Nadu	11.498	79.776	644	MW137480
Crenimugil buchanani	Vellar, Tamil Nadu	11.498	79.776	644	MW137481
Crenimugil cf. *seheli* sp. A	Muthalapozhi, Kerala	8.631	76.783	582	MN728321
Crenimugil cf. *seheli* sp. A	Muthalapozhi, Kerala	8.631	76.783	582	MN728322
Crenimugil cf. *seheli* sp. B	Cochin, Kerala	9.9667	76.2333	676	MW137486
Crenimugil cf. *seheli* sp. B	Munambam, Kerala	10.178	76.169	582	MN728320
Ellochelon sp. B	Vellar, Tamil Nadu	11.498	79.776	645	MW137487
Ellochelon sp. B	Vellar, Tamil Nadu	11.498	79.776	644	MW137488
Ellochelon sp. B	Vellar, Tamil Nadu	11.498	79.776	643	MW137489
Mugil cf. *cephalus*	Vellar, Tamil Nadu	11.498	79.776	627	MW137496
Mugil cf. *cephalus*	Vellar, Tamil Nadu	11.498	79.776	645	MW137497
Mugil cf. *cephalus*	Vellar, Tamil Nadu	11.498	79.776	644	MW137498
Mugil cf. *cephalus*	Vellar, Tamil Nadu	11.498	79.776	645	MW137499
Mugil cf. *cephalus*	Vellar, Tamil Nadu	11.498	79.776	646	MW137500
Mugil cf. *cephalus*	Cochin, Kerala	9.9667	76.2333	676	MW137495
Mugil cf. *cephalus*	Cochin, Kerala	9.9667	76.2333	644	MW137494
Mugil cf. *cephalus*	Poyya, Kerala	10.221	76.234	582	MN728324
Mugil cf. *cephalus*	Korapuzha, Kerala	11.396	75.745	582	MN728325
Mugil cf. *cephalus*	Poyya, Kerala	10.221	76.234	582	MN728326
Mugil cf. *cephalus*	Kattampally, Kerala	11.937	75.376	582	MN728327

Table 1. *Cont.*

Species Name	Fishing Locations	Latitude (N)	Longitude (E)	*COI* Sequence Length (bp)	GenBank Accession
Osteomugil cf. *perusii*	Ashtamudi, Kerala	8.959	76.577	582	MN728329
Osteomugil cf. *perusii*	Poyya, Kerala	10.221	76.234	582	MN728330
Osteomugil cf. *perusii*	Fort Kochi, Kerala	9.963	76.237	582	MN728331
Osteomugil cf. *perusii*	Madakkara, Kerala,	12.21672	75.130238	582	MN728332
Osteomugil sp. D	Vellar, Tamil Nadu	11.498	79.776	676	MW137515
Osteomugil sp. D	Vellar, Tamil Nadu	11.498	79.776	653	MW137514
Osteomugil sp. D	Vellar, Tamil Nadu	11.498	79.776	655	MW137516
Osteomugil sp. D	Thrikkaripur, Kerala	12.138	75.16	582	MN728328
Planiliza cf. *macrolepis* sp. H	Vellar, Tamil Nadu	11.498	79.776	679	MW137525
Planiliza cf. *macrolepis* sp. H	Vellar, Tamil Nadu	11.498	79.776	658	MW137524
Planiliza cf. *macrolepis* sp. H	Vellar, Tamil Nadu	11.498	79.776	658	MW137523
Planiliza cf. *macrolepis* sp. H	Vellar, Tamil Nadu	11.498	79.776	658	MW137522
Planiliza cf. *macrolepis* sp. H	Paravoor, Kerala	8.835	76.667	582	MN728317
Planiliza cf. *macrolepis* sp. H	Paravoor, Kerala	8.835	76.667	582	MN728318
Planiliza cf. *macrolepis* sp. H	Vellar, Tamil Nadu	11.418	75.734	582	MN728319
Planiliza sp. D	Vellar, Tamil Nadu	11.498	79.776	652	MW137518
Planiliza sp. D	Cochin, Kerala	9.9667	76.2333	678	MW137535
Planiliza sp. D	Cochin, Kerala	9.9667	76.2333	644	MW137544
Planiliza sp. D	Cochin, Kerala	9.9667	76.2333	644	MW137540
Planiliza sp. D	Cheruvathur, Kerala	12.203	75.129	582	MN728302
Planiliza sp. D	Valapattanam, Kerala	12.04	75.49	582	MN728303
Planiliza sp. D	Kayamkulam, Kerala	9.161	76.46	582	MN728304
Planiliza sp. D	Korapuzha, Kerala	11.396	75.745	582	MN728305
Planiliza sp. D	Thrikkaripur, Kerala	12.141	75.172	582	MN728306
Planiliza sp. D	Korapuzha, Kerala	11.396	75.745	582	MN728307
Planiliza sp. D	Korapuzha, Kerala	11.396	75.745	582	MN728308
Planiliza sp. D	Edakochi, Kerala	9.916	76.291	582	MN728309
Planiliza sp. D	Korapuzha, Kerala	11.396	75.745	582	MN728310
Planiliza sp. D	Korapuzha, Kerala	11.396	75.745	582	MN728311
Planiliza sp. D	Korapuzha, Kerala	11.396	75.745	582	MN728312
Planiliza sp. D	Kayamkulam, Kerala	9.161	76.46	582	MN728313
Planiliza sp. D	Cochin, Kerala	9.863	76.32	582	MN728314
Planiliza sp. D	Cochin, Kerala	9.863	76.32	582	MN728315
Planiliza sp. D	Fort Kochi, Kerala	9.963	76.237	582	MN728316
Planiliza subviridis	Vellar, Kerala	11.498	79.776	652	MW137547
Planiliza subviridis	Cochin, Kerala	11.498	79.776	644	MW137546
Planiliza subviridis	Cochin, Kerala	11.498	79.776	665	MW137545
Planiliza subviridis	Cochin, Kerala	11.498	79.776	644	MW137548
Planiliza subviridis	Vellar, Tamil Nadu	11.498	79.776	643	MW137551
Planiliza subviridis	Vellar, Tamil Nadu	11.498	79.776	644	MW137550
Planiliza subviridis	Vellar, Tamil Nadu	11.498	79.776	644	MW137566
Planiliza subviridis	Vellar, Tamil Nadu	11.498	79.776	652	MW137549
Planiliza subviridis	Cochin, Kerala	9.863	76.32	646	MW137552
Planiliza subviridis	Kattampally, Kerala	11.937	75.376	582	MN728289
Planiliza subviridis	Varapuzha, Kerala	10.074	76.239	582	MN728290
Planiliza subviridis	Cheliya, Kerala	12.04	75.49	582	MN728291
Planiliza subviridis	Thrikkaripur, Kerala	12.138	75.16	582	MN728292
Planiliza subviridis	Kayamkulam, Kerala	9.161	76.46	582	MN728293
Planiliza subviridis	Korapuzha, Kerala	11.396	75.745	582	MN728294
Planiliza subviridis	Paravoor, Kerala	8.835	76.667	582	MN728295
Planiliza subviridis	Thrikkaripur, Kerala	12.138	75.16	582	MN728296
Planiliza subviridis	Fort Kochi, Kerala	9.963	76.237	582	MN728297
Planiliza subviridis	Poyya, Kerala	10.221	76.234	582	MN728298

Table 1. *Cont.*

Species Name	Fishing Locations	Latitude (N)	Longitude (E)	*COI* Sequence Length (bp)	GenBank Accession
Planiliza subviridis	Kattampally, Kerala	11.937	75.376	582	MN728299
Planiliza subviridis	Korapuzha, Kerala	11.396	75.745	582	MN728300
Planiliza subviridis	Kayamkulam, Kerala	9.161	76.46	582	MN728301
Plicomugil labiosus	Agatti, Lakshadweep	10.857	72.1959	582	MN728323

The Bayesian information criterion (BIC) was used for *COI* data to infer phylogenetic relationships. We performed a partition model analysis [52], where one allows each partition to have its own model. The best partition scheme was chosen using ModelFinder [53], in IQ-TREE. Of the seven partition schemes, the best-fit partition model was obtained by optimizing TPM2 + F + I + G4 parameters for partition part1 + part2 + part3. The optimal log-likelihood (ln L) score of the best tree is −6624.878. The ML tree was subsequently used as a basis for species delimitation using the Automatic Barcode Gap Distance (ABGD) [54], using 100,000 Markov chain Monte Carlo (MCMC) generations, a thinning parameter of 100, and a burn-in of 0.1. The output tree was viewed using FigTree v1.4.2. [55].

The accuracy of the Molecular Operational Taxonomic Unit (MOTU) delineations identified using ABGD was evaluated by comparing MOTUs to BINs provided by BOLD [56] obtained from http://www.barcodinglife.org (accessed on 5 June 2023), and based on the RESL algorithm [57] and ASAP (Assemble Species by Automatic Partitioning analysis) [58]. Species delimitation was carried out using the online ABGD version (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with default parameters, applying the Kimura K80 (ts/tv 2.0) substitution model, and for ASAP (https://bioinfo.mnhn.fr/abi/public/asap/), we performed the analysis using the Kimura K80 (ts/tv 2.0) substitution model with a recursive split probability of 0.01. Pairwise genetic distances were calculated by employing the Kimura 2-parameter model [59] using MEGA X [60].

Relevant information on type species and type localities follows the Catalog of Fishes [10] and Kottelat [61], and nomenclature follows Durand and Borsa [11]. When a species was identified morphologically, and its DNA barcode matched (>99% of sequence homology) a sequence published and labelled by Durand and Borsa [11], we mentioned the species name by a "cf." as well as the interim nomenclature proposed by Durand and Borsa [11].

3. Results

The phylogenetic tree (Figure 2; Supplementary Figure S1) represents eight genera of Mugilidae, viz., *Crenimugil* Schultz 1946, *Ellochelon* Whitley 1930, *Mugil* Linnaeus, 1758, *Minimugil* Durand, Chen, Shen, Fu, and Borsa, 2012, *Osteomugil* Luther 1982, *Planiliza* Whitley 1945, *Plicomugil* Schultz 1953, and *Rhinomugil* Gill 1863, each of which were confirmed by the presence of topotypic sequences of the type species (marked with # after the sequence accession number in Supplementary Table S1 and information on topotypic and other reference sequences given in Supplementary Table S2). These eight groups include all known mugilid genera occurring in India and close to one-third of all known mugilid genera (26 genera) in the world.

Species delimitation based on the ABGD, RESL, and ASAP analysis confirmed the occurrence of twenty-one Molecular Operational Taxonomic Units (MOTU) of mullets in India. The phylogenetic tree depicting the genetic diversity of Indian mullets is represented in Figure 2. Furthermore, these 21 species are effectively delineated based on the pairwise genetic distance analysis (Figure 3). The phylogenetic tree with newly generated sequences is presented in Figure 4, and a detailed phylogenetic tree showing comprehensive analysis is provided in Supplementary Figure S1. We restrict our results and discussion to the species diversity within southern peninsular India, though our results are largely applicable to the species diversity of the Indian subcontinent and entire Indo-Pacific.

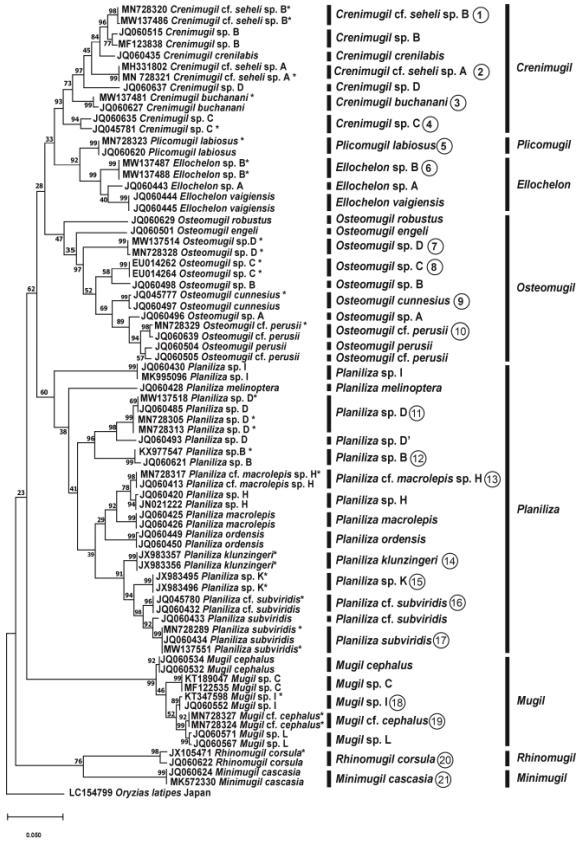

Figure 2. The maximum likelihood phylogenetic tree of the family Mugilidae from India (Arabic numerals inside the circles represent "species groups" found in India, and these same numbers are also depicted in the matrix diagram (Figure 3). Sequences originating from the Indian region are denoted with "*" in the tree).

1	**1**																		
2	0.08	**2**																	
3	0.08	0.12	**3**																
4	0.08	0.12	0.08	**4**															
5	0.16	0.18	0.16	0.13	**5**														
6	0.16	0.18	0.16	0.14	0.13	**6**													
7	0.2	0.2	0.2	0.19	0.2	0.17	**7**												
8	0.23	0.23	0.18	0.2	0.21	0.21	0.17	**8**											
9	0.19	0.19	0.17	0.16	0.19	0.17	0.13	0.16	**9**										
10	0.23	0.21	0.17	0.17	0.21	0.22	0.14	0.1	0.09	**10**									
11	0.15	0.17	0.17	0.14	0.16	0.15	0.19	0.23	0.18	0.19	**11**								
12	0.17	0.18	0.18	0.15	0.2	0.2	0.21	0.23	0.19	0.18	0.12	**12**							
13	0.16	0.17	0.16	0.14	0.18	0.19	0.19	0.22	0.19	0.18	0.13	0.07	**13**						
14	0.19	0.19	0.17	0.18	0.16	0.19	0.21	0.23	0.21	0.2	0.17	0.15	0.15	**14**					
15	0.17	0.18	0.17	0.17	0.18	0.19	0.19	0.21	0.21	0.2	0.13	0.13	0.11	0.15	**15**				
16	0.18	0.19	0.17	0.17	0.18	0.17	0.2	0.2	0.18	0.2	0.14	0.13	0.14	0.15	0.1	**16**			
17	0.19	0.2	0.19	0.2	0.2	0.18	0.2	0.22	0.2	0.21	0.15	0.14	0.14	0.16	0.1	0.07	**17**		
18	0.25	0.25	0.24	0.21	0.21	0.23	0.24	0.22	0.22	0.2	0.22	0.2	0.21	0.22	0.22	0.22	0.25	**18**	
19	0.23	0.23	0.23	0.21	0.2	0.22	0.23	0.21	0.22	0.21	0.21	0.2	0.22	0.21	0.22	0.23	0.24	0.02	**19**
20	0.19	0.21	0.21	0.18	0.18	0.2	0.21	0.21	0.17	0.21	0.2	0.22	0.21	0.21	0.21	0.21	0.22	0.2	0.2	**20**
21	0.23	0.25	0.22	0.22	0.22	0.24	0.24	0.23	0.22	0.22	0.19	0.23	0.23	0.28	0.21	0.2	0.21	0.21	0.21	0.17

Figure 3. Matrix diagram representing pairwise genetic distances among *COI* sequences of Indian species groups. (Arabic numerals in the green squares are the same species group number depicted in Figure 2, decimal numbers in the yellow squares represent the fraction of the maximum possible genetic distance between the corresponding species groups).

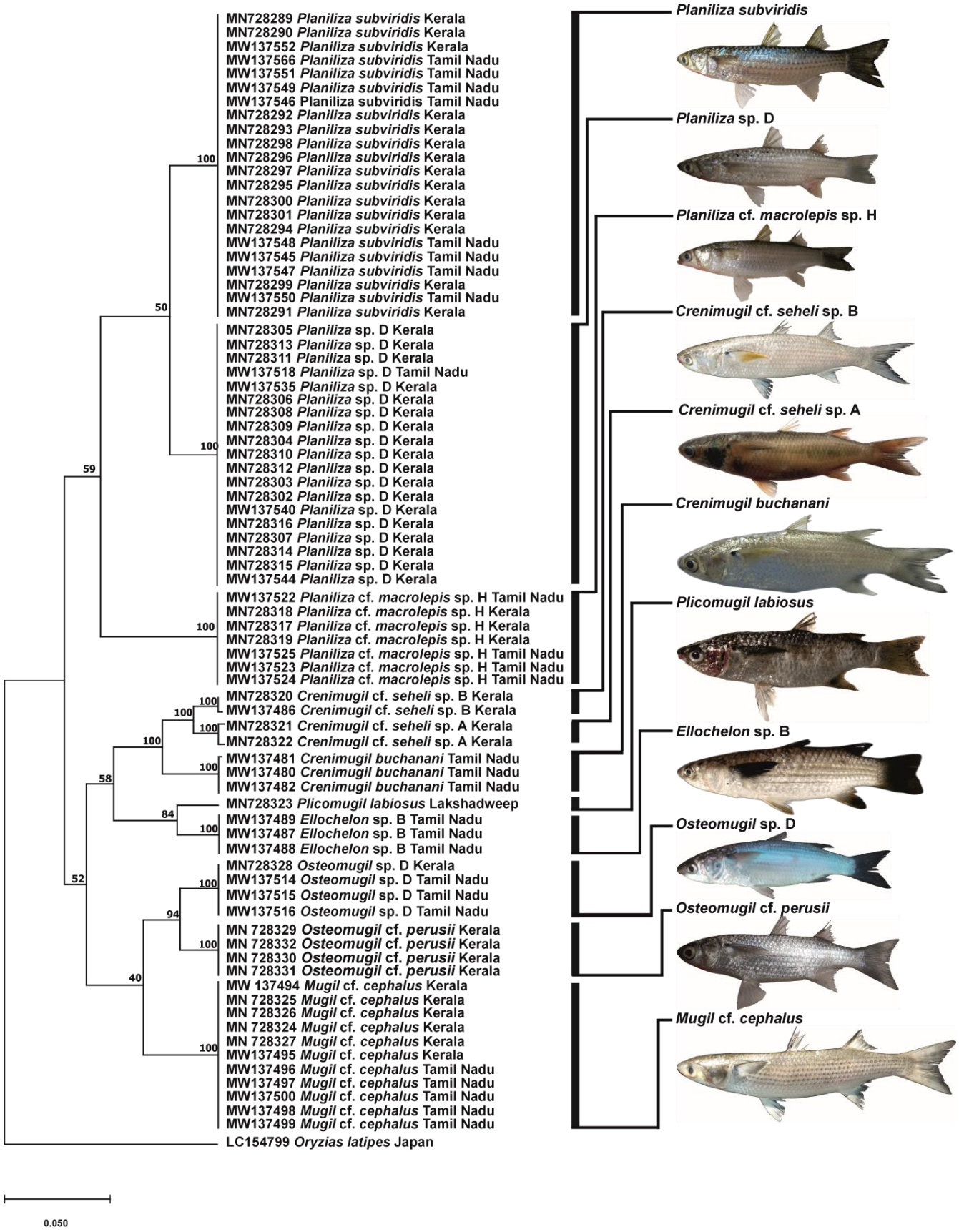

Figure 4. The maximum likelihood phylogenetic tree constructed using the newly generated sequences as part of our study.

Details on the species diversity within each genus are provided below.

1. *Crenimugil* Schultz, 1946—Type species: *Crenimugil crenilabis* (Forsskål 1775) (JQ060435) ("A" in Supplementary Figure S1)

Four species of *Crenimugil* occur in India including *Crenimugil* cf. *seheli* sp. B (Kerala), *Crenimugil* cf. *seheli* sp. A (Kerala, Andaman Islands), *C. buchanani* (eastern coast, including West Bengal and Tamil Nadu), and "*Crenimugil* sp. C" sensu Durand and Borsa (2015), represented by the sequence JQ045781 from the Vellar Estuary on the southeastern coast.

2. *Plicomugil* Schultz, 1953—Type species: *Plicomugil labiosus* (Valenciennes 1836) (topotype: MN728289) ("G" in Supplementary Figure S1)

The presence of *Plicomugil* in the Indian subcontinent was identified based on a sequence generated from Agatti Island in the Laccadive archipelago, the Arabian Sea.

3. *Ellochelon* Whitley, 1930—Type species: *Ellochelon vaigiensis* (Quoy & Gaimard 1825) (topotype: JQ060444) ("F" in Supplementary Figure S1)

Our analysis revealed that the species of *Ellochelon* occurring in the Vellar Estuary is likely to represent an undescribed species, distinct from topotypic *E. vaigiensis*, or the species "*Ellochelon* sp. A" sensu Durand and Borsa (2015), based on the results of ABGD, ASAP, and RESL.

4. *Osteomugil* Schultz, 1946—Type species: *Osteomugil cunnesius* (Valenciennes 1836) (topotype: JQ045777) ("E" in Supplementary Figure S1)

Our analysis delineated ten "species groups" within the genus *Osteomugil*, of which four occur in India. These include (i) "*Osteomugil* sp. D" sensu Shen and Durand (2016), found in Yanam and the Vellar Estuary on the southeastern coast, and the Thrikkaripur estuary on the southwestern coast; (ii) "*Osteomugil* sp. C" sensu Shen and Durand (2016), distributed in the coastal and estuarine waters of the eastern coast of India; (iii) *O. cunnesius* in the Vellar Estuary; and (iv) *Osteomugil* cf. *perusii* found in Andhra Pradesh (Kakkinada), Tamil Nadu (Vellar), and throughout the estuarine systems of Kerala.

5. *Planiliza* Whitley, 1945—Type species: *Planiliza ordensis* (*Whitley 1945*) (topotype: JQ060449–50) ("H" in Supplementary Figure S1)

Of the fourteen species of *Planiliza* delineated in our analysis (ABGD, RESL, and ASAP), seven are known to occur in India. These include (i) "*Planiliza* sp. D" sensu Durand and Borsa (2015), one of the most common and abundant species of *Planiliza* found throughout the estuaries and backwaters of the southwestern state of Kerala; (ii) "*Planiliza* sp. B" sensu Durand and Borsa (2015), a species recorded first from Malaysia (topotypic sequence KX223926), which is also distributed on the eastern coast of India in the states of West Bengal and Andhra Pradesh; (iii) "*Planiliza* cf. *macrolepis* sp. H" phylogenetically identical to "*Planiliza* sp. H" sensu Durand and Borsa (2015), a species that is often confused in the literature from India (e.g., Sarojini [34]; Kurup [62]; Fischer and Bianchi [63]) and other regions in the Indo-Pacific (e.g., Sri Lanka, the Maldives, the Philippines, and Japan) as being *Planiliza macrolepis*, but is distinct from *Planiliza macrolepis* (Smith 1846) sensu stricto (type locality: South Africa; putative topotypes: JQ060425–26; see Durand and Borsa [11]) and is distributed in the estuaries and backwaters of both the southeast (Vellar) and southwest coasts (Cochin, Ashtamudi) of India; (iv) *P. klunzingeri*, described as being from Mumbai and distributed along the northwestern coast of India (JX983355–57); (v) "*Planiliza* sp. K" sensu Shen and Durand [39], which occurs in the Bharuch estuary on the northwestern coast (misidentified as *Minimugil cascasia* in GenBank—JX983495 and JX983496); (vi) "*Planiliza* cf. *subviridis*" specimens morphologically identical to *P. subviridis* but belonging to another MOTU (BIN BOLD:ABY5947), identified in India based on two sequences (JQ045780, JQ045782) from the Vellar Estuary, on the southeastern coast. It is phylogenetically close to *P. subviridis* and another specimen from Indonesia (JQ060433) identified as *Planiliza subviridis* but forming a third MOTU (BOLD: ACC0823); and (vii) *P. subviridis*, a species described as coming from the erstwhile British Indian region of Malabar (current day northern

Kerala State/Southern Karnataka State) represented by topotypic sequences (MG495936, MN728289, MN728299, MN728294, MN728292, MN728296) and several additional matching sequences confirming their wide distribution throughout the coastal and estuarine waters of the entire eastern (West Bengal, Andhra Pradesh, and Tamil Nadu), as well as southwestern coasts (Kerala).

6. *Mugil* Linnaeus, 1758—Type species: *Mugil cephalus* Linnaeus 1758 (topotypes: KY683176, JQ060529, JQ060532–34) ("C" in Supplementary Figure S1)

The species delimitation analysis using ABGD, ASAP, and RESL delineated the *Mugil cephalus* clade into five "species groups" or lineages. Two species groups within the "*Mugil cephalus* complex" are likely to occur in India. The first species ("species group" number "3" in Supplementary Figure S1) did not match any lineages highlighted by Durand et al. [7] or Durand and Borsa [11]. It comprised sequences originating from both the southeastern and southwestern coasts of India. Outside India this MOTU (BOLD: ADZ0803) has been observed in Bangladesh, Pakistan, and Iran (BOLD, consultation on 02/08/2023). It is phylogenetically close to the species provisionally named as "*Mugil* sp. L" by Durand and Borsa [11], and observed in the Pacific from Taiwan to New Caledonia and Fiji [64].

A likely second species group within the "*Mugil cephalus* complex" could occur in India based on three sequences (KT347596–98) from the Vellar Estuary, on the southeastern coast. This species has been identified as "*Mugil* sp. I" by Durand and Borsa [11]. With the exception of the three specimens from the Vellar Estuary, it is only observed in the Northwest Pacific from Japan to Vietnam and the Philippines [64].

7. *Rhinomugil* Gill, 1863—Type species: *Rhinomugil corsula* (topotype: JX105471) ("A" in Supplementary Figure S1)

Only one species, *Rhinomugil corsula*, occurs in the Indian subcontinent, confirmed through a topotypic sequence available in GenBank (JX105471). The distribution range of this species includes the estuarine and riverine systems on the eastern and western coasts of India with confirmed genetic data from the states of Assam, West Bengal, Andhra Pradesh, and Gujarat.

8. *Minimugil* Durand, Chen, Shen, Fu, and Borsa, 2012—Type species: *Minimugil cascasia* (Hamilton 1822) (topotypes: JQ060624 and MK572330) ("B" in Supplementary Figure S1)

No sequence of this species was generated as part of this study, and, also, no sequences are available in GenBank from the Indian region that match putative topotypic sequences from Bangladesh (JQ060624 and MK572330). Given the geographic proximity of Bangladesh to the northeastern coast of India (West Bengal), it is highly likely that this species is distributed in the estuaries of India, but this needs to be confirmed by future studies.

4. Discussion

Mullets are major contributors to brackish-water and estuarine fisheries along both the western and eastern coasts of India [35], and are also emerging candidates for brackish-water aquaculture diversification [65,66]. This has made species identification a priority, so as to inform conservation, management, trade, and policy. To the best of our knowledge, there have been no previous studies from the Indian subcontinent, where mullet species have been delineated and identified using topotypic material and/or sequences—a knowledge gap we address in our study.

The taxonomy and nomenclature of mullets is in flux, and recent research, particularly using molecular genetic information, has challenged two hundred years of morphology-based taxonomy (e.g., [7,11]). This is because previous studies on Indian species of mullets were mainly based on conventional taxonomy, and identification was mainly based on the personal perceptions of authors. Since the 1950s, most authors who working on mullet taxonomy have (i) provided conflicting opinions and statements regarding the morphology and meristics of the various "species" recorded from India (See Supplementary Table S3), (ii) used diagnostic characters that have subsequently been proved as unreliable for identi-

fication (e.g., those that change during ontogenic development—adipose eyelids, types of scales, mandibular angle, third anal fin spine) [22,67], and (iii) erroneously assigned species to various genera [18,22,35,68–71], without proper justification.

Most historic records (1800s to the early 20th century) of mullets in the Indian literature have assigned the species to either of the two genera, i.e., *Mugil* or *Liza* [21,22,33,67,68]. Generic-level revisions carried out since then have significantly changed our understanding of mullet taxonomy and nomenclature, but the absence of systematic genetic studies on mullets in India makes the comparison of our results difficult.

Our analysis reveals that three monotypic genera, *Rhinomugil* (*R. corsula*), *Plicomugil* (*P. labiosus*) and *Minimugil* (*M. cascasia*) occur in India. Of these, the occurrence of *Plicomugil* was confirmed based on a topotypic sequence from the Laccadive archipelago and *Rhinomugil* from a topotypic sequence from Assam. Previous records on *Plicomugil labiosus* are available from Lakshadweep waters [72]. The likely occurrence of *Minimugil* in Indian waters is suggested based on sequences collected from neighboring Bangladesh (as no sequences are as yet available from India), as this location is also part of the Indian subcontinent. *Ellochelon*, which has been considered monotypic [10], is also likely to contain additional species, as evident from our species delimitation, and as suggested by Durand and Borsa 2016 [11], and more recent studies [73,74].

Apart from *Crenimugil* cf. *seheli* sp. A (in Kerala) and *C. buchanani* (eastern coast including West Bengal and Tamil Nadu), two other species of the genus occur in Indian waters. One species, which we identify as *Crenimugil* cf. *seheli* sp. B, distributed along the southwestern coast and in the Andaman and Nicobar Islands, may be considered to be a distinct species named as *Crenimugil* sp. B sensu Durand & Borsa [11] since two species delimitation methods separate Indian specimens from other specimens named *Crenimugil* sp. B. However, this result needs to be strengthened using a larger dataset able to improve inter- and intraspecies diversity estimation, and thus the barcode gap detection. The second unnamed species, which we consider as "*Crenimugil* sp. C", occurs in the Vellar Estuary on the southeastern coast of India.

The *Osteomugil* clade (clade E in Supplementary Figure S1) comprises *Osteomugil cunnesius*, *Osteomugil* cf. *perusii*, and two other species, which we list as "*Osteomugil* sp. D" (southeastern and southwestern coasts) and "*Osteomugil* sp. C", (eastern coast). *Osteomugil cunnesius* is confirmed based on the topotypic sequence (JQ045777) from the Vellar Estuary. *Osteomugil perusii* is associated with four BINs (Supplementary Table S1), but only three MOTUs are considered following species delimitation analyses (ABGD, RESL, and ASAP). In India, the two BINs (AEZ8463 and AAW7354) converge into a single MOTU. The geographic distribution of the three BINs linked to the three MOTUs is as follows: BIN BOLD: ACC0135 is observed in New Caledonia, Papua New Guinea, and Indonesia; BIN BOLD: AAW7354 is found in Indonesia, India, the Philippines, Malaysia, and Sri Lanka; and BIN BOLD: ACC0061 is present in Malaysia, Indonesia, China, and Taiwan. These three BINs exhibit some overlap in Indonesia, as mentioned in Delrieu-Trottin [8], although this overlap appears to be partial. This leads to the suggestion that BIN BOLD: ACC0135 represents *Osteomugil perusii* sensu stricto, given the species' type locality is in Vanikoro Island and Santa Cruz Islands in the southwestern Pacific. The remaining two BINs may be considered sister species, with one primarily inhabiting the Indian Ocean (species group number "10" in Figure 2) and the other in the NW Pacific. The identity of the species found in India may be considered as *Mugil amarulus* Valenciennes, 1836, a synonym of *Osteomugil perusii* [10], but requires further studies for confirmation. To assign a species name to *Osteomugil* sp. C and *Osteomugil* sp. D, more detailed morphological investigations are needed.

Planiliza is probably the most species-rich mullet genus in the Indo-Pacific, represented by at least fourteen "species groups" (clade H in Supplementary Figure S1), of which seven are known to occur in India. The species found in India, identified and validated by matching topotypic sequences, include *P. klunzingeri* and *P. subviridis*. The second putative species closely related to *Planiliza subviridis* and "*Planiliza* cf. *subviridis* [BOLD: ACC0823]"

(JQ060433 from Indonesia) designated as "*Planiliza* cf. *subviridis*" in Table 1 and assigned to the BIN BOLD: ABY5947 occurs in the Vellar Estuary, on the southeastern coast of India. This BIN ABY5947 includes specimens from eastern Asia including from China and Taiwan. The presence of this species in the natural waters of India is also doubtful similar to the occurrence of "*Mugil* sp. I", and could probably correspond to fish imports [75]. To confirm the identity of four additional species occurring in Indian waters (*Planiliza* sp. B, D, H, and K), detailed taxonomic studies are required. Two of these species have a distribution primarily on the west coast of India: "*Planiliza* sp. D" (Cochin backwaters to the Bharuch estuary in Gujarat) and "*Planiliza* sp. K" (Bharuch estuary in Gujarat). *Planiliza* sp. H is closely allied to *P. macrolepis*—a species described as coming from the rivers and lakes of South Africa [76], and currently known throughout the Indo-West Pacific. The clade comprising the sequences JQ060425–26 originating from South Africa and the Seychelles is considered topotypic for the present analysis, and is distinct from the specimens misidentified as *P. macrolepis* in India—which we designated as "*Planiliza* cf. *macrolepis* sp. H" since no significant divergence was observed between specimens from Sri Lanka and the Maldives named by Durand and Borsa [11] as *Planiliza* sp. H. It can be noticed in Figure 2 (also in Supplementary Figure S1) that specimens named by Durand and Borsa [11] as *Planiliza* sp. H form two distinct MOTUs irrespective of the species delimitation method used (BINs BOLD: ACC0087 and BOLD:AAC0697). However, Durand and Borsa [11] did not acknowledge this genetic differentiation despite evident geographic disconnection in the BIN's geographic distribution since genetic divergence evaluated using three mitochondrial markers is not significant (see Figure 1 in Durand and Borsa [11]). Three of these putative species (*Planiliza* sp. K, H, and D) occur in the estuarine waters of the west coast (two also on the east coast) where several names, which are now considered synonyms of *P. macrolepis*, are available. For example, *Mugil cunnumboo* Day, 1865 (type locality: Malabar, India), and *Mugil olivaceus* Day, 1876 (type locality: Seas of India ascending rivers), could be assigned to "*Planiliza* cf. *macrolepis* sp. H" pending detailed taxonomic studies, and the conspecificity of *Planiliza mandapamensis* (Thomson, 1997) can be determined with specimens of either "*Planiliza* sp. K" and "*Planiliza* sp. D".

The cosmopolitan *Mugil cephalus*, which has been shown to be a species complex [14,64,77,78], is one of the most commonly listed mullet species in the Indian literature, recorded from both coasts of the region. Though the *Mugil cephalus* clade (clade C in Supplementary Figure S1) was well-resolved in the mitochondrial *COI* phylogeny, and the subsequent ABGD, RESL, and ASAP analysis delineated at least five groups despite limited sampling of this species complex (in Supplementary Figure S1). We consider these as "putative species", with two of them represented in Indian waters. The first putative species has a distribution on the eastern and western coasts of India where a name, *Mugil cephalotus* Valenciennes, 1836 (type locality: Malabar and Pondicherry, India), is already available. *Mugil cephalotus* is currently considered to be a synonym of *M. cephalus*, and has been described as coming from erstwhile provinces of Malabar (west coast/Arabian sea) and Pondicherry (east coast/Bay of Bengal) in southern peninsular India [20]. The presence of a genetically distinct species within the genus *Mugil* from both the southwestern and southeastern coasts of India necessitates the need for a separate name. The name *M. cephalotus* can be tentatively used to represent this genetically distinct lineage, as it is the oldest available name for a species aligned to *M. cephalus* from southern India. The revalidation of this name should, however, be carried out after detailed morphological examination using topotypic specimens and available syntypes, representative of a larger geographic sampling to determine the exact distribution range of this species.

The sequence FJ384686, identified as *Mugil cephalus* in GenBank, is also part of this group, but the location provided (11.48° N and 76.76° E) points to the Nilgiri hills in the Western Ghats Mountain range, at an altitude of >2000 m above sea level. It is highly unlikely that a species of the *Mugil cephalus* complex occurs at such a high altitude, and the location and coordinates provided by the authors could therefore be erroneous. The second putative species in the *Mugil cephalus* complex occurs in the Vellar Estuary, on the

southeastern coast of India. This lineage, which was designated as "*Mugil* sp. I" in Durand and Borsa [11], includes specimens from eastern Asia (e.g., Vietnam, China, Taiwan, and Japan). This fish (i.e., "*Mugil* sp. I") has also been observed in the markets of Bangladesh (E. Hasan, pers. comm.). The presence of this species in the natural waters of India is doubtful, and could likely correspond to fish imports from countries such as China [76] where "*Mugil* sp. I" (or Northwest Pacific 2 sensu Shen et al., 2011) is largely reared in aquaculture for the production of bottarga (a local delicacy made from the roe) [79].

Although mullets have been recorded in checklists and other compilations from major estuaries on the eastern and western coasts of India, the available sequences in GenBank mainly originate from three major areas: the Vellar Estuary in Tamil Nadu (18 sequences), the Bharuch estuary in Gujarat (8 sequences), and the Cochin Backwaters (10 sequences). Despite adding 78 new sequences from the coastal and estuarine waters of the southwestern coast of India as part of this study, there remain many geographical areas that are known for their mullet diversity (e.g., Chilika Lake in Odisha, Pulicat Lake and Mandapam backwaters in Tamil Nadu, and the Zuari and Mandovi estuaries in Goa) where intensive surveys complemented by barcoding will be required in the future. This is in addition to the need for integrative taxonomic studies (morphology, anatomy, and genetics) for genera such as *Planiliza, Osteomugil*, and the *Mugil cephalus* species complex, to identify their true diversity and fine-scale distribution.

5. Conclusions

Our results, based on a comprehensive mitochondrial phylogeny and species delimitation of 238 *COI* sequences, confirmed the presence of 21 species of mullets within eight genera in India. This includes one species each under the genera *Rhinomugil, Minimugil, Plicomugil*, and *Ellochelon*; seven species of *Planiliza*; four species of *Crenimugil*; four species of *Osteomugil*; and two species of the *Mugil cephalus* complex. Overall, the results of our study will help improve the knowledge of the diversity and distribution of one of the most important groups of brackish-water and coastal finfish species of the Indian subcontinent.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15121193/s1, Supplementary Figure S1: Maximum likelihood phylogenetic tree of family Mugilidae from the Indo-Pacific and their species delimitation using ABGD, ASAP, and RESL; Supplementary Table S1: Details of species, locations, GenBank accession numbers, and BIN ID (BOLD) for the mullet sequences included in the phylogenetic tree presented in Supplementary Figure S1; Supplementary Table S2: Details of topotypic and other reference sequences obtained from GenBank; Supplementary Table S3: Discrepancies observed in morphological characters used in previous studies on Indian mullets. Refs. [80–85] are cited in SM file.

Author Contributions: R.R. (Rahul Rajan) and R.R. (Rajeev Raghavan) conceived the study. R.R. (Rahul Rajan), B.X. and M.A.U.R. undertook field surveys, carried out morphological examinations, and contributed to laboratory work. J.-D.D., L.T. and A.S. carried out the laboratory work. J.-D.D., A.S., R.R. (Rajeev Raghavan), R.R. (Rahul Rajan) and B.X. performed the phylogenetic analysis. M.A.U.R. and J.-D.D. contributed sequences and other resources. R.R. (Rahul Rajan), J.-D.D. and R.R. (Rajeev Raghavan) co-wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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