“DNA Barcoding Of Fishes Under The Family Pomacentridae From Lakshadweep Island, Arabian Sea India”

DISSERTATION SUBMITTED TO ST. TERESA'S COLLEGE, ERNAKULAM IN PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR THE AWARD OF

DEGREE OF MASTER OF SCIENCE IN ZOOLOGY

SUBMITTED BY,

SWETHA K S

REG. NO.: SM21ZOO015

DEPARTMENT OF ZOOLOGY

ST TERESA'S COLLEGE (AUTONOMOUS), ERNAKULAM

KOCHI-682011

2021-2023

CERTIFICATE

This is to certify that the dissertation entitled **"DNA Barcoding Of Fishes Under The Family Pomacentridae From Lakshadweep Island, Arabian Sea India"** is an authentic record of original project work carried out by **"SWETHA K S" (Reg. No.:SM21ZOO015)**, during the academic year **2021-2023**, under the external guidance of Dr. S Sureshkumar, Deen FOSC, Kerala University of Fisheries and Oceanographic Studies,Panangad, Kochi and the internal guidance of Dr. Helvin Vincent, Assistant Professor, Department of Zoology, St. Teresa's College, for the partial fulfillment of the requirement of the Degree of Masters of Science in Zoology from St. Teresa's College, Ernakulam.

Dr. Helvin Vincent

Assistant Professor

St. Teresa's College (Autonomous)

Ernakulam

Dr. Soja Louis

Head of zoology department

St. Teresa's College

(Autonomous)

Ernakulam

Place : ERNAKULAM

Date : 17/05/23

External examiners

1. Dr. Smitha S. Sacred Heart College

2. 17/05/23 Mrs. Indu Vasudevan.

ST. TERESA'S COLLEGE ERNAKULAM DEPARTMENT OF ZOOLOGY

KERALA UNIVERSITY OF FISHERIES AND OCEAN STUDIES
കേരള ഫിഷറീസ്-സമുദ്രപഠന സർവ്വകലാശാല
PANANGAD P.O., KOCHI 682 506, KERALA, INDIA

☎0484- 2703782, Fax: 91-484-2700337; e-mail: ulypanangad@kufos.ac.in, registrar@kufos.ac.in website: www.kufos.ac.in

Prof. S. Suresh Kumar, PhD.
Dean, Faculty of Ocean Science and Technology

11.01.2023

CERTIFICATE

This is to certify that the dissertation entitled **"DNA Barcoding of fishes under the Family Pomacentridae from Lakshadweep Islands, Arabian Sea, India"** is a bonafide record of the original research work done by **Ms. Swetha K.S**, St. Teresa's College, Ernakulam in partial fulfilment of the requirement for the award of Degree of Master of Science in Zoology. The work was done under my direct supervision and guidance, also reported work has not formed the basis for the award of any other Degree/Diploma/Associateship/ Fellowship or another similar title to any candidate of any University.

The work was conducted at the Kerala University of Fisheries and Ocean Studies under the Faculty of Ocean Science and Technology. This work is carried out under the SSR scheme of the DST-SERB- CORE Project CRG/2020/004498. The work mentioned in the project is the sole property of the Principal Investigator, and the work cannot be published in any journals without the written consent of the supervising guide.

Dr. S. Suresh Kumar

Prof. S. SURESH KUMAR Ph.D.
Dean
Faculty of Ocean Science and Technology
Kerala University of Fisheries and Ocean Studies
Panangad, Kochi 682 506, Kerala, India

ST.TERESA'S COLLEGE (AUTONOMOUS) ERNAKULAM

Certificate of Plagiarism Check for Thesis

Author Name	Swetha. K. S
Course of Study	MSc. Zoology
Name of Guide	Dr. Helvin Vincent
Department	Post Graduate Zoology
Acceptable Maximum Limit	20%
Submitted By	library@teresas.ac.in
Paper Title	DNA Barcoding of fishes under the family Pomacentridae from Lakshadweep island, Arabian sea INDIA
Similarity	0%
Paper ID	719731
Submission Date	2023-04-05 16:06:46

Signature of Student

Signature of Guide

Checked By
College Librarian

ST.TERESA'S COLLEGE LIBRARY ERNAKULAM

* This report has been generated by DrillBit Anti-Plagiarism Software

DECLARATION

I hereby declare that this dissertation entitled " **DNA Barcoding Of Fishes Under The Family Pomacentridae From Lakshadweep Island, Arabian Sea India**" submitted to Mahatma Gandhi University, Kottayam in the partial fulfillment for the award of Master of Science in Zoology, is a record of original project work done by me, and no part thereof has been submitted to any other course. To the best of my knowledge, this project does not include any content that has been previously published or written by someone else, unless proper acknowledgement has been given to the original source.

SWETHA.K.S

ACKNOWLEDGEMENT

Foremost, I am grateful to god almighty for the opportunity to pursue this endeavor, for the guidance and wisdom that I believe will come from a higher power, and for the strength, perseverance, peace of my mind and good health in order to finish this dissertation.

I owe my deep gratitude to my Dr. Helvin Vincent, Assistant Professor,Department of Zoology, St. Teresas College (Autonomous), Ernakulam, my internal guide having taken a keen interest in my research and guiding me along to build a good project work, by providing all the necessary information, the best available resources and moreover for supporting me throughout the study.

I would like to extend my gratitude towards Dr. S Sureshkumar, Deen FOSC, Kerala University of Fisheries and Oceanographic Studies, Panangad, Kochi, my external guide for allowing me to be part of the project work and his immense support and guidance throughout the project work. I would like to express my sincere thanks to Ms. Nayana Narayanankutty, Research fellow,and Ms. Minu Thomas, project assistant of the School of ocean science and Technology,KUFOS for their continuous support and efforts, patience, motivation, enthusiasm, andimmense knowledge. Their guidance helped a lot in completion of my project work. Also thank the rest of the KUFOS faculty, the facilitators and the institution for providing all the necessary requirements to make the project a success.

Beside my mentors, I would like to express my heartfelt gratitude to Dr. Soja Louis, Head of the Department of Zoology, St. Teresa's College (Autonomous), Ernakulum, for her constant support, guidance and inspiration to complete my dissertation.

I profusely thank Mrs. Indu Vasudevan , Dr. Damaris Benny and Mrs.Gayathri.R for their valuable reinforcement and suggestion throughout. Also, I extend my gratitude to my parents for the moral and emotional support.

Last but not the least, my profound thanks to all my friends and non- teaching staffs of St. Teresa's College for the heartening support to complete my dissertation.

SWETHA.K.S

LIST OF ABBREVIATIONS

%	:	Percentage
&	:	And
°C	:	Degree Celsius
g	:	Gram
hrs	:	Hours
min	:	Minutes
mL	:	Milliliter
Mm	:	Millimolar
DNA	:	Deoxyribo nucleic acid
PCR	:	Polymerase Chain Reaction
pH	:	Potential of Hydrogen
µl	:	Microliter
S	:	Seconds
COI gene	:	Cytochrome oxidase subunit 1
spp	:	Species
rpm	:	Revolutions per minute
NJ	:	Neighbor-joining
ML	:	Maximum likelihood

LIST OF FIGURES

CONTENTS

ABSTRACT

The Pomacentridae, or damselfish, is a significant family of marine reef fishes living in various biological niches in the world's oceans. In coral reefs, damselfish exhibit vast biodiversity. To better understand such diversity, morphological and molecular studies of damselfish have been studied in sixteen different species of Pomacentridae family collected from Kadmat, Kavaratti, Amini, Suheli, Agatti and Parali islands of Lakshadweep.

From the morphological and phylogenic study of *Pomacentrus pavo, Dascyllus aruanus, Chromis weberi, Chromis viridis, Chromis dimidiata, Abudefduf sordidus, Abudefduf vaigiensis, Dascyllus carneus, Dascyllus trimaculatus, Plectroglyphidodon dickii, Stegastus nigricans, Amblyglyphidodon leucogaster, Chrysiptera caeruleolineata, Chrysiptera glauca, Amphiprion clarkii*, and *Amphiprion nigripes,* it was observed all 16 species show high variability in their body size, shape, and color and even though some belong to the same genus they completely differed morphologically from each other. DNA barcoding of 16 species of Pomacentridae family has done for species identification and phylogenetic study

From the study, it was observed that the Family Pomacentridae has four subfamilies Chrominae, Glyphisodontinae, Pomacentrinae, and Microspathodontinae. Phylogenetic interpretation of three subfamily Chrominae, Glyphisodontinae, and Pomacentrinae were examined and based on the analysis it was observed that genus Abudefduf remained as a separate clade which shows that they belongs to a separate subfamily Glyphisodontinae. Our observations contradicts the earlier classification of Nelson *et al* (2006)which states that genus Abudefduf belong to the Pomacentrinae subfamily and validates the recent work of Cooper *et al.* (2021).

INTRODUCTION

The oceans and seas, which make up about 71 percent of the planet's surface, are the most significant and dominant natural phenomena. This environmental element, with its enormous size and sheer volume, is extremely significant to humanity and provides a reliable source of income for coastal communities and fishers (Radhakrishnan *et al*., 1998). Yet, little is known about the biological variety, abundance, and distribution of marine creatures or the structure and function of the marine ecosystem as a whole, with the exception of hotspots including coastal wetlands, mangroves, and coral reefs. The most endangered marine species are corals, sponges, oysters, octopus, porpoises, whales, ornamental fish, sea turtles, and dugongs. Because they are maritime, India's Lakshadweep Islands are home to significant marine vegetation and fauna. (Tripathy, 2002)

In the Arabian Sea, between 08° 00' and 12° 30' N latitude and 71° 00' and 74° 00' E longitude, is the Indian archipelago of Lakshadweep. It consists of six islands, twelve atolls, five underwater banks, and three reefs, eleven of which are inhabited (Sinha, 1994). Kavaratti, Agatti, Amini, Kadmat, Kiltan, Chetlat, Bitra, Androth, Kalpeni, Bangaram, and Minicoy are the inhabited islands and atolls. As a result of these islands' isolation from the mainland, less research has been done on their fish species than has been done on India's main land.

Corals are the core species of coral reef ecosystems and the main forces behind reef formation and expansion. They also make up the majority of the structural habitat (Jones *et al*., 1994; Reaka Kudla, 1997). The most prominent of these are coral reef fishes, a remarkably diverse collection of species characterised by their close relationship with coral reefs.

Marine ornamental fish species are found in India's Gulf of Munnar/Palk Bay, Gulf of Kutch along the mainland coast, and the reefs surrounding the Andaman & Nicobar and Lakshadweep Islands. 400 marine ornamental fishes from 175 species were reported to be from India (Krishnan, 2008). Normal habitats for marine ornamental fish include rocky, coral, seaweed, seagrass, and debris areas. Due to the abundance of these habitats, the lagoons of Lakshadweep are home to one of the Indo-Pacific region's most diverse populations of ornamental fish. The fishes from the Lakshadweep Islands were not well recognised despite the fact that there are several publications devoted to the marine fishes of India, the other nations bordering the Arabian Sea and the Western Indian Ocean.

DNA barcoding promises to resolve issues and disputes around taxon identification and biodiversity assessment that are arising as a result of many cutting-edge scientific methodologies such as morphology taxonomy analysis and sister species identification, among others (Blaxter, 2003).

Pomacentridae, a rich family of reef fishes with many species, are most prevalent on the coral reefs. There are currently about 400 recognised damselfish species, which are grouped into the Pomacentridae family and distributed among 29 genera (Fricke *et al*., 2020). New species are continuously being described. Despite the fact that the majority of damselfishes are omnivorous and consume a range of plankton, benthic algae, and benthic invertebrates, their diets and trophic strategies are very diverse.

Pomacentrids exhibit a diversity of mating behaviours, reproductive techniques, and social structures. Pomacentrids exhibit various forms of parental care (such as nest building, egg guarding, and brooding) by either the male alone or both parents, depending on the species. Although all pomacentrids are oviparous, their adhesive eggs are often laid on the substrate. Like many marine fishes, the larvae of almost all damselfish species go through a dispersive, pelagic phase. This pelagic step is completely absent in the species of Acanthochromis and Altrichthys, which makes them unique among pomacentrids Because of that life history, they are also the only damselfishes to exhibit brooding behavior, where the parents care for and defend the young through the recruitment phase.

Among damselfishes, the anemonefishes possess the most well-known life histories. All anemone fishes share an obligate symbiotic relationship with sea anemones. This unique life history likely contributed to their distinctive reproductive biology and social structure: anemonefishes are protandrous serial hermaphrodites that form long-term monogamous pairs

Pomacentridae family has been subdivided into four subfamilies Amphiprioninae, Chrominae, Lepidozyginae, and Pomacentrina to produce the final recognised classification (Allen, 1975a: 34). Based on the evolutionary links identified by Cooper *et al*., the Pomacentridae family is divided recently into the Chrominae, Glyphisodontinae, Microspathodontinae, and Pomacentrinae subfamilies (2021).

The present study is an attempt to analyse morphological characters of sixteen species from the Pomacentridae family. Further molecular understanding is analysed using DNA Bar coding of COI gene of mitochondrial DNA of the Pomacentridae family and also phylogenetic

interpretation of three subfamilies Chrominae, Glyphisodontinae and Pomacentrinae has also been studied.

Aim and Objectives

AIM

The present study is to generate the DNA Barcoding of fishes under the family Pomacentridae from Lakshadweep island, Arabian sea INDIA

Objectives

- Morphological Analysis of family Pomacentridae
- DNA Bar-coding of family Pomacentridae
- Phylogenic interpretation of three subfamily Chrominae, Pomacentrinae and Glyphisodontinae under family pomacentrida

Relevance of the work

Corals are the foundation species of coral reef ecosystems, forming the predominant structural habitat and are the foremost contributors to reef development and growth. The most prominent of these are coral reef fishes, a remarkably diverse collection of species characterised by their strong relations to coral reefs. Coral reef communities around the world have been in decline since humans began to exploit them, the degradation of reefs has rapidly accelerated over the past several decades. This crisis has made the development of new and more effective approaches to coral reef conservation a high priority .The sensitive coral habitat of Lakshadweep is deteriorating as a result of both natural and artificial causes. On these islands, immediate conservation measures must be implemented in order to protect and preserve these habitats for scientific, cultural, and commercial purposes. Since there are few molecular-level research in the Lakshadweep islands, various misidentification problems arise. Molecular-level research is essential for resolving each of these challenges The present study attempts to identify and conserve the marine biodiversity of Lakshadweep island..

REVIEW OF LITERATURE

Reliable baseline estimations of fish species diversity based on precise species identification are necessary for conserving and managing fish biodiversity. Fish species are typically identified based on morphology (Dayrat, 2005; Triantafyllidis *et al*., 2011). Because that fish shape varies and frequently changes concurrently with the developmental stage, morphological identification demands much experience (Leis & Carson-Ewart, 2000; Wang *et al*., 2018). DNA barcoding is more trustworthy for identifying species (Hebert *et al*., 2003; Hebert & Gregory, 2005). The taxonomic study took on a new dimension using molecular techniques and quantitative morphometric comparisons (Vogler & Monaghan, 2007).

MtDNA loci that are known to differ between species. The hyper-variable displacement loop (D-Loop) and cytochrome b (cyt b) genes are most widely applied in the identification of species, while other genes have also been successful Prieto *et al* (2003). Compared to nuclear DNA, mitochondrial genes have a higher copy number, which makes it possible to extract more mtDNA from trace samples (Carracedo *et al* (2000). Moreover, mtDNA genes often do not undergo recombination, which encourages the loss or fixation of mtDNA haplotypes. This decreases within-species diversity and makes it possible to identify different species Savolainen *et al* (2005). The marker has been used to identify different fish, bird, insect, and primate species. Because of the growing demand for COI data, it has evolved into an important taxonomic identification tool. (Altschul *et al.*, 1997).

Studies on morphology support a limited molecular identification system (Will & Rubinoff, 2004). To facilitate quick species identification, molecular and morphological taxonomists should collaborate (Summerbell *et al*, 2005). Instead of species determination needing more research, DNA barcodes may offer helpful information about taxa (Krishnamurthy & Francis, 2012). The claim is that DNA barcoding can provide valuable taxonomic data but must be utilized cautiously to prevent it from being applied inappropriately (Pilgrim *et al*, 2011).

However, this methodology should be used in conjunction with other techniques for successful conservation efforts. DNA barcoding is an increasingly popular new concept that has inspired confidence in improving biodiversity estimates of the effectiveness of COI in identifying links between taxa. Moreover, in general, it establishes that the information capacity of COI is sufficient to enable the placement of organisms in the deepest taxonomic ranks; COI-based

identification systems can also facilitate the initial delineation of species. For species- and family-level analyses, other mtDNA, Cytb, and ND2 gene sequences are employed (Johns & Avise, 1998; Kartavtsev & Lee, 2006; Naylor *et al*., 2012)

Adopting a common COI threshold to direct species determination is interesting in circumstances where a past taxonomic study has been sparse. Nonetheless, testing this method by figuring out the crucial points that separate species from other taxonomic and geographic categories. (Naylor *et al*., 2012) For groups with different features, such as generation length or dispersal regime, that are likely to change the rate of molecular evolution or the degree of population subdivision, thresholds must be determined specifically (Hebert *et al.* 2003). Also, since COI sequences from museum specimens may be retrieved without destroying them, it will be possible for groups that do not currently have the authority to regain taxonomic competency (Hebert *et al*., 2003).

Examining the levels of sequence divergence within and between species and establishing a threshold of divergence for species designations are the strategies promoted by Hebert *et al.* (2003). In some circumstances, the ranges will overlap, making some divergence values uncertain. Ranges of sequence variation vary among taxa, as mentioned by Hebert *et al.* (2003b); hence thresholds should be estimated independently for each taxonomic group.

DNA Barcoding can result in the discovery of new species and the identification of existing ones.(Hebert *et al,*2004) This has happened due to the collecting of barcode sequences; occasionally, sequences from specimens of the same nominal species are discovered to be as diverse as those from recognised species pairs (Hebert *et al.*, 2004; Holland *et al.*, 2004).

Fishes are among the easiest animals to produce DNA barcode data because they are the largest and most diversified vertebrates. More than half of the recognised extant vertebrates in the world are represented by them (Nelson, 2006). Around 30,000 species are thought to exist, with 300 new species being described annually (Eschmeyer *et al*., 2010; Fricke & Eschmeyer, 2010).

The current increase in fish species can be ascribed to more expeditions and investigations in deeper waters and newer locations, the use of molecular taxonomy, and increased awareness of the value of biodiversity and its cataloguing. Several geographic areas have carried out fish DNA barcoding initiatives, most of which are approaching complete taxonomic coverage in marine, freshwater, or very biodiverse environments (Aquilino *et al.*, 2011).

Marine fish are vertebrates. Some individuals have cartilage-filled vertebrae, whereas others have bone vertebrae. About 15,000 of the 24,000 recognised species of fish are marine species. FISH-BOL and SHARKBOL are the two primary global barcoding initiatives for fish.(Stienke *et al*,2009a,b)) Additionally, it offers a search engine that returns the status of any taxon or species' barcoding, a map of where to gather any species' barcodes, a global fish checklist (derived from FishBase), several quick news items, and links to other pertinent databases (Holmes *et al.*, 2009). Not only is DNA barcoding helpful for identifying whole fish, but it can also be used to identify larvae, eggs, fillets, fins, and other body pieces that are challenging to identify based just on morphology.

Coral reefs are the most diverse of marine communities and many of their species remain undescribed. One of the most conspicuous species in a coral reef's ecosystem is fish. Coral reef fishes stand out for their small breeding pools, isolated populations, stationary, non-migratory lifestyles, and associations with particular ecosystems. Coral reef areas provide a range of habitats, some of which may be rich or low in species within or across habitats, supporting distinctly diverse fish populations, some of which may be cosmopolitan. Other variables that are known to restrict the dispersion of reef fish include dependence on a certain food source, behavioural interactions, and depths across reefs,Vijay anandh (1994)

The interest in coral reef fishes peaked in the 1960s and 1970s, according to (Sale 1991a), who published an important review on the ecology of fishes on coral reefs. Publications on coral reef fishes originate from the middle and late 1950s and 1970. Since then, Ehrlich (1975), Goldman and Talbot (1976), Sale (1980 a.), and Doherty and Williams (1988) all published noteworthy critiques. Admittedly, India has yet to make much progress in this area. The most thorough description of reef fishes was provided by Jones and Kumaran (1980), who described 503 fish species from the Lacadive Archipelago. Day (1878), Herre (1906), and others provided significant descriptions of reef fishes that reside in other coral reef habitats. Evidence suggests that many more reef species have yet to receive appropriate descriptions.

Many fish species have been discovered in India's various coral reef environments. In contrast to most marine fish, the fish that live in coral reef zones have very distinct features, especially in terms of color pattern and habitat preferences. Additionally, specific coral reef sub-habitats are known to be associated with particular distinct assemblages of fish species (de Boer: 1978: Luckhurst, 1978a; Bell and Galzin, 1984: Bouchon-Navara *et al*., 1985: Roberts and Ormond, 1987: Sale. 1991). There is not a complete list of fish species that live in India's various coral

reef environments, even though many species have been described by Day (1878), Herre (1338), Jones and Kumaran (1980), Talwar and Kacker (1984), and others.

The Lakshadweep Islands in India are home to significant marine flora and fauna. According to the history of Lakshadweep, fish surveys started by Pillai in (1981). Lakshadweep's lagoons and reef flats are rich in coral, with 104 species in 37 genera (Pillai, 1989). Perches, gar-fishes, half-beaks, scarids, goat-fishes, carangids, grey mullets, antherinids, spyraenids, polynemids, balistids, blennies, and globe-fishes are the common fish species found in the coralline niches of the lagoon (Balan, 1958; Kumaran *et al*., 1989). The Laccadive archipelago is home to 603 different species of fish, according to Jones and Kumaran (1980). Fish like seer fish, sharks, sailfish, tunnies, flying fish, carangids, and ribbon fish make up offshore fisheries. The water in the Lakshadweep is also frequently home to rays and skates. The offshore seas of Lakshadweep are home to various fish, including *Crenimugil crenilabis, Polynemus sexfilis, Naso tuberosus, Naso unicornis, Gomphousus varius, Novacutichthys taeniurus, and Anampses diadematus* (James *et al.,* 1989).

More than 131 years ago, ichthyological research in the Lakshadweep waters began. Since then, new records of fish have been continually published. In the most recent study on the new records of reef fishes in Lakshadweep, Rajan *et al.* (2021), accounted for an extra 15 new records of reef fishes among the 856 species in the catalogue. previously unknown and unregistered twelve species from lakshadweep waters were set downby Sreeram *et al,* (2022) which include koumansetta hectori, cheilo dipterus macrodon, cirrhitichthys oxycephalus, gunnellichthys monostigma, plagiotremus phenax chilomycterus reticulatus, meiacanthus smithi, plectorhinchus chaetodonoides, pterocaesio trilineata amblyglyphidodon indicus and siganus guttatus, bringing the entire known species from lakshadweep to 868

Among the fishes of Lakshadweep islands, those of ornamental fishes are exceptionally high: From the 601 marine fish species, representing 126 families, that have been recorded from these islands (Jones & Kumaran, 1980), at least 300 species, representing more than 40 families, are ornamental fish species. Murty *et al.* (1989) provided the first account of the ornamental fishes of Lakshadweep. In 1990, Vijayanand and Varghese provided some notes on these Lakshadweep fish. However, until Madanmohan *et al.* (1987) described the biology of Chromis caeruleus from Minicoy, there had never been a study on the biology of the

ornamental fishes Lakshadweep. The research on Dascyllus aruanus, Acanthurus triostegus, and Abudefduf glaucus by Pillai and Madanmohan (1987b, 1988, and 1989, respectively) came next (1990). Pillai *et al.* studied the distribution of Ctenochaetus strigosus, Chromis caeruleus, and Dascyllus aruanus (1983,1987 a, 1992). Most of the ornamental fish species examined by COI gene analysis from coral reef locales belonged to separate barcode clusters (Steinke *et al*., 2009a,b).

Family Pomacentridae, the third highest species-rich group of organisms, have existed in coral reef ecosystems for at least 50 Myr (Bellwood, 1996; Bellwood *et al*., 2015). Family on contemporary reefs, following Labridae and Gobiidae, live in tropical and temperate near-shore seas all over the world, with coral reefs hosting the majority of their species diversity. (Allen, 1991; Jones, 1992; Bellwood and Hughes, 2001; Ceccarelli *et al.*, 2001 Eagle and Jones, 2004; Alwany *et al*., 2005.

This family's diversity of trophic strategies and ecomorphological traits can be attributed to convergent radiations throughout its phylogenetic history. Closely related species' molecular phylogenetic investigations shed light on their relationships and enable verification of their morphological taxonomic classification. These radiations may have been sparked by competition, functional limitations, and the regionalisation of coral reefs (Frederich *et al.*, 2013).

Moreover, consistent rates of cladogenesis appear to be mediated by this repeated ecological diversification (predictable patterns in the evolution of phenotypic features; Losos, 2011) among clades in the Pomacentridae (Frederich *et al*., 2013). Pomacentridae has undergone transitions independently during the family's evolutionary history, retaining stable cladogenesis rates over time, albeit with considerable variance among crown lineages (Frederich *et al.*, 2013). (Cowman & Bellwood, 2011). Pieter Bleeker, who conducted research in Indonesia and South-East Asia in the 19th century (Bleeker, 1847, 1875-1878; Bleeker,1877), and Gerald Allen, who has studied damselfishes all over the world for the past three and a half decades, have produced the greatest contributions to pomacentrid taxonomy. Allen has described four genera and about one-fourth of all species known to science (e.g. Allen, 1975, 1991, 1999; Santini and Polacco, 2006). He created the Amphiprioninae, Chrominae, Lepidozyginae, and Pomacentrinae pomacentrid subfamilies in 1975 and assigned the known Indo-Pacific damselfish species to them.

Pomacentridae is a monophyletic family, and there are four subfamilies within it. A clade of Chrominae-Pomacentrinae is sister to the subfamily Glyphisodontinae. The relationships between the members of each subfamily and the composition of these clades are generally consistent with the conclusions drawn from earlier phylogenetic studies (Tang, 2001; Tang *et al*., 2004; Cooper *et al*., 2009; Cowman and Bellwood, 2011; Betancur-R. *et al*., 2013a, 2015, 2017; Frederich *et al*., 2013; Rabosky *et al*., 2013, 2018; Lobato *et al*., 2014; DiBattista *et al.*, 2016; Mirande, 2016; Gaboriau *et al*., 2018; Delrieu-Trottin *et al.*, 2019). First record of the large caerulean damselfish, pomacentrus caeruleopunctatus (actinopterygii: perciformes: pomacentridae), from reunion island, south-west indian ocean by Bourjon *et al* (2019)

Tang *et al* (2021) recent study recognize 29 genera in the Pomacentridae family , taking into account that there are nameless Chrysiptera lineages that probably need new genus-group labels and also identified Glyphisodontinae as Pomacentrinae's sibling group. They found differences in some significant ways between the connections within Pomacentrinae. Another distinction is that they identified Hoplochromis, a clade related to Chromis, as the sister group to the remainder of the Chrominae. Minor variations in where specific species or tiny clades should be placed are the main sources of disagreement. The position of Lepidozygus, which they also discovered to be the sister group of Stegastes sensustricto inside the Microspathodontinae, is the most striking resemblance in their phylogeny; this classification differs significantly from the earlier publications (e.g., Cooper *et al*., 2009; Cooper and Santini, 2016)

Materials and methods

Sample collection

The specimens were captured using a scoop net from the intertidal and lagoon area during the survey period from March 2021 to April 2021 and February 2022 to March 2022 at the Lakshadweep islands Kadmat, Suheli, Kavaratti, Amini, and Parali. Fish tissue samples were collected near the upper lateral line tissue and the pectoral fin from the right side. Tissue removed from the fish was stored at 100% alcohol for molecular analysis and kept at -4 ° C. All specimens were fixed in formalin and preserved in 5% formalin for further morphological identification. All the specimens were vouchered at the Department of Science and Technology, Lakshadweep. All specimens were identified systematically based on original descriptions, published literature, and keys (Iwatsuki *et al*., 2001; Anderson & Allen, 2001; Allen & Erdmann, 2012). Systematic characteristics were checked in WORMS, FishBase, and species nomenclature and type locality following the Catalog of Fishes (Fricke *et al*., 2022).

Fig1:Islands where fish samples collected

DNA isolation

The genomic DNA was extracted from the fish body using the DNA extraction kit Invitrogen by Thermo Fisher Scientific. The genomic DNA extract was isolated from the specimen's preserved tissue. The extraction kit contains the following chemicals:

1. Genomic Digestion buffer

2. Genomic lysis buffer

3. Proteinase K

4. RNase A

5. DNA wash buffer 1

6. DNA wash buffer 2

7. Elution buffer

PROTOCOL FOR THE DNA ISOLATION:

•The preserved tissue samples were taken out of the vial, and a portion (<25mg) of the sample was transferred to a sterile microcentrifuge tube and labelled.

• 180µL of Genomic Digestion Buffer was added to the centrifuge tube, followed by 20µL of Proteinase K.

•The mixture was vortexed (occasional vortex is done until lysis is complete with homogenising the contents and incubated in a thermomixer at 55°C for 1-4 hours until the whole tissue was completely digested.

• To remove particulate materials, the lysate was centrifuged at maximum speed for 3 minutes at room temperature, and the supernatant was transferred to a new, sterile microcentrifuge tube.

• 20 µL RNase A was added to the lysate, mixed well by brief vortexing, and incubated at room temperature for 2 min, followed by adding 200 µL Binding Buffer and well vortexed.

• 200µL of 96–100% ethanol was added to it and then mixed by vortexing for 5 seconds.

• The lysate of the centrifuge tube was then transferred into labelled spin columns in collection tubes from the package.

•The columns were placed in a balanced configuration and centrifuged for 1 minute at 7600 rpm at room temperature.

.Collection tubes were discarded, and the spin column was then placed into the new collection tube.

• 500µL of Buffer 1 prepared with ethanol was added to the column and centrifuged at room temperature at 10,000 rpm for 1 min.

• The collection tube was discarded, and the spin column was placed into the new collection tube..

• 500µL Wash Buffer 2 was added to the column tube and centrifuged at maximum speed for 3 minutes at room temperature.

•The spin column was placed in a sterile 1.5-mL microcentrifuge tube, and 30µL of Elution Buffer was added to the column.

• Incubated at room temperature for 1 minute and then centrifuged for 1 minute at maximum speed at room temperature. The tubes contained genomic DNA.

•To recover more DNA, a second elution step using the same elution buffer was performed.

• Centrifuged at 1.5 minutes at maximum speed at room temperature.

•Buffer elutes the DNA from the spin column membrane into the centrifuge tube. The

eluted DNA in the labelled centrifuge microtube was stored at -20°C.

The concentration of isolated DNA was estimated using NanoPhotometer-N60.

Amplification and sequencing

The COI gene was amplified using the PCR method.

PCR was carried out in reaction volume 25µl containing 12.5µl Emerald Amp GT PCR Master Mix (2X Premix) of TaKaRa PCR buffer -including Distilled water-9µl, Mastermix-12.5µl, Forward primer -1.25µl, Reverse primer-1.25µl, DNA-1µl. The primers used for the amplification of the COI gene were FishF1 – 5 ‘TCAACCAACCACAAAGACATTGGCAC 3‘ as forward primer and FishR1-5‘ TAGACTTCTGGGTGGCCAAA GAATCA 3‘ as reverse primer.

The thermocycling program consisted of an initial step of denaturation at 95^0C for 5 min, followed by 35 cycles of Denaturation II 95 ^{0}C for 0.45 m, annealed at 58^0C for 1.05 m, First Extension at 72^0 C for 1 min 20s, final extension at 72^0 C for 7min and finally hold and store at -4^0 C.

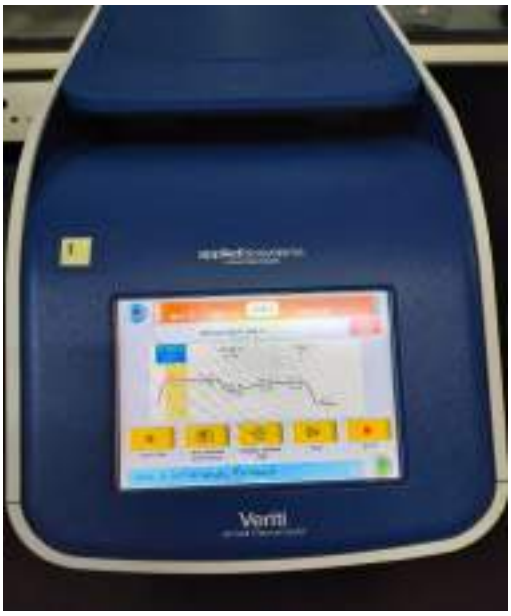
The PCR products were visualised on 0.8% agarose gels, and bands developed were observed in GelDoc using image lab software.

PCR primer was used for the conventional Dideoxy Sangers method of sequencing. Editing of the resulting sequence was performed using the peak clarities of the chromatogram. The BLAST program was performed to obtain a similar sequence through the NCBI database. The sequence was submitted using sequin to the NCBI GenBank.

Sequence alignment and phylogenetic analyses.

To prevent sequencing errors, the COI gene sequences were manually assembled, trimmed, and aligned using Bioedit. By comparing the obtained sequence results to the reference sequence using the BLAST (Basic Local Alignment Search Tool) application, which can be downloaded from https://www.ncbi.nlm.nih.gov, the sequence results were then determined. Fish COI gene sequences that were readily available from their type. The MUSCLE algorithm in MEGA X aligned the trimmed sequence and the retrieved COI sequences. Both character-based Maximum Likelihood (ML) trees and distance-based Neighbour-Joining (NJ) trees were used to create phylogenetic trees utilising complementary nucleotides.

Fig2:INVITROGEN KIT **Fig3:INSTUMENTS USED FOR DNA ISOLATION**



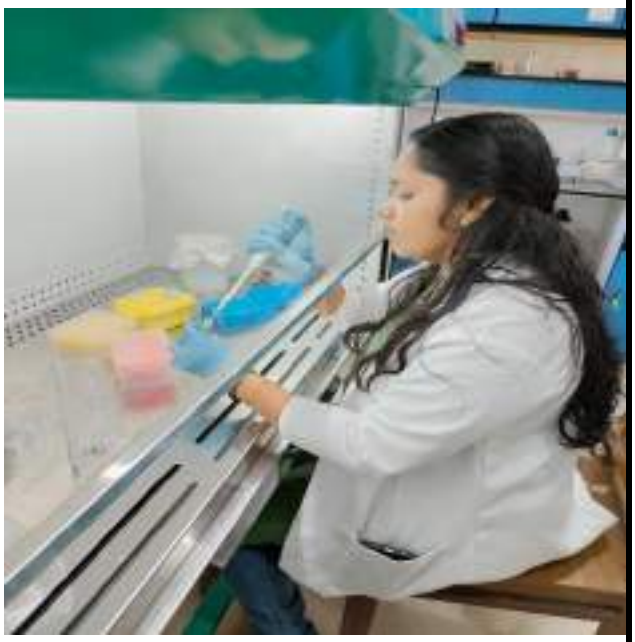


Fig4: PCR AMPLIFICATION CYCLE **Fig5:PCR COMPONENTS MIXING**

RESULT

MORPHOLOGICAL ANALYSIS

Pomacentrus pavo (Bloch, 1787)

TYPE LOCALITY:

East Indies.

COMMON NAMES

Peacock damsel, Saffier-nooientjie, Bluedamsel, Sapphire damsel, pachakkotti

SYNONYM:

Chaetodon pavo, Holocentrus diacanthus, Pomacentrus polynem, Pomacentrus furcatus, Pomacentrus suvarovensis, Pomacentrus notatus, Pomacentrus caudovittatus, Pomacentrus hainanensis

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Pomacentrus

SPECIES: Pomacentrus pavo

Distribution:

Indo-West Pacific: KwaZulu-Natal (South Africa), East Africa (Mozambique, Tanzania, Kenya), Seychelles, Comoros, Mozambique Channel, Madagascar and Mascarenes (Mauritius) east to Marshall Islands, Gilbert Islands (Kiribati), Marquesas Islands and Tuamotu Archipelago (French Polynesia), north to Kerama Islands (southern Japan), south to Western Australia, Lord Howe Island (Australia), Norfolk Island (Australia) and Tonga.

Fig6: ***Pomacentrus pavo***

MORPHOLOGY:

Dorsal spines :XII; Dorsal soft rays :13; Anal spines: II; Anal soft rays: 12.Body from metallic green to light blue. A small and usually distinct greenish 'ear' spot. Tail yellowish. Head with vermiculations blue.

VOUCHER NO:

MTRLOST-135-0721

COLLECTION DETAILS:

Specimen was collected on 3/10/2022 from Kavaratti Island, Lakshadweep

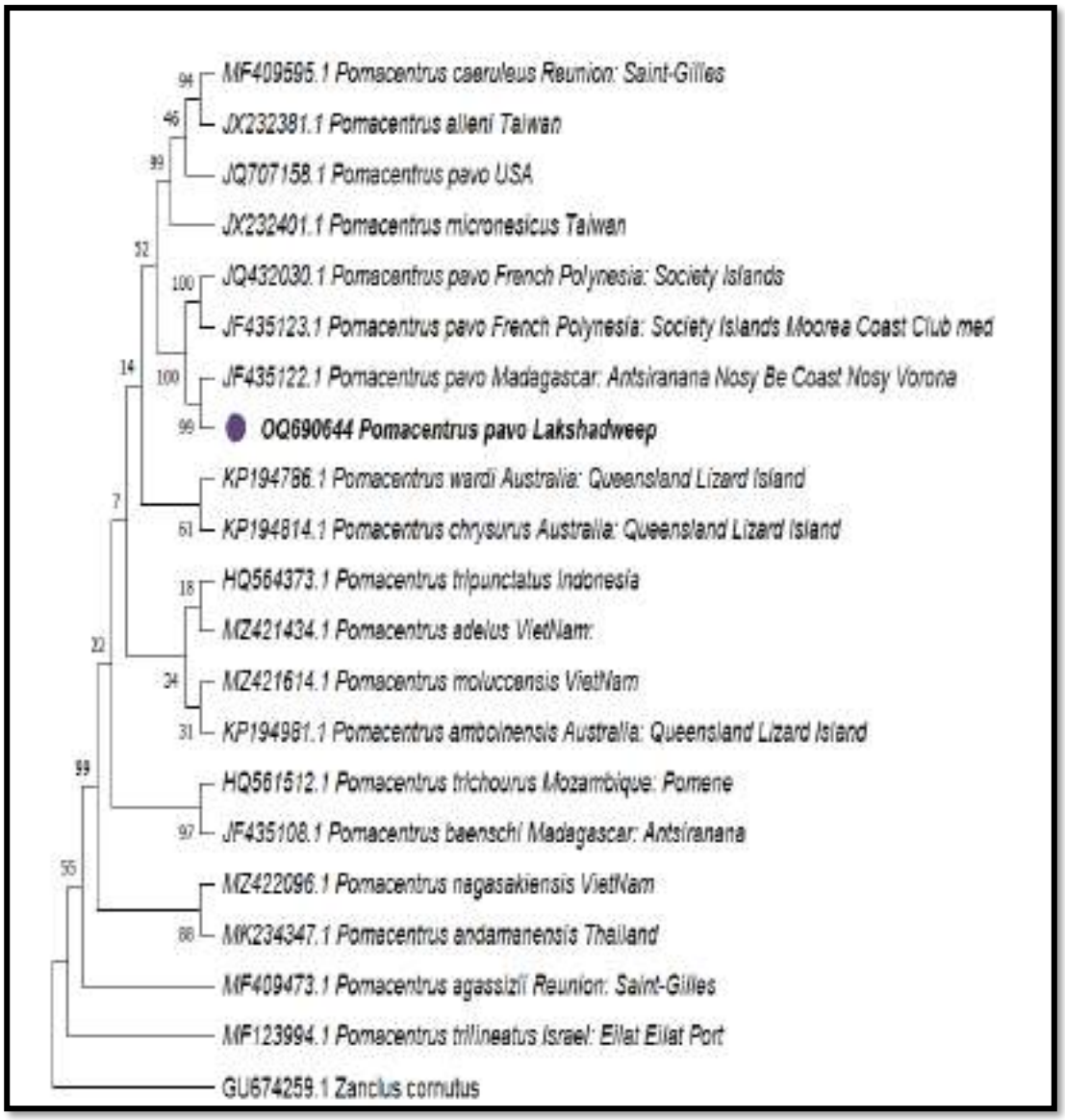


Fig7:Genus tree of Pomacentrus

The given species from the Pomacentridae family belong to genus Pomacentrus .The fish species is Pomacentrus pavo according to the phylogenic tree.The sequence from the study with accession number OQ690644 clade with sequence of fish from Madagascar with accession number JF435122.

***Stegastes nigricans* (Lacepède, 1802)**

TYPE LOCALITY:

Probably Mauritius, southwestern Indian Ocean

COMMON NAMES:

Dusky farmer fish, Blackdamsel, Swart nooientjie, Dusky Gregory, Padatham

SYNONYM:

Holocentrus nigricans, Eupomacentrus nigricans, Parapomacentrus nigricans, Pomacentrus nigricans, Pomacentrus scolopseus, Pomacentrus taeniops, Pomacentrus scolopsis, Pomacentrus subniger, Abudefduf tamaii, Pomacentrus lividus

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Stegastes

SPECIES: *Stegastes nigricans*

Distribution:

Red Sea; Indo-West Pacific: East Africa, Mozambique Channel, Seychelles, Comoros, Madagascar and Mascarenes (La Réunion, Mauritius, Rodrigues) east to Wake Atoll and Tuamotu Archipelago (French Polynesia), north to Ryukyu Islands (Japan), south to Western Australia, New Caledonia and Tonga

Fig8:*Stegastes nigricans*

MORPHOLOGY:

Dorsal spines :XII; Dorsal soft rays :15;Anal spines: II; Anal soft rays: 14.Adults generally brown; dorsal part of head and nape darker, grading to tan on lower part of head and breast. The scales have darker brown margins.The suborbital and pectoral fin includes yellow patches. The median and pelvic fins are brown; the pectorals are dusky; a well-defined dark brown or blackish spot is sometimes present at base of posteriormost dorsal rays, which distinguishes it from *S. lividus* where the spot is diffuse.

VOUCHER NO:

MTRLOST-135-0727

COLLECTION DETAILS:

Specimen was collected on 13/02/2022 from Kavaratti Island, Lakshadweep

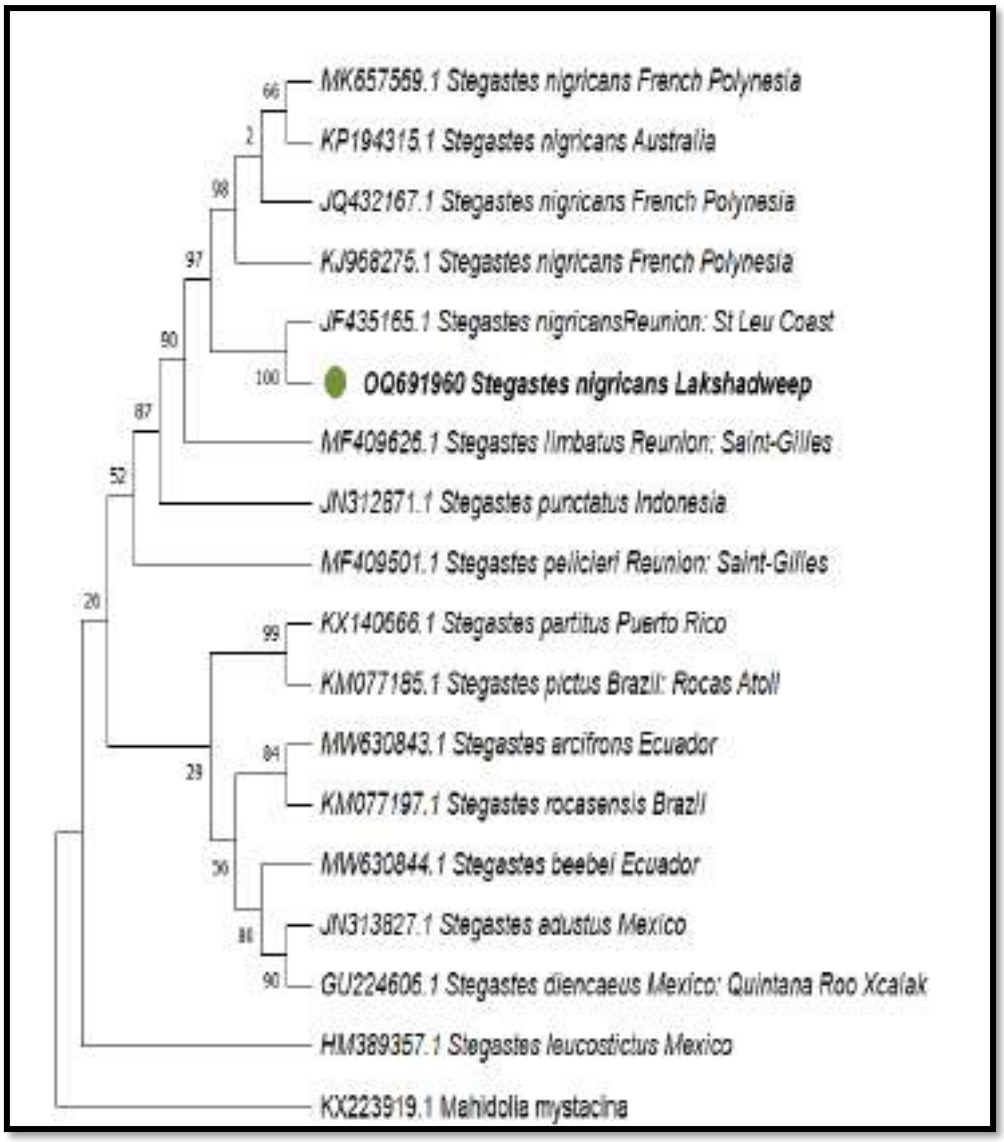


Fig9:Genus tree of Stegastes

The given species from the Pomacentridae family belong to genus Stegastes .The fish species is Stegastes nigricans according to the phylogenic tree. The sequence from the study with accession number OQ691960 clade with sequence of fish from Reunion: St Leu Coast with accession number JF435165.

Amblyglyphidodon leucogaster **(Bleeker, 1847)**

TYPE LOCALITY:

Java,Indonesia

COMMON NAMES:

Yellowbelly damselfish, Whitebelly devil, Yellowbelly devil, White-breasted sergeant, Bombin,

SYNONYM:

Abudefduf leucogaster, Amblygliphidodon leucogaster, Glyphidodon leucogaster ,Glyphisodon leucogaster, Plectroglyphidodon leucogaster

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Amblyglyphidodon

SPECIES: *Amblyglyphidodon leucogaster*

Distribution:

Indo-West Pacific: East Africa east to Marshall Islands and Samoa, north to Kagoshima Prefecture (southern Japan), south to Western Australia and Queensland (Australia).

Fig10*: Amblyglyphidodon leucogaster*

MORPHOLOGY:

Dorsal spines :XIII; Dorsal soft rays : 13; Anal spines: II; Anal soft rays: 14. This species is distinguished by the following characters: colouration, silvery grey with paler scales and yellow belly, the anterior half of anal fin and anterior margin of soft dorsal fin and the upper and lower margins of caudal fin is blackish; upper pectoral fin base with a distinct wedge-shaped black mark.

VOUCHER NO:

MTRLOST -135-0684

COLLECTION DETAILS:

Specimen was collected on 05/03/2022 from Parali Island, Lakshadweep

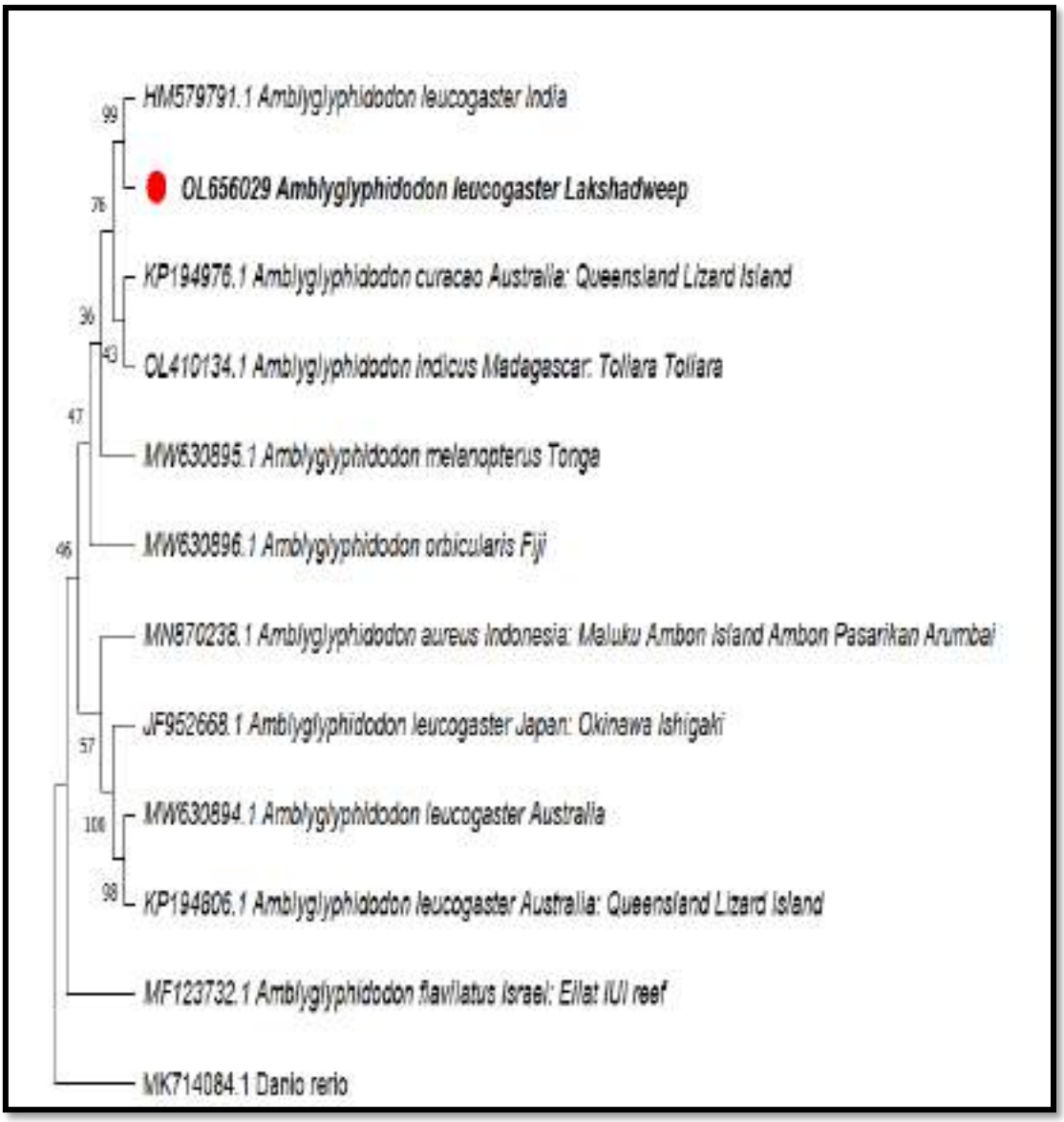


Fig11:Genus tree of Amblyglyphidodon

The given species from the Pomacentridae family belong to genus Amblyglyphidodon .The fish species is Amblyglyphidodon leucogaster according to the phylogenic tree. The sequence from the study with accession number OL656029 clade with sequence of fish from India with accession number HM579791

Plectroglyphidodon dickii **(Lienard, 1839)**

TYPE LOCALITY:

Mauritius, Mascarenes, southwestern Indian Ocean

COMMON NAMES:

Blackbar devil,Dick's damsel,Narrow bar damsel,Palata,Bombin

SYNONYM:

Abudefduf dicki, Glyphidodon dickii, Glyphidodon unifasciatus, Glyphisodon dickii, Glyphisodon unifasciatus, Paraglyphidodondickii, Plectoglyphidodon dickii,

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: *Plectroglyphidodon*

SPECIES**:** *Plectroglyphidodon dickii*

Distribution:

Indo-West Pacific: KwaZulu-Natal (South Africa), East Africa, Socotra (Yemen), Seychelles, Madagascar and Mascarenes (La Réunion, Mauritius, Rodrigues) east to Wake Atoll, Marquesas Islands and Tuamotu Archipelago (French Polynesia), north to Amami Islands (southern Japan), south to Rottnest Island (Western Australia), Noosa (Queensland, Australia), Lord Howe Island (Australia), New Caledonia and Tonga.

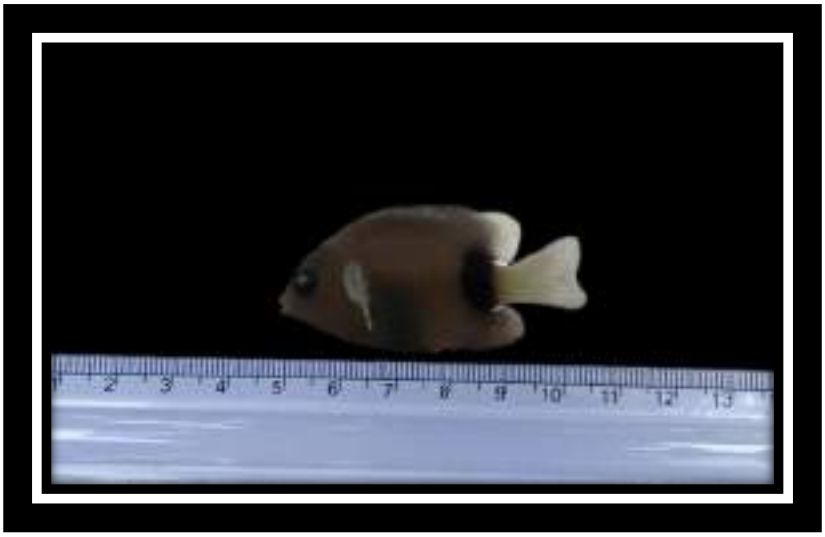


Fig12: *Plectroglyphidodon dickii*

MORPHOLOGY:

Dorsal spines : XII; Dorsal soft rays : 16; Anal spines: II; Anal soft rays: 16. Overall yellowish light brown except tail, vertical bar black broad posterior. Easily identified by the white tail. Lips swollen

VOUCHER NO:

MTRLOST -135-6713

COLLECTION DETAILS:

Specimen was collected on 13/02/2022 from Kavaratti Island, Lakshadweep

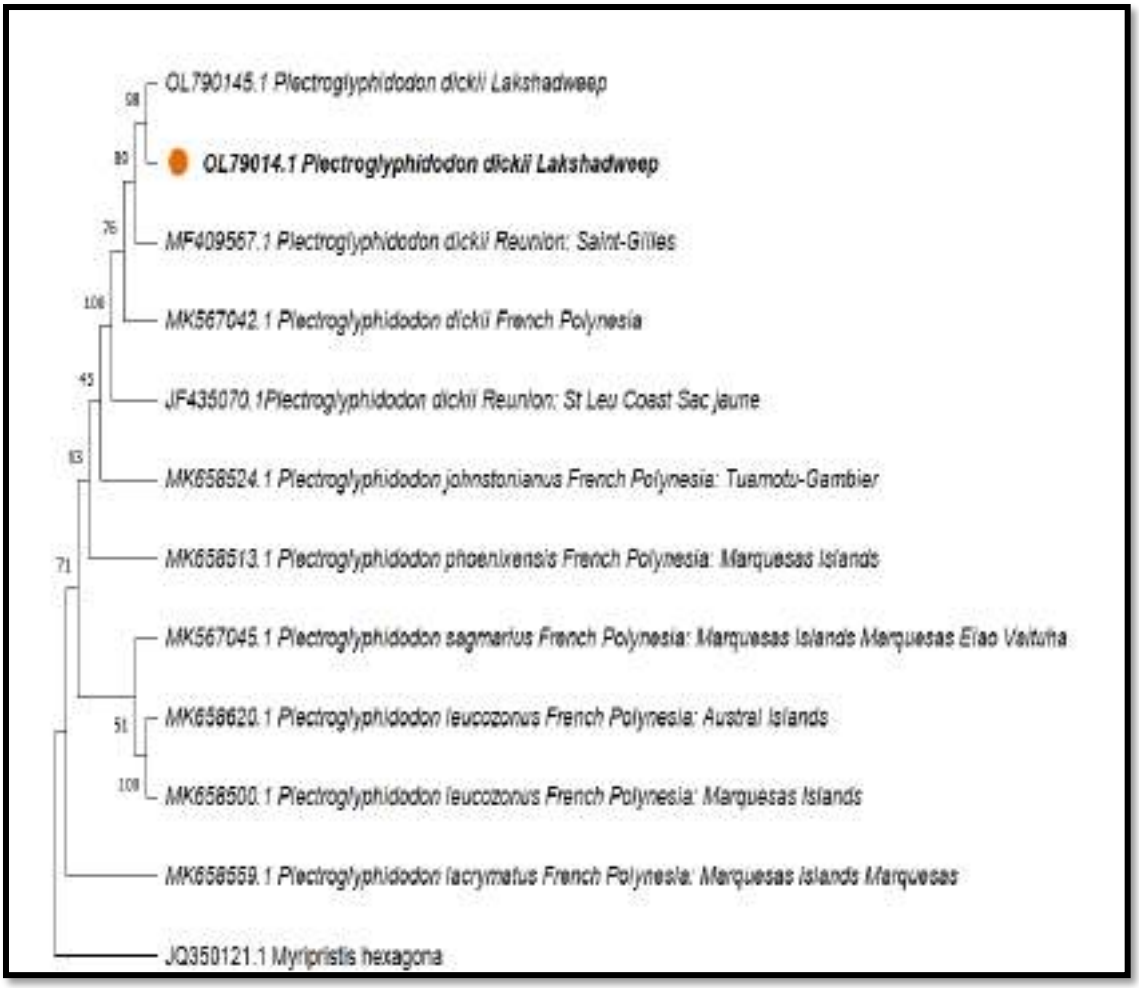


Fig 13:Genus tree of Plectroglyphidodon

The given species from the Pomacentridae family belong to genus Plectroglyphidodon .The fish species is Plectroglyphidodon dickii according to the phylogenic tree. The sequence from the study with accession number OL79014 clade with sequence of fish from Reunion:Saint Gilles with accession number MF409567

*Chromis dimidiata (*Klunzinger, 1871)

TYPE LOCALITY

Al-Qusair, Red Sea Governorate, Egypt, Red Sea

COMMON NAMES:

Chocolate dip damselfish, Bicolor damselfish, Padatham, Half and half chromis, Moong suzumedai.

SYNONYM:

PycnoChromis dimidiate, Chromis dimidiatus, Heliastes dimidiatus

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Pycnochromis

SPECIES*:* **:** *Pycnochromis dimidiatus*

Distribution:

Red Sea endemic.

Fig14: *Chromis dimidiata*

MORPHOLOGY:

Dorsal spines : XII; Dorsal soft rays : 12; Anal spines: II; Anal soft rays: 12Anterior 2/3 of the body dark brown To purple ; posterior 1/3 pearly white

VOUCHER NO:

MTRLOST -135-0690

COLLECTION DETAILS:

Specimen was collected on 09/04/2022 from Amini Island, Lakshadweep

Chromis viridis (Cuvier, 1830)

TYPE LOCALITY:

Red Sea; Guam, Mariana Islands, western Pacific

COMMON NAMES:

Blue-green puller,Palata,Green chromis,Green damsel, Blue green damselfish,Blue green chromis

SYNONYM:

Dascyllus cyanurus, Heliases frenatus, Heliases lepisurus, Pomacentrus viridis, Glyphisodon bandanensis,

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Chromis

SPECIES**:** *Chromis viridis*

Distribution:

Red Sea; Indo-West Pacific: East Africa, Seychelles, Comoros, Mozambique Channel, Madagascar and Mascarenes (La Réunion, Mauritius, Rodrigues) east to Wake Atoll, Marquesas Islands and Tuamotu Archipelago (French Polynesia), north to southern Japan, south to Western Australia, Middleton Reef, New Caledonia and Tonga

Fig 15: *Chromis viridis*

MORPHOLOGY:

Dorsal spines : XII; Dorsal soft rays : 9; Anal spines: II; Anal soft rays: 10.Body overall pale green to light blue

VOUCHER NO:

MTRLOST -135-0695

COLLECTION DETAILS:

Specimen was collected on 10/03/2021 from Suheli Island, Lakshadweep

Chromis weberi (Fowler & Bean,1928)

Type Locality:

Jakarta, Java, Indonesia

COMMON NAMES:

Weber's chromis, Darkbar chromis, Weber's puller, Kapal, Gombing-puyu Weber

SYNONYM:

Chromis simulanis, *Chromis simulans*, *Pomacentrus weberi*

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Chromis

SPECIES**:** *Chromis weberi*

Distribution:

Red Sea; Indo-West Pacific: KwaZulu-Natal (South Africa), East Africa, Persian Gulf, Socotra (Yemen), Madagascar and western Mascarenes (La Réunion) east to Line Islands (Kiribati), north to southern Japan, south to Jervis Bay (A.C.T., Australia) and New Caledonia

Fig16: *Chromis weberi*

MORPHOLOGY:

Dorsal spines : XIII; Dorsal soft rays : 11; Anal spines: 2; Anal soft rays: 12. Overall olivaceous to bluish gray, the scale edges dark brown. The edges of the opercle and preopercle are dark brown. The tips of the caudal fin lobes are black. A black spot is at the upper base and axil of the pectoral fins

VOUCHER NO:

MTRLOST -135-0696

COLLECTION DETAILS:

Specimen was collected on 25/02/2022 from Kavaratti Island, Lakshadweep

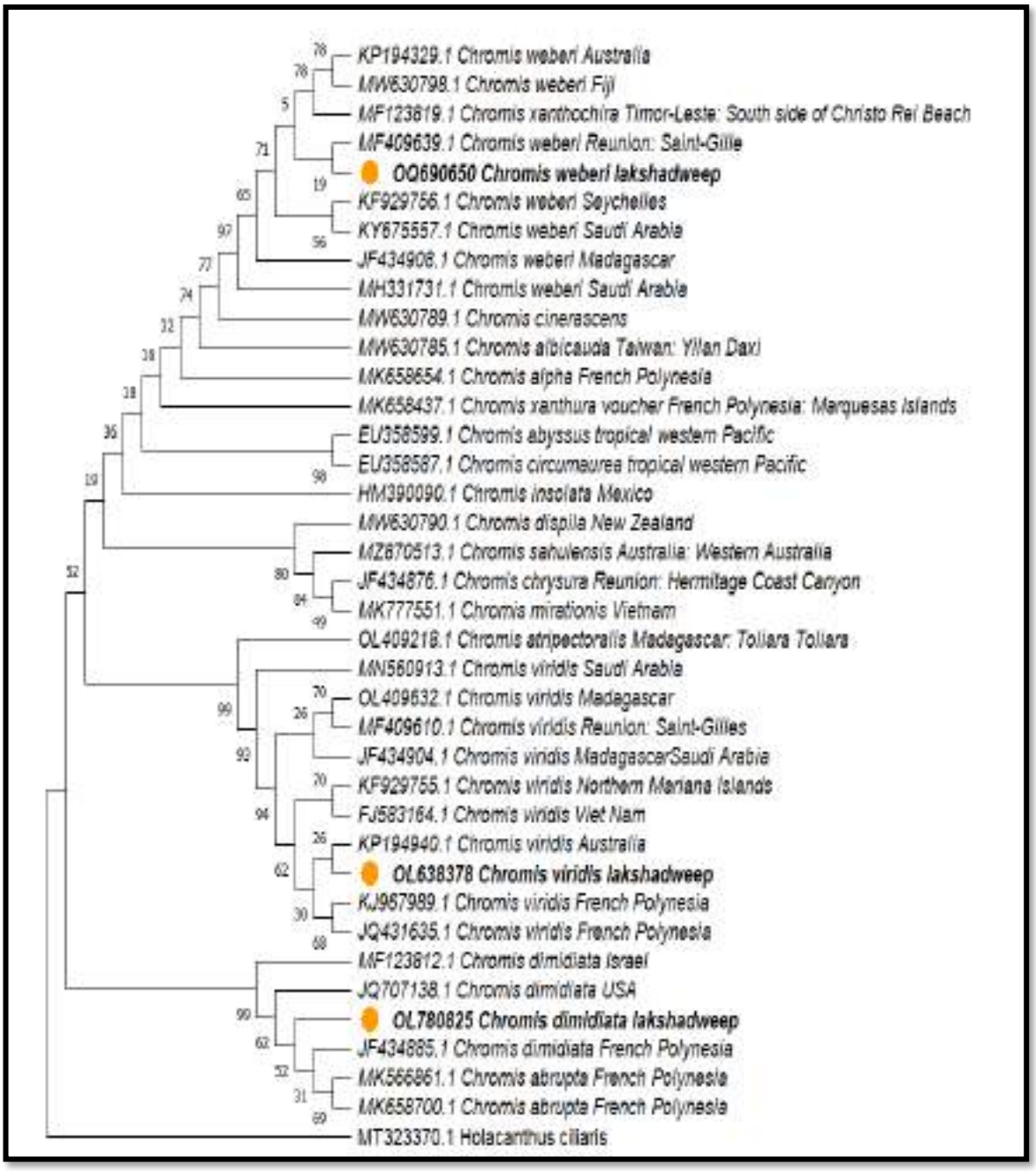


Fig17: Genus tree of *Chromis*

The given species from the Pomacentridae family belong to genus Chromis .The fish species is Chromis weberi,Chromis viridis and Chromis dimidiata according to the phylogenic tree. The sequence from the study with accession number OQ690650 clade with sequence of fish from Reunion:Saint Gilles with accession number MF409639,other sequence with accession number OL638378 clade with sequence of fish from Australia with accession number KP194940 and the last sequence with accession number OL780825 is closest to sequence of fish from USA with accession number JQ707138

***Abudefduf vaigiensis*(Quoy & Gaimard,1825)**

TYPE LOCALITY:

Pulau Waigeo, Papua Barat, Indonesia, western Pacific

COMMON NAMES:

Yellowback sergeant ,Oyabiccha**,** Keping, Kepal laut, Gombing jalur lima, Bombin, Indo-Pacific sergeant, , Palata, Kukumanali, Shabbar, Sergeant major

SYNONYM:

Abudefduf quinquelineatus, Abudefduf quinquilineatus, Abudefduf vargensis, Abudefduf caudobimaculatus ,Glyphisodon quadrifasciatus, Glyphisodon rahti, Glyphisodon vaigiensis,. Chaetodontyrwhitti.

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Abudefduf

SPECIES*: Abudefduf vaigiensis*

Distribution:

Red Sea; Indo-West Pacific: Eastern Cape and KwaZulu-Natal (South Africa), East Africa, Persian Gulf, Socotra (Yemen), Seychelles, Comoros, Madagascar and western Mascarenes (La Réunion) east to Samoa and Tonga, north to southern Korea and central Japan, south to Rottnest Island (Western Australia), Mallacoota (Victoria, Australia), and northern New Zealand; Mediterranean Sea (Red Sea immigrant); Hawaiian Islands

Fig18:*Abudefduf vaigiensis*

MORPHOLOGY:

Dorsal spines : XIII; Dorsal soft rays : 12; Anal spines: II; Anal soft rays: Body compressed, oval .11 - 13colour of body blue-green dorsally, shading to silvery white ventrally; five broad bluish black bars the first just behind the head, the narrow fifth on caudal peduncle, the third to fifth extending into the dorsal fin; dorsal part of body between the first and third dark bars often yellow; caudal fin without dark bands

VOUCHER NO:

MTRLOST -135-0683

COLLECTION DETAILS:

Specimen was collected on 10/03/2022 from Suheli Island, Lakshadweep

***Abudefduf sordidus*(Forsskal,1775)**

Type locality

Djiddae [Jeddah, Saudi Arabia, Red Sea]

COMMON NAMES:

Makhrev, Amangpang, SenMoong Sergeant, Greydamselfish, Padatham, Bombin, Karameamea, Black spot sergeant, Spotdamsel

SYNONYM:

Abudefduf tridentatus, Chaetodon sordidus, Glyphidodon adenensis, Glyphidodon leucopleura, Glyphisodon geant, Glyphisodon gigas, Glyphisodon sordidus ·

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Abudefduf

SPECIES **:** *Abudefduf sordidus*

Distribution:

Red Sea; Indo-West Pacific: astern Cape and KwaZulu-Natal (South Africa), East Africa, Socotra (Yemen), Seychelles, Comoros, Madagascar and Mascarenes (La Réunion, Mauritius, Rodrigues) east to Hawaiian Islands (U. S. A.), Wake Atoll and Pitcairn Group, north to southern Korea and central Japan, south to Rottnest Island (Western Australia), Montague Island (New South Wales, Australia), Lord Howe Island (Australia), Tonga and Rapa (French Polynesia).

Fig19 :*Abudefduf sordidus*

MORPHOLOGY:

Dorsal spines : XII; Dorsal soft rays :14:Anal spines: II; Anal soft rays: 15.Head and body white, vertical bars six grey broad , first band at predorsal to pectoral axil; the last *band* on caudal peduncle .Prominent black spot (saddle) on upper caudal peduncle**.** Caudal fin forked with round tips.

VOUCHER NO:

MTRLOST -135-0682A

MTRLOST -135-0682B

COLLECTION DETAILS:

Specimen was Collected on 13/02/2022 and 04/02/2022 from Kadmat Island and Kavaratti Island, Lakshadweep

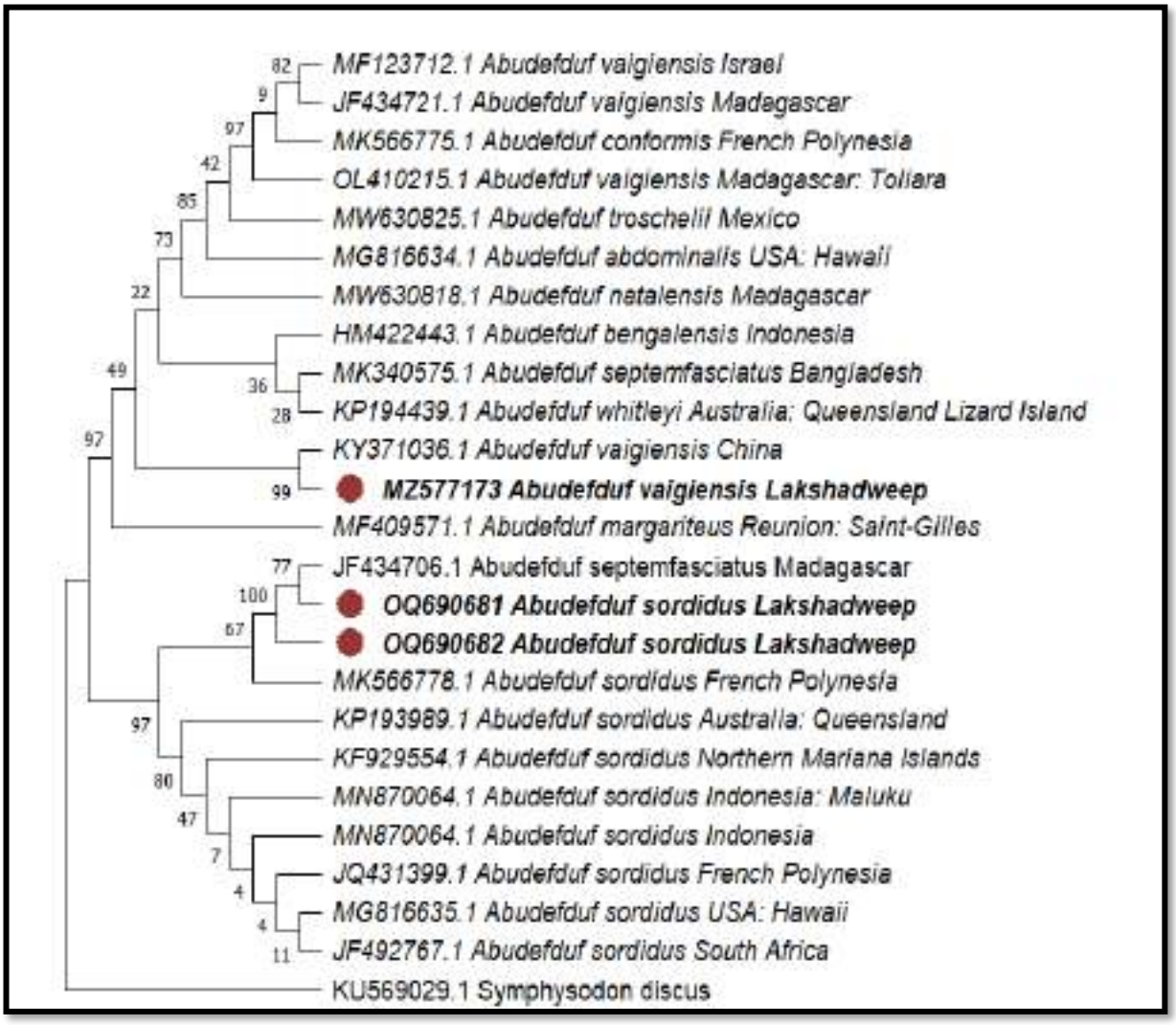


Fig20: Genus tree of Abudefduf

The given species from the Pomacentridae family belong to genus Abudefduf .The fish species is Abudefduf vaigiensis and Abudefduf sordidus according to the phylogenic tree. The sequence from the study with accession number MZ577173 clade with sequence of fish from China with accession number KY371036,other sequence with accession numbers OQ690681 and OQ690682 is closest to sequence of fish from Madagascar with accession number JF434706

***Dascyllus aruanus* (Linnaeus, 1758)**

TYPE LOCALITY:

Aru Islands, Molucca Islands, Indonesia

COMMON NAMES:

Black and white damselfish,Zebra humbug,sebra-humbug,Three striped damsel,Humbug dascyllus,Banded humbug,Gombing belang,Kallikkotti

SYNONYM:

Abudefduf caroli ,Chaetodon arcuanus ,Chaetodon aruanus Dascyllus arnanus,Dascyllus blochii Pomacentrus devisi Pomacentrus emamo ,Tetradrachmum arcuatum ,Tetradrachmum aruanum ,Pomacentrus trifasciatus

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Dascyllus

SPECIES**:** *Dascyllus aruanus*

Distribution:

Eastern Indian Ocean, western Pacific: Indonesia east to Wake Atoll, Marquesas Islands and Gambier Islands (French Polynesia), north to southern Japan, south to Western Australia, Lord Howe Island (Australia), New Caledonia and Rapa (French Polynesia).

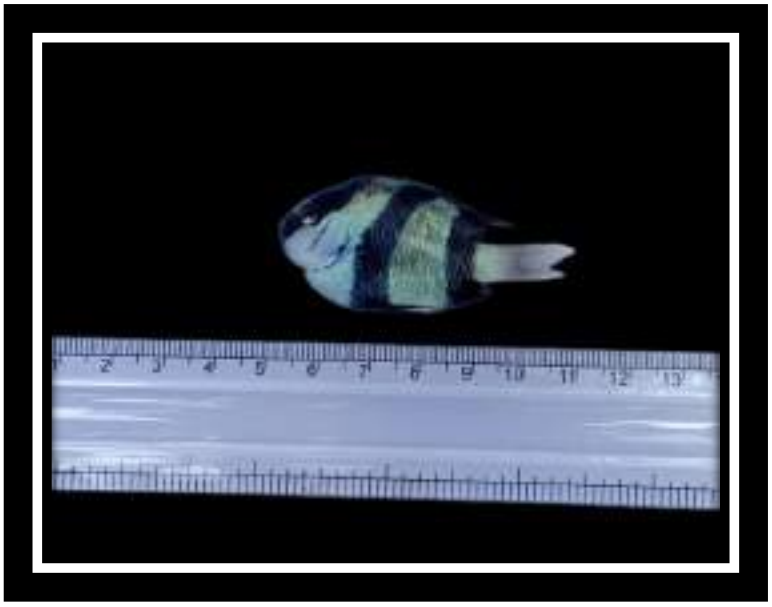


Fig21:*Dascyllus aruanus*

MORPHOLOGY:

Dorsal spines XII; Dorsal soft rays:13; Anal spines: II; Anal soft rays: 13. Color in life white with 3 black bars; a large brown spot on dorsal part of snout and interorbital; lips dusky or white; caudal fin pale; pelvic fins black; pectorals transparent. Margins of preorbital, suborbital, and preoperculum finely serrated.

VOUCHER NO:

MTRLOST -135-0704

COLLECTION DETAILS:

Specimen was collected on 16/02/2022 from Kavaratti Island, Lakshadweep

***Dascyllus trimaculatus*(Rüppell,1829)**

Type locality:

Aru Islands, Molucca Islands, Indonesia

COMMON NAMES:

White spot humbug, Three spot dascyllus,Three spot humbug ,Guru ,Tebukubuki,Karipatatam

SYNONYM:

Dascyllus axillaris, Dascyllus unicolor, Dascyllus trimaculatum , Dascyllus niger Pomacentrus nuchalis ,Pomacentrus trimaculatus , Sparus nigricans .

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Dascyllus

SPECIES**:** *Dascyllus trimaculatus*

Distribution:

Red Sea; Indo-West Pacific: Eastern Cape and KwaZulu-Natal (South Africa), East Africa (Mozambique, Tanzania, Kenya, Somalia, Djibouti), Persian Gulf, Socotra (Yemen), Seychelles, Comoros, Madagascar and Mascarenes (La Réunion, Mauritius, Rodrigues) east to Marshall Islands and Pitcairn Group, north to southern Korea and southern Japan, south to Rottnest Island (Western Australia), Wollongong (New South Wales, Australia), Lord Howe Island (Australia), New Caledonia and Austral Islands (French Polynesia)

Fig22: ***Dascyllus trimaculatus***

MORPHOLOGY:

Dorsal spines : XII; Dorsal soft rays (total): 14-16; Anal spines: 2; Anal soft rays: 14 - 15; no spot on forehead; spot on upper sides very reduced; head and fins normally black; scales with black margins. Margins of preorbital, suborbital and preoperculum finely serrated

VOUCHER NO:

MTRLOST -135-0707

COLLECTION DETAILS:

Specimen was collected on 08/04/2022 from Kadmat Island, Lakshadweep

Dascyllus carneus **(Fischer, 1885)**

TYPE LOCALITY:

Mozambique, western Indian Ocean

COMMON NAMES:

Blue spotted dascylus, , Indian hambug, Cloudy dascyllus,Whitetail damselfish,Tweebalk-humbag

SYNONYM:

Dascyllus nigripinnis

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Dascyllus

SPECIES**:** *Dascyllus carneus*

Distribution:

Indian Ocean: KwaZulu-Natal (South Africa), East Africa (Mozambique, Tanzania, Kenya), Socotra (Yemen), Seychelles, Comoros, Madagascar and Mascarenes (La Réunion, Mauritius, Rodrigues) east to Andaman Sea and Java (western Indonesia).

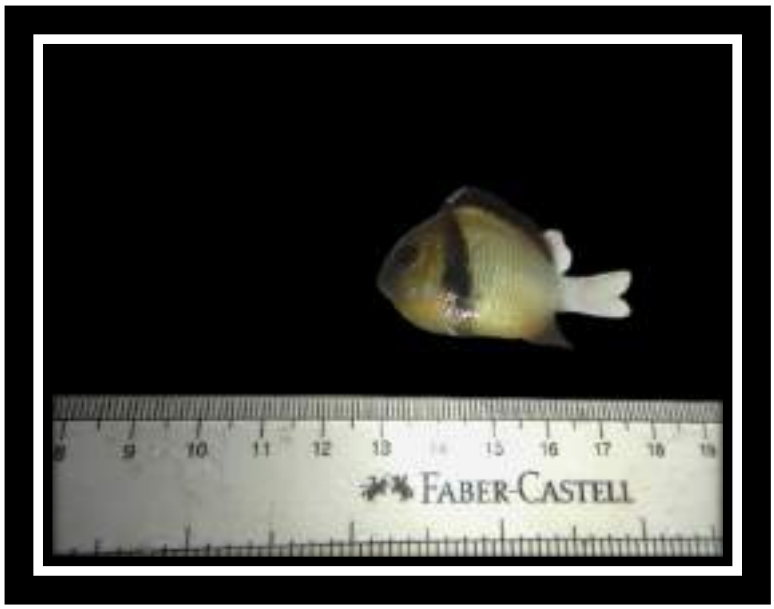


Fig23: ***Dascyllus carneus***

MORPHOLOGY:

Dorsal spines :XII; Dorsal soft rays: 14; Anal spines: II; Anal soft rays: 14. Body white with vertical bars two behind head and before caudal peduncle, blackish diffuse, spots blue numerous on head and breast.

VOUCHER NO:

MTRLOST -135-0705

COLLECTION DETAILS:

Specimen was collected on 11/03/2021 from Suheli Island, Lakshadweep

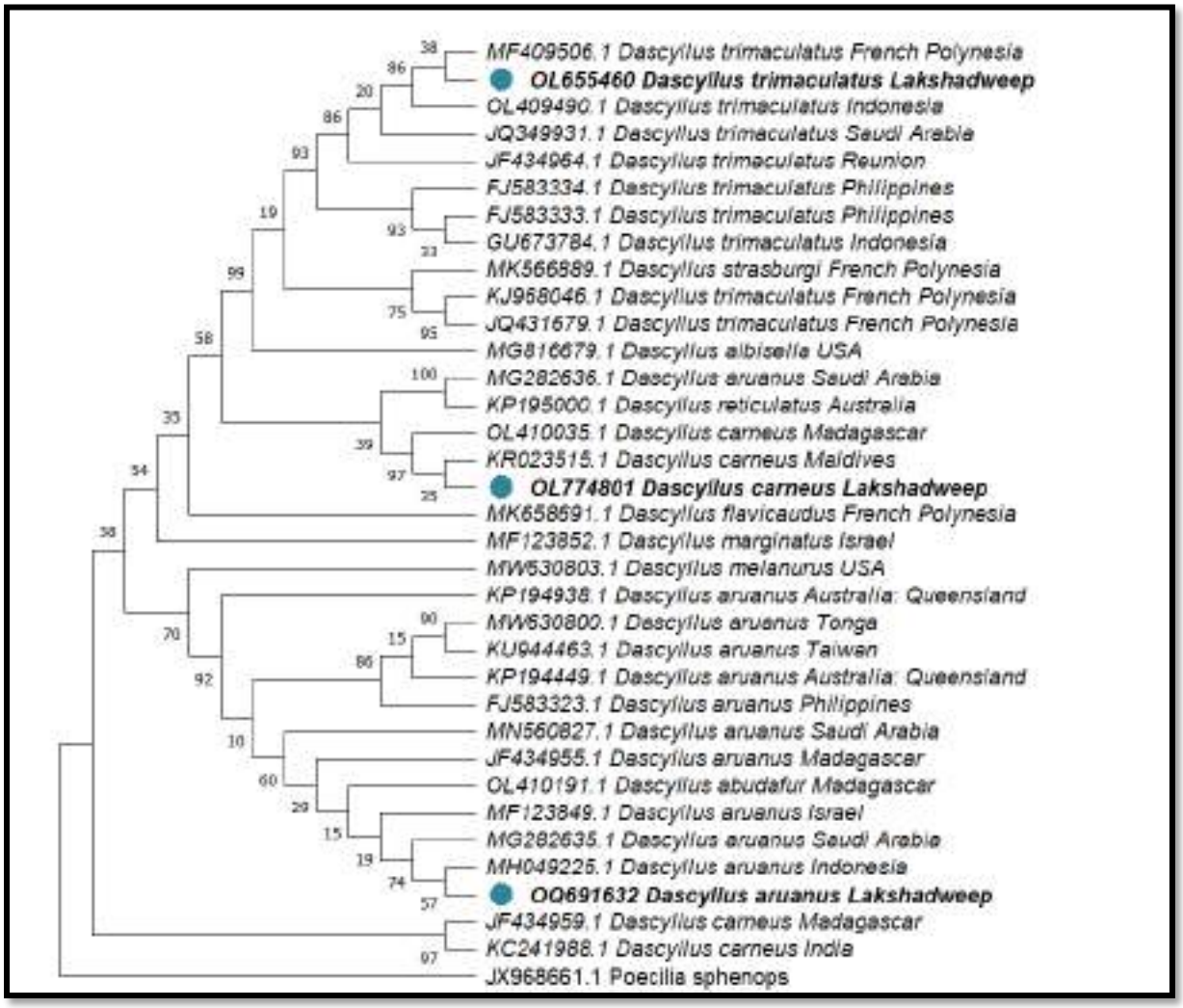


Fig24:Genus tree of Dascyllus

The given species from the Pomacentridae family belong to genus Dascyllus .The fish species is Dascyllus trimaculatus, Dascyllus carneus and Dascyllus aruanus according to the phylogenic tree. The sequence from the study with accession number OL655450 clade with sequence of fish from French Polynesia with accession number MF409506,other sequence with accession number OL774801 clade with sequence of fish from Maldives with accession number KR023515 and the last sequence with accession number OQ691632 clade with sequence of fish from Indonesia with accession number MH049225

Chrysiptera caeruleolineata **(ALLEN, 1973)**

TYPE LOCALITY:

New Guinea

COMMON NAMES:

Nimmis, Neon damsel, Blueline demoiselle, Bluelineddemoiselle, Aosuji-suzumedai

SYNONYM:

Abudefduf caeruleolineatus, Chryseptera caeruleolineata, Chrysiptera caeruleolineatus, Glyphidodontops caerulineatus

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Chryseptera

SPECIES*: Chryseptera caeruleolineata*

Distribution:

Eastern Indian Ocean, western Pacific: Indonesia east to Micronesia, Fiji and Samoa, Micronesia, north to Kagoshima Prefecture (southern Japan), south to Western Australia.

Fig25: ***Chryseptera caeruleolineata***

MORPHOLOGY:

Dorsal spines :XIV; Dorsal soft rays : 14; Anal spines: II; Anal soft rays: 14.Body and fins pale yellow, head and anterior of body bluish with a broad deep-blue-edged neon blue horizontal stripe from snout extending across the back up to dorsal fin rear portion.

VOUCHER NO:

A) MTRLOST -135-0700A

B) MTRLOST -135-0700B

COLLECTION DETAILS:

Specimen was collected on 28/01/2022 and 21/03/2022 from Kavaratti Island and Agatti Island, Lakshadweep

Chrysiptera glauca (Cuvier, 1830)

TYPE LOCALITY:

Guam, Mariana Islands, western Pacific

COMMON NAMES

Sombre damsel-fish,Grey demoiselle,Grey damsel,Pale-blue damsel, Blue damsel

SYNONYM:

Chrysiptera hollisi ,*Glyphidodon modestus* , *Glyphidodontops glaucus* ,*Glyphisodon phaiosoma* *Glyphidodon pallidus*, *Abudefduf caesio* ,*Abudefduf glaucus* ,

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Chrysiptera

SPECIES**:** *Chrysiptera glauca*

Distribution:

Indo-West Pacific: KwaZulu-Natal (South Africa), East Africa, Socotra (Yemen), Seychelles, Madagascar and Mascarenes (La Réunion, Mauritius, Rodrigues) east to Wake Atoll, Marquesas Islands and Tuamotu Archipelago (French Polynesia), north to Amami Islands (southern Japan), south to Rottnest Island (Western Australia), Noosa (Queensland, Australia), Lord Howe Island (Australia), New Caledonia and Tonga.

Fig26: ***Chryseptera glauca***

MORPHOLOGY:

Dorsal spines : XIII; Dorsal soft rays : 12; Anal spines: II; Anal soft rays: 13.

Overall bluish grey, body scales streaks bluish, head spots blue,Dorsal part whtish yellow

VOUCHER NO:

MTRLOST -135-0702

COLLECTION DETAILS:

Specimen was collected on 14/02/2022 from Kavaratti Island, Lakshadweep

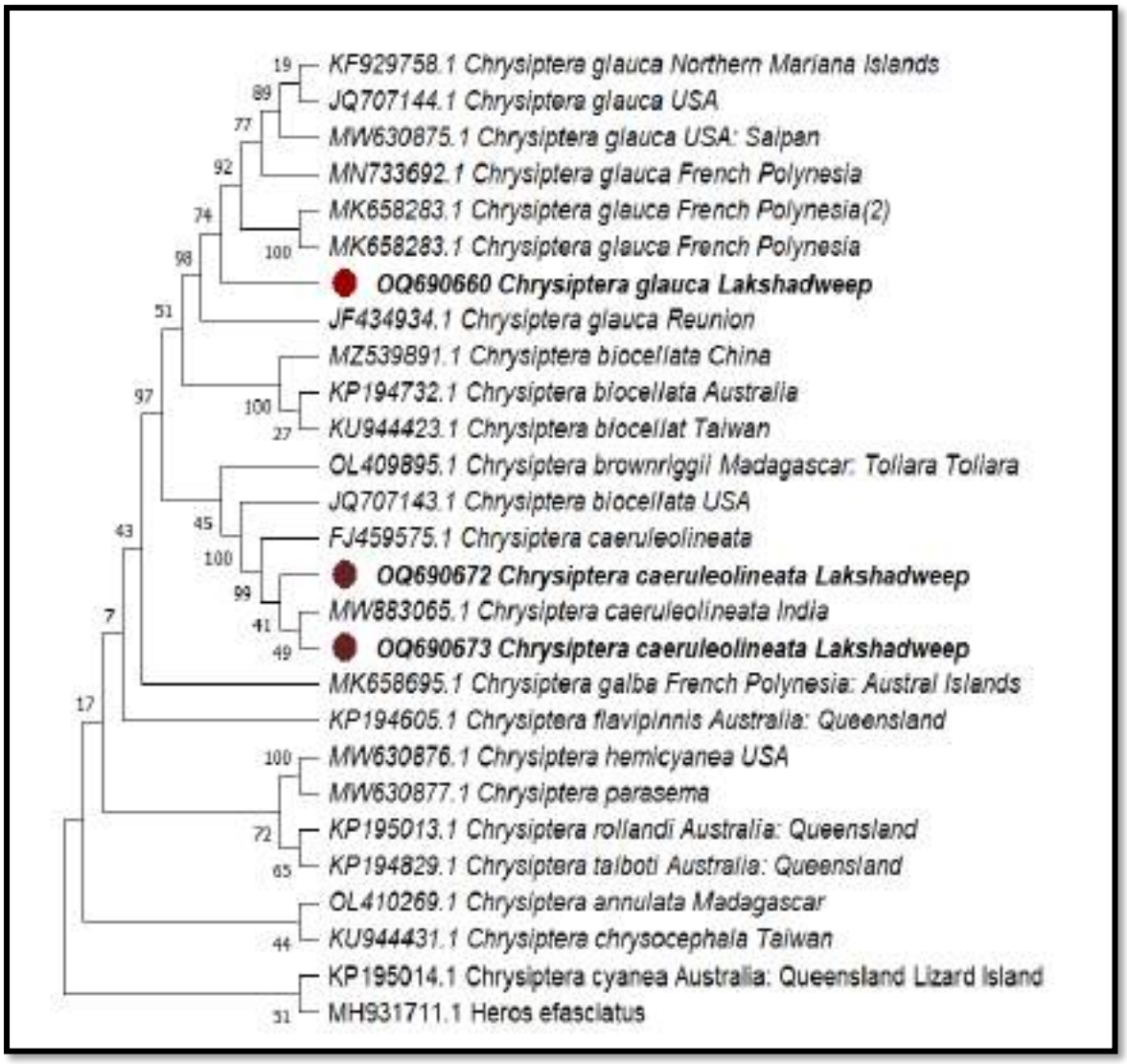


Fig27:Genus tree of Chryseptera

The given species from the Pomacentridae family belong to genus Chrysiptera .The fish species is Chrysiptera glauca and Chrysiptera caeruleolineata according to the phylogenic tree. The sequence from the study with accession number OQ690660 clade with sequence of fish from French Polynesia with accession number MK658283,other sequence with accession numbers OQ690672 and OQ690673 clade with sequence of fish from India with accession number MW883065

Amphiprion clarkii **(Bennett, 1830)**

TYPE LOCALITY:

Southern coast of Sri Lanka

COMMON NAMES:

Clark's anemonefish, Mørk klovnfisk, Chocolate clownfish,Clarki;s clown, Clown fish, Damsel fish, sea bee, Two banded anemone fish, Kumanomi, Poisson-clown de Clark,Yellow tail clownfish, Kumanomi, Black clown.

SYNONYM:

Amphiprion boholensis ,Amphiprion clarkia ,Amphiprion japonicus ,Amphiprion melanostolus ,Amphiprion papuensis ,Amphiprion snyderi ,Amphiprion xanthurus ,Anthias clarkii

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Amphiprion

SPECIES: *Amphiprion clarkii*

DISTRIBUTION:

Indo-West Pacific: Persian Gulf and Oman east to Caroline Islands (Federated States of Micronesia) and Tuamotu Islands (French Polynesia), north to Korea and central Japan, south to northern Australia.

Fig28: ***Amphiprion clarkii***

MORPHOLOGY:

Highly variable in colour and several geographical and localized forms. Body is black with two white bands, one behind the eye and one above the anus. Caudal fin yellowish, lighter than rest of the body. Caudal peduncle with another white bar, the rest of median fins variable in colour, dorsal fin is black in colour but anal and ventral fins are in yellowish orange in colour. Snout is also yellowish orange in colour

VOUCHER NO:

MTRLOST -135-0687

COLLECTION DETAILS:

Specimen was Collected on 08/04/2022 from Kadmat Island, Lakshadweep

***Amphiprion nigripes* (Regan,1908)**

TYPE LOCALITY:

Maldives, Indian Ocean

COMMON NAMES :

Maldivisk klovnfisk, Maagandu mas, pushpachala, Maldive anemonefish, Poisson-clown des Maldives

SYNONYM:

Amphiprion nigripes

TAXONOMY:

KINGDOM: Animalia

PHYLUM: Chordata

CLASS: Teleostei

FAMILY: Pomacentridae

GENUS: Amphiprion

SPECIES: *Amphiprion nigripes*

Distribution:

Indian Ocean: Maldives and Sri Lanka.

Fig29: *Amphiprion nigripes*

MORPHOLOGY:

Dorsal spines :XI; Dorsal soft rays : 18; Anal spines: II; Anal soft rays: 15. It is oval-bodied and laterally compressed characterized by its rusty orange color with a single white stripe running vertically just behind the eye. It has black pelvic and anal fins and a variable area on its belly can be more or less black. some fishes can be orangeish-yellow with an ventral which is not black but the same color as the body.

VOUCHER NO:

MTRLOST -135-0687

COLLECTION DETAILS:

Specimen was Collected on 19/02/2022 from Kavaratti Island, Lakshadweep

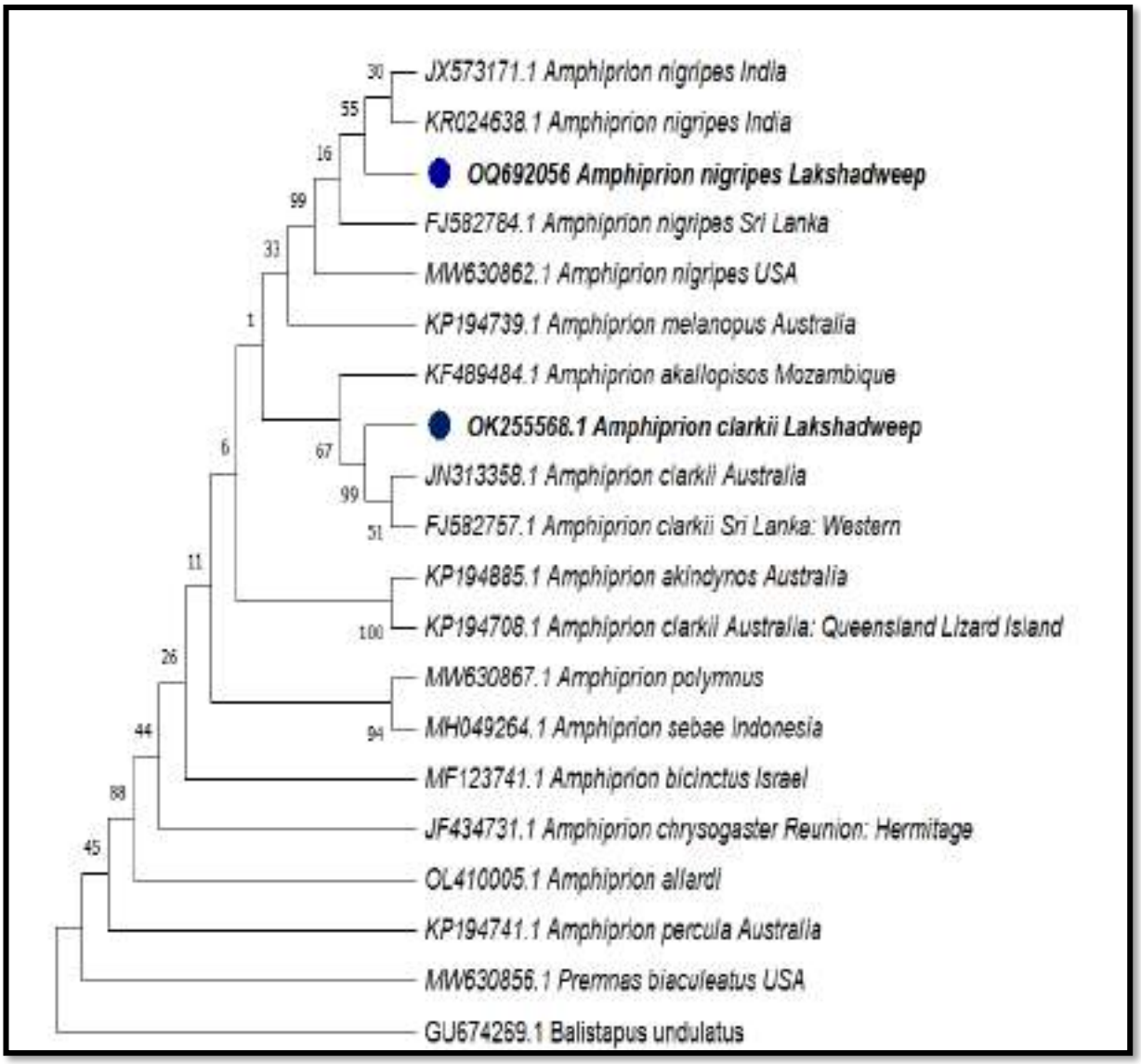


Fig30:Genus tree of Amphiprion

The given species from the Pomacentridae family belong to genus Amphiprion..The fish species is Amphiprion nigripes and Amphiprion.clarkiiaccording to the phylogenic tree. The sequence from the study with accession number OQ692056 is closest to sequence of fish from India with accession number KR024638,other sequence with accession numbers OK255568 clade with sequence of fish from Australia with accession number JN313358

DNA Barcoding of family Pomacentridae from Lakshadweep.

A total of 19 COI gene sequences were generated from 16 species. The analysis was conducted in 661 bp in the final dataset. The sequence analysis revealed average nucleotide frequencies as A = 23.60%, T = 29.10%, G = 17.70% and C = 29.5%.

All nine genera considered for COI gens Maximum Likelihood method and Neighbor-Joining trees are monophyletic with more than 50% bootstrap values. Chromis and Dascyllus belong to the same clade with bootstrap support of 50%. Chrysiptera, Amblyglyphidodon and Amphiprion belong to the same clade support with 60%the bootstrap value, and Pomacentrus is also joined with this clade with a 73% other bootstrap value. Abudefduf, Stegastes and Plectroglyphidodon stay as sinagle clade.

The intraspecies distance for the COI genes is 0-1% for all species under consideration. The interspecific distance between the species of all the genera for the COI gene is above 14%. Our result concluded as Family Pomacentridae is a divergent group, and the species under this family are monophyletic.

Table:Mean genetic distance of genus under Pomacentridae.

		1	2	3	4	5	6	7	8
1	Chromis								
2	Dascyllus	0.18							
3	Stegastes	0.19	0.22						
4	Chrysiptera	0.21	0.25	0.22					
5	Amphiprion	0.2	0.21	0.21	0.18				
6	Amblyglyphidodon	0.19	0.22	0.21	0.16	0.14			
7	Plectroglyphidodon	0.19	0.2	0.19	0.21	0.2	0.18		
8	Abudefduf	0.2	0.22	0.2	0.2	0.22	0.19	0.2	
9	Pomacentrus	0.2	0.21	0.21	0.18	0.18	0.19	0.23	0.19

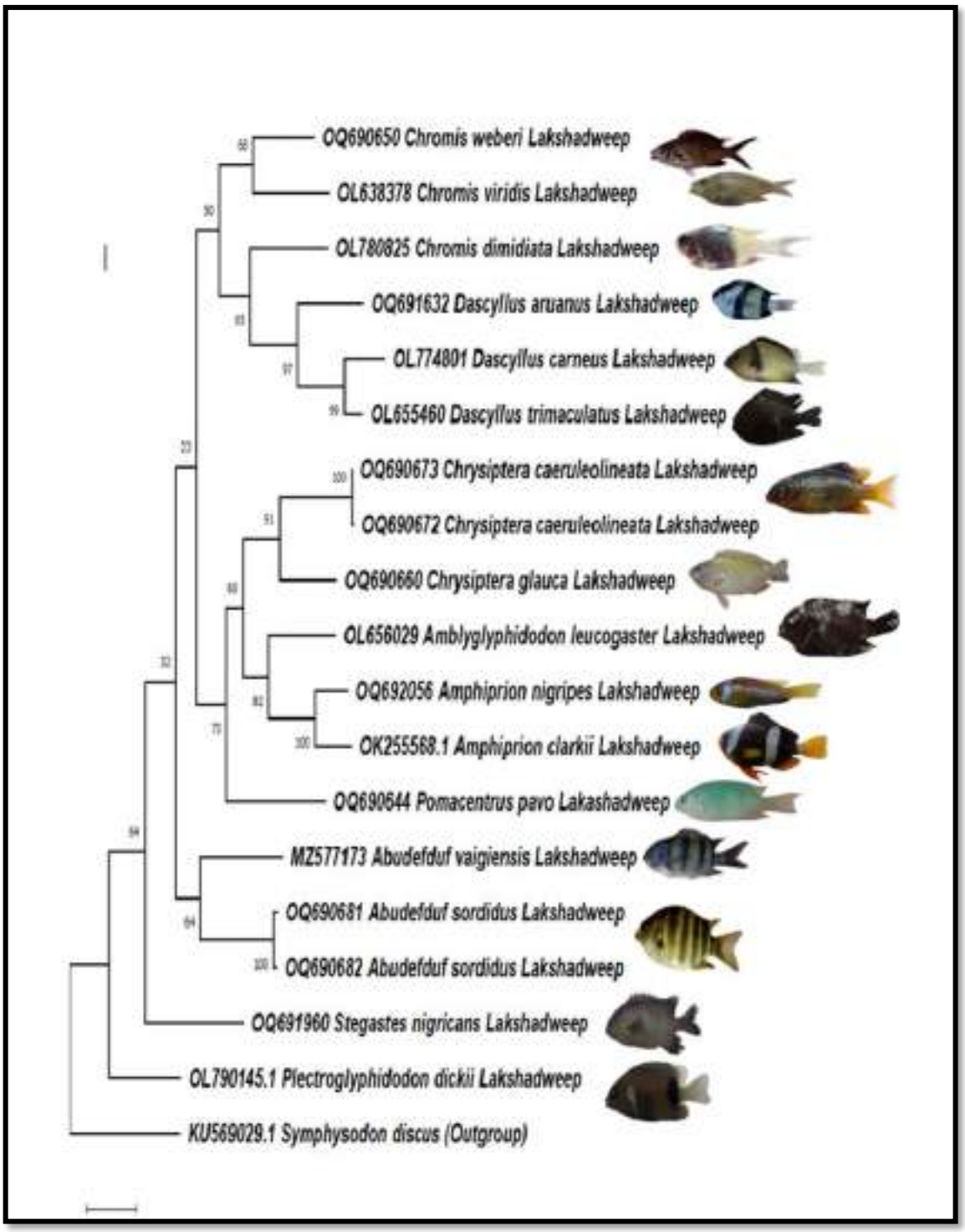


Fig31:Phylogenic tree of Pomacentridae

Phylogenetic interpretation of subfamily chrominae, Glyphisodontinae and Pomacentrinae

Our phylogenetic interpretation used three subfamilies of subfamily Chrominae, Glyphisodontinae and Pomacentrinae. From the analysis, the three subfamilies are monophyletic clades. Subfamily Chrominae includes two genera, Chrominae and Dascyllus. Form Our phylogenetic tree shows generated sequences and retrieved sequences from NCBI joint together with the same clade. The clade was monophyletic because all of them were clustered with the same genus only. Based on molecular analysis, we concluded in this clade that Chromis and Dascyllus are sister groups. , but there is no morphological similarity. In the phylogenetic analysis, they may have evolved from the same ancestors and become sister clades. Subfamily Glyphisodontinae includes only one genus, Abudefduf. In the phylogenetic analysis genus, Abudefduf shows that monophyly consists of a strong bootstrap value (89%). And also the Subfamily Pomacentrinae, we select the genus Chrysiptera for the analysis. It shows the monophyly support with a bootstrap value of 72%. Earlier classification Nelson, 2016, the genus Abudefduf and Chrysiptera include the same subfamily Pomacentrinae. A recent study by Cooper et al., 2021, revised the classification of the genus Abudefduf under the subfamily Glyphisodontinae. Our study also proves the separation of the genus Abudefduf from promacentrinae.

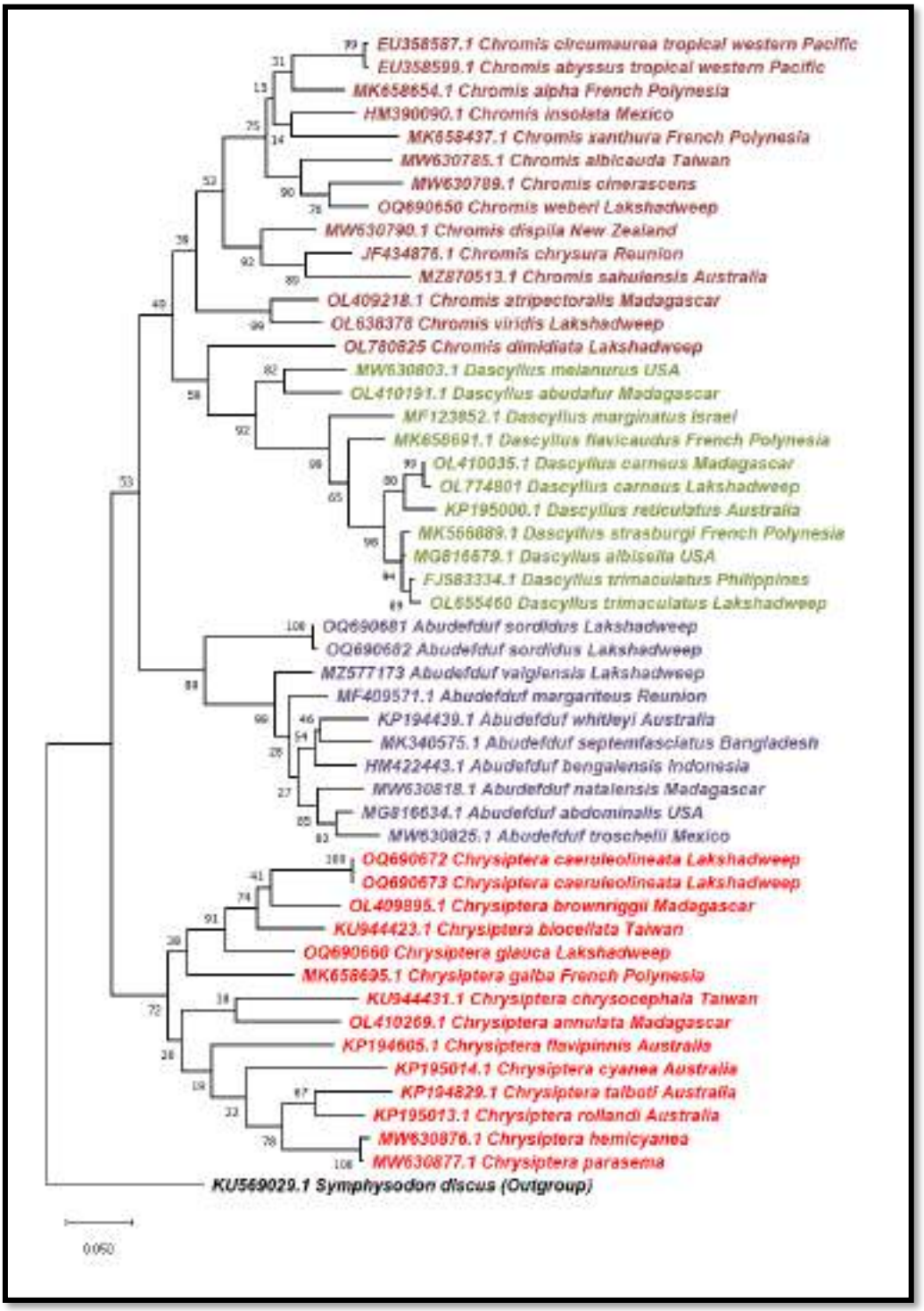


Fig32: Phylogenic tree of subfamily of Chrominae, Glyphisodontinae and Pomacentrinae

DISCUSSION

Pomacentridae is a large family of marine fishes found in tropical oceans, where they represent a significant part of reef habitats. Currently, there are about 400 identified damselfish species, which are grouped among 29 genera and belong to a single family, Pomacentridae where new species are continuously being described.

In the current work, 16 species of the Pomacentridae family were used to better understand their morphological characteristics, identify and analyse the pomacentridae phylogeny using DNA barcoding, and conduct further phylogenetic interpretation of three pomacentridae subfamilies.

Further details regarding the pomacentridae family were provided by the morphological study, which confirms that even within species of the same genus, pomacentridae exhibit significant morphological differences in their size, shape, and colour.

According to A. E Edwards species identification guide 2016 it is mentioned that the diagnostic characters of pomacentridae is that they are small fishes with about 6-30 cm in total. Most have deep, laterally compressed bodies with 14 to 10 dorsal fin spines, and anal fin always with two spines; They show bright and highly varied body colour; the above characters are shown by the fish sample which confirms their morphological characters.

These body variations were identified by Floeter *et al.* (2017), and mentioned the cause of variations primarily due to their habitat and diet, with benthic feeders showing smaller optimal sizes than pelagic and intermediate feeders as a result. This suggests that multiple convergent radiations may have been shaped by common ecological selection pressures.

DNA barcoding was used to conduct the phylogenetic analysis and species confirmation, mtDNA COI gene was used as a taxonomic identification tool. The Pomacentridae family is confirmed to be separated into 4 subfamilies by the molecular phylogenetic tree. They are Chrominae, Glyphisododon, Pomacentrinae, and Microspathodontinae. These analyses are in line with the recent study by Cooper *et al.* (2021), which identified four subfamilies in our samples that involves Chrominae with the genus Dascyllus and Chromis. The Glyphisododon subfamily includes a single gene, Abudefduf. Pomacentrinae consists of the three genus Chrysiptera, Amphiprion, and Pomacentrus. The genus Stegastus and the genus

Plectroglyphidodon show sister lineage while analysing the phylogeny of the subfamily Microspathodontinae; Stegastus and Plectroglyphidodon remain as two separate clade and are not monophyletic to each other.

This project's final goal was to interpret the phylogeny of three subfamilies of the Pomacentridae family Chromina, Glyphisodontinae, and pomacentrinae . The result was in compatible with a recent study by Cooper *et al.* (2021), which identified Dascyllus and Chromis as monophyletic clades in the Chomrinae family that have evolved from a common ancestor to become sister clades despite their lack of morphological similarity.

Cooper *et al.* confirmes the taxonomy of genus Abudefduf within the Glyphisodontinae subfamily, contradicting a previous study by Nelson (2016) that claimed the genus Abudefduf and Chrysiptera comprise a single subfamily, the Pomacentrinae. Our study of phylogenic interpretation, which demonstrates that Chrysiptera and Abudefduf belong to two different subfamilies, corroborated Cooper et al(2021) .'s identification.

CONCLUSION

The aforementioned result analysis provided valid information about the morphology of the Pomacentridae family. Most have a length total of 15 cm or less, with a maximum of 35 cm, , deep and laterally compressed body with high colour variations They show high variability in their morphological characters even though they belong to the same genus. Their feeding environment or ecosystem may be the cause of these morphological alterations.

With the use of DNA barcoding of the mtDNA COI genes as the taxonomic identification tool, a deeper understanding of the family, Pomacentridae, and molecular confirmation of each species were analysed.

The resulting phylogenic tree provided information about 4 subfamilies of Pomacentridae,which include the Chrominae with the genera Dascyllus and Chromis. The Glyphisododon subfamily includes a single gene, Abudefduf. Pomacentrinae has the largest clade, which consists of the genera Chrysiptera, Amphiprion, and Pomacentrus. While considering the phylogeny of subfamily Microspathodontinae, the genus Stegastus is not monophyletic with Plectroglyphidodon; Stegastus has a sister lineage to the genus Plectroglyphidodn.

And to conclude the study, the phylogenetic interpretation of three subfamilies of the Pomacentridae was studied. From the observations, it was determined that each clade was monophyletic within the same genus. Chromis and Dascyllus are sister groups without morphological similarities that belong to the subfamily Chrominae.

The Glyphisodontinae include only a single genus, Abudefduf, and from the phyloigenic interpretations it was confirmed that Abudefduf and Pomacemtrinae are of 2 different clades, hence they are of 2 different subfamily, which supports the recent study of Cooper *et al.* 2021.

REFERENCE

1. Ahrens, D., Monaghan, M. T., & Vogler, A. P. (2007). DNA-based taxonomy for associating adults and larvae in multi-species assemblages of chafers (Coleoptera: Scarabaeidae). *Molecular Phylogenetics and evolution*, *44*(1), 436-449.
2. Allen, G. R. (1975). *Damselfishes of the south seas*. TFH publications.
3. Anderson, G. R. V., Ehrlich, A. H., Ehrlich, P. R., Roughgarden, J. D., Russell, B. C., & Talbot, F. H. (1981). The community structure of coral reef fishes. *The American Naturalist*, *117*(4), 476-495.
4. Aquilino, S. V., Tango, J. M., Fontanilla, I. K., Pagulayan, R. C., Basiao, Z. U., Ong, P. S., & Quilang, J. P. (2011). DNA barcoding of the ichthyofauna of Taal Lake, Philippines. *Molecular Ecology Resources*, *11*(4), 612-619.
5. Balan, V. (1958). Notes on a visit to certain islands of the Laccadive Archipelago, with special reference to fisheries.
6. Bamaniya, D. C., Pavan-Kumar, A., Gireesh-Babu, P., Sharma, N., Reang, D., Krishna, G., & Lakra, W. S. (2016). DNA barcoding of marine ornamental fishes from India. *Mitochondrial DNA Part A*, *27*(5), 3093-3097.
7. BELLWOOD, D. R., & SORBINI, L. (1996). A review of the fossil record of the Pomacentridae (Teleostei: Labroidei) with a description of a new genus and species from the Eocene of Monte Bolca, Italy. *Zoological Journal of the Linnean Society*, *117*(2), 159-174.
8. Bhattacharya, M., Sharma, A. R., Patra, B. C., Sharma, G., Seo, E. M., Nam, J. S., ... & Lee, S. S. (2016). DNA barcoding to fishes: current status and future directions. *Mitochondrial DNA Part A*, *27*(4), 2744-2752.
9. Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R., & Abebe, E. (2005). Defining operational taxonomic units using DNA barcode data. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1935-1943.
10. Bourjon, P., Crochelet, E., & Fricke, R. (2019). First record of the large caerulean damselfish, Pomacentrus caeruleopunctatus (Actinopterygii: Perciformes: Pomacentridae), from Reunion Island, south-west Indian Ocean. *Acta Ichthyologica et Piscatoria*, *49*(1), 59-63
11. Bourjon, P., Crochelet, E., & Fricke, R. (2019). First record of the large caerulean damselfish, Pomacentrus caeruleopunctatus (Actinopterygii: Perciformes:

Pomacentridae), from Reunion Island, south-west Indian Ocean. *Acta Ichthyologica et Piscatoria*, *49*(1), 59-63.

12. CMFRI, K. (1989). Marine Living Resources of the Union Territory of Lakshadweep: An Indicative Survey with Suggestions for Development. *CMFRIBulletin*, *43*, 1-116.
13. Cole, A. J., Pratchett, M. S., & Jones, G. P. (2008). Diversity and functional importance of coral-feeding fishes on tropical coral reefs. *Fish and Fisheries*, *9*(3), 286-307.
14. Cooper, W. J., Smith, L. L., & Westneat, M. W. (2009). Exploring the radiation of a diverse reef fish family: phylogenetics of the damselfishes (Pomacentridae), with new classifications based on molecular analyses of all genera. *Molecular Phylogenetics and Evolution*, *52*(1), 1-16.
15. Dawnay, N., Ogden, R., McEwing, R., Carvalho, G. R., & Thorpe, R. S. (2007). Validation of the barcoding gene COI for use in forensic genetic species identification. *Forensic science international*, *173*(1), 1-6.
16. Dayrat, B. (2005). Towards integrative taxonomy. *Biological journal of the Linnean society*, *85*(3), 407-417.
17. Delrieu-Trottin, E., Maynard, J., & Planes, S. (2014). Endemic and widespread coral reef fishes have similar mitochondrial genetic diversity. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1797), 20141068.
18. Dhaneesh, K. V., Kumar, T. T. A., & Kumar, A. B. (2015). Barcoding, phylogeography and species boundaries in clownfishes of the Indian Ocean. *DNA Barcodes*, *3*(1), 5-16.
19. Dragičević, B., Fricke, R., Soussi, J. B., Ugarković, P., Dulčić, J., & Azzurro, E. (2021). On the occurrence of Abudefduf spp.(Pisces: Pomacentridae) in the Mediterranean Sea: a critical review with new records. *BioInvasions Record*, *10*(1).
20. Eschmeyer, W. N., Fricke, R., Fong, J. D., & Polack, D. A. (2010). Marine fish diversity: history of knowledge and discovery (Pisces). *Zootaxa*, *2525*(1), 19-50.
21. Fahmi, M. R., Kusrini, E., Hayuningtiyas, E. P., Sinansari, S., & Gustiano, R. (2020). DNA barcoding using coi gene sequences of wild betta fighting fish from Indonesia: phylogeny, status and diversity. *Indonesian Fisheries Research Journal*, *26*(2), 97-105.
22. Ferri, G., Alu, M., Corradini, B., Licata, M., & Beduschi, G. (2009). Species identification through DNA "barcodes". *Genetic testing and molecular biomarkers*, *13*(3), 421-426.

23. Frederich, B., Pilet, A., Parmentier, E., & Vandewalle, P. (2008). Comparative trophic morphology in eight species of damselfishes (Pomacentridae). *Journal of Morphology*, *269*(2), 175-188.
24. Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S., & Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS biology*, *2*(10), e312.
25. Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313-321.
26. Hughes, L. C., Ortí, G., Huang, Y., Sun, Y., Baldwin, C. C., Thompson, A. W., ... & Shi, Q. (2018). Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. *Proceedings of the National Academy of Sciences*, *115*(24), 6249-6254.
27. James, P. S. B. R. (2011). Lakshadweep: Islands of ecological fragility, environmental sensitivity and anthropogenic vulnerability. *Journal of Coastal Environment*, *2*(1), 9-25. Rajan
28. James, P. S. B. R., Kumaran, M., Pillai, C. G., & Lal Mohan, R. S. (1989). Development of fisheries in Lakshadweep recommendations. *CMFRI Bulletin Marine living resources of the union territory of Lakshadweep An Indicative Survey With Suggestions For Development*, *43*, 253-256.
29. Jones, C. G., Lawton, J. H., & Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, 373-386.
30. Jones, S., & Kumaran, M. (1980). *Fishes of the Laccadive archipelago*. The Nature Conservation and Aquatic Sciences Service.
31. Jordan, D. S., & Herre, A. W. (1906). A review of the herring-like fishes of Japan.
32. Koh, J. R., & Park, Y. C. (2007). Species identification and molecular phylogenetic position of Korean damselfishes (Pomacentridae: Chrominae) based on DNA bioinformation. *Korean Journal of Ichthyology*, *19*(4), 274-285.
33. Krishnan, L. (2008). Marine ornamental resources and Management Strategies.
34. Kumar, R., Dineshbabu, A. P., Jaiswar, A. K., Shenoy, L., Kumar, A. P., Rahangdale, & Damodaran, D. (2019). New Distributional Records for Cardinalfishes (Perciformes/Apogonidae) from North East Arabian Sea, Western Indian Ocean. *Thalassas: An International Journal of Marine Sciences*, *35*, 341-346.

35. Lahaye, R., Van der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., ... & Savolainen, V. (2008). DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences*, *105*(8), 2923-2928.
36. Leis, J. M., & Carson-Ewart, B. M. (Eds.). (2000). *The larvae of Indo-Pacific coastal fishes: an identification guide to marine fish larvae* (Vol. 2). Brill.
37. Leprieur, F., Pellissier, L., Mouillot, D., & Gaboriau, T. (2021). Influence of historical changes in tropical reef habitat on the diversification of coral reef fishes. *Scientific Reports*, *11*(1), 20731.
38. Lessios, H. A., Allen, G. R., Wellington, G. M., & Bermingham, E. (1995). Genetic and morphological evidence that the eastern Pacific damselfish Abudefduf declivifrons is distinct from A. concolor (Pomacentridae). *Copeia*, 277-288.
39. Lobato, F. L., Barneche, D. R., Siqueira, A. C., Liedke, A. M., Lindner, A., Pie, M. R., ... & Floeter, S. R. (2014). Diet and diversification in the evolution of coral reef fishes. *PLoS One*, *9*(7), e102094.
40. Luckhurst, B. E., & Luckhurst, K. (1978). Analysis of the influence of substrate variables on coral reef fish communities. *Marine Biology*, *49*, 317-323.
41. Maheswarudu, G., Radhakrishnan, E. V., Pillai, N. N., Arputharaj, M. R., Ramakrishnan, A., Mohan, S., & Vairamani, A. (1998). Tagging experiments on searanched Penaeus indicus in the Palk Bay, southeast coast of India. *Indian Journal of Fisheries*, *45*(1), 67-74.
42. McCord, C. L., Nash, C. M., Cooper, W. J., & Westneat, M. W. (2021). Phylogeny of the damselfishes (Pomacentridae) and patterns of asymmetrical diversification in body size and feeding ecology. *PLoS One*, *16*(10), e0258889
43. Mohan, M., Livingston, P., & Kunhikoya, K. K. (1985). Fishery and bionomics of tunas at Minicoy Island. *CMFRI Bulletin*, *36*, 122-137.
44. Mohan, M., Pillai, C. G., & Kunhikoya, K. K. (1986). Biology of the bluepuller, Chromis caeruleus (Cuvier), from Minicoy Atoll. *Indian Journal of Fisheries*, *33*(4), 457-470.
45. Murty, V. S. (1996). Marine ornamental fishes of India. In *Proceedings of the Seminar on Fisheries-A Multibillion Dollar Industry, Madras, Aug 17-19, 1995* (pp. 23-34). Aquaculture Foundation of India & The Fisheries Technocrats Forum.
46. Murty, V. S. (2001). Ornamental fish resources of Lakshadweep. *Geological Survey of India Special Publication*, (56), 103-111.

47. Nelson, J. S., Grande, T. C., & Wilson, M. V. (2016). *Fishes of the World*. John Wiley & Sons Tripathy, 2002
48. Patzner, R. A. (2008). Ecological studies on two Amphiprion species in Maldivian coral reefs. *The Open Fish Science Journal*, *1*(1).
49. Pilgrim, E. M., Jackson, S. A., Swenson, S., Turcsanyi, I., Friedman, E., Weigt, L., & Bagley, M. J. (2011). Incorporation of DNA barcoding into a large-scale biomonitoring program: opportunities and pitfalls. *Journal of the North American Benthological Society*, *30*(1), 217-231.
50. Quenouille, B., Bermingham, E., & Planes, S. (2004). Molecular systematics of the damselfishes (Teleostei: Pomacentridae): Bayesian phylogenetic analyses of mitochondrial and nuclear DNA sequences. *Molecular phylogenetics and evolution*, *31*(1), 66-88.
51. Rajan, R., Rajan, P. T., Mishra, S. S., CN, A. R., & AT, D. (2021). Fishes of Lakshadweep archipelago: new records, review and a revised checklist. *Marine Biodiversity Records*, *14*(1), 1-13.
52. Ramadan, H. A., & Baeshen, N. A. (2012). Biological Identifications through DNA barcodes. *Biodiversity conservation and utilization in a diverse World, InTech Publisher, Republika Hrvatska*, 109-129.
53. Randall, J. E., & DiBattista, J. D. (2013). A new species of damselfish (Pomacentridae) from the Indian Ocean. *aqua*, *19*, 1-16.
54. Reaka-Kudla, M. L. (1997). The global biodiversity of coral reefs: a comparison with rain forests. *Biodiversity II: Understanding and protecting our biological resources*, *2*, 551.
55. Sandra, B., Anto, A., Sreeram, M. P., Sreenath, K. R., Aju, K. R., Sreekumar, K. M., ... & Joshi, K. K. (2022). New Distributional Records of Twelve Reef Fishes from Lakshadweep Waters, India. *Thalassas: An International Journal of Marine Sciences*, 1-13.
56. Sen, A., Sreeraj, C. R., Raghunathan, C., Chandra, K., & Sreeraj, C. (2021). First report of the fish family Pomacentridae (Damsel fishes) from Sunderban biosphere reserve, India. *Int. J. Fish. Aquat. Stud*, *9*(1), 142-145.
57. Sinha, B. (1994). *Geo-economic survey of Lakshadweep*. Concept Publishing Company.
58. Talwar, P. K., & Kacker, R. K. (1984). Commercial sea fishes of India.

59. Tang, K. L., McNyset, K. M., & Holcroft, N. I. (2004). The phylogenetic position of five genera (Acanthochromis, Azurina, Chrysiptera, Dischistodus, and Neopomacentrus) of damselfishes (Perciformes: Pomacentridae). *Molecular Phylogenetics and Evolution*, *30*(3), 823-828.
60. Tang, K. L., Stiassny, M. L., Mayden, R. L., & DeSalle, R. (2021). Systematics of damselfishes. *Ichthyology & Herpetology*, *109*(1), 258-318.
61. Triantafyllidis, A., Bobori, D., Koliamitra, C., Gbandi, E., Mpanti, M., Petriki, O., & Karaiskou, N. (2011). DNA barcoding analysis of fish species diversity in four north Greek lakes. *Mitochondrial DNA*, *22*(sup1), 37-42.
62. Tripathy, B. (2002). Marine biodiversity of Lakshadweep: An overview. *Kachappa*, *7*, 14-19.
63. Trivedi, S., Aloufi, A. A., Ansari, A. A., & Ghosh, S. K. (2016). Role of DNA barcoding in marine biodiversity assessment and conservation: an update. *Saudi journal of biological sciences*, *23*(2), 161-171.
64. Vijay Anand, P. E., & Pillai, N. G. K. (2003). Habitat distribution and species diversity of coral reef fishes in the reefslope of the Kavaratti atoll, Lakshadweep, India. *Journal of the Marine Biological Association of India*, *45*(1), 88-98.
65. Vijay Anand, P. E., & Pillai, N. G. K. (2005). Community organization of coral reef fishes in the rubble sub-habitat of Kavaratti Atoll, Lakshadweep, India. *Journal of the Marine Biological Association of India*, *47*(1), 77-82.
66. Wang, A. R., Ran, C., Ringø, E., & Zhou, Z. G. (2018). Progress in fish gastrointestinal microbiota research. *Reviews in Aquaculture*, *10*(3), 626-640.
67. Ward, R. D., Hanner, R., & Hebert, P. D. (2009). The campaign to DNA barcode all fishes, FISH-BOL. *Journal of fish biology*, *74*(2), 329-356.
68. Weigt, L. A., Driskell, A. C., Baldwin, C. C., & Ormos, A. (2012). DNA barcoding fishes. *DNA barcodes: Methods and protocols*, 109-126
69. Zemlak, T. S., Ward, R. D., Connell, A. D., Holmes, B. H., & Hebert, P. D. (2009). DNA barcoding reveals overlooked marine fishes. *Molecular Ecology Resources*, *9*, 237-242.