

**MOLECULAR TAXONOMY OF DEEP-SEA FISHES OFF  
THE SOUTHERN COAST OF INDIA**

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*Under*

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*By*

**BINEESH K.K  
(Reg. No. 3479)**



भारतीय कृषि अनुसंधान परिषद  
Indian Council of Agricultural Research  
केन्द्रीय समुद्री मात्स्यिकी अनुसंधान संस्थान  
**Central Marine Fisheries Research Institute**



April 2015

## *Certificate*

This is to certify that this thesis titled **MOLECULAR TAXONOMY OF DEEP-SEA FISHES OFF THE SOUTHERN COAST OF INDIA** is an authentic record of the research work carried out by **Mr. Bineesh K.K (Reg. No. 3479)** under my co-guidance and joint supervision in the Central Marine Fisheries Research Institute, Kochi, in partial fulfillment of the requirement for the award of Ph.D. degree in Faculty of Marine Sciences, Cochin University of Science and Technology, Kochi, Kerala and that no part of this thesis has previously formed basis for the award of degree/associateship, in any University or Institution.

**Dr. N.G.K. Pillai**  
(Supervising Guide)  
ICAR Emeritus Scientist  
Central Marine Fisheries Research Institute, Kochi

Kochi

April 2015

## *Declaration*

I, **Bineesh K.K.**, do hereby declare that the thesis entitled **MOLECULAR TAXONOMY OF DEEP-SEA FISHES OFF THE SOUTHERN COAST OF INDIA** is a genuine record of research work carried out by me under the guidance of **Dr. N.G.K. Pillai** (Former Head, Pelagic Fisheries Division, Central Marine Fisheries Research Institute, Kochi, India) in partial fulfilment for the award of Ph.D. degree under the Faculty of Marine Sciences, Cochin University of Science and Technology, Kochi and no part of the work has previously formed the basis for the award of any degree, diploma, associateship, or any other title or recognition from any University/Institution.

**BINEESH K.K**

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*Dedicated to My Family*

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## ABBREVIATION

FISH-BOL	<i>Fish Barcode of Life</i>
12SrRNA	<i>12S ribosomal ribonucleic acid</i>
16SrRNA	<i>16S ribosomal ribonucleic acid</i>
A	<i>Adenine</i>
AFLP	<i>Amplified Fragment Length Polymorphism</i>
Ant	<i>Antorbital</i>
AO	<i>Anal organs</i>
AOa	<i>Anal organs anterior</i>
AOp	<i>Anal organs posterior</i>
Ap spA	<i>Apristurus sp. A</i>
Be fi	<i>Benthoosema fibulatum</i>
Be pt	<i>Benthoosema pterotum</i>
BIOEDIT	<i>Biological Sequence Alignment Editor</i>
BLAST	<i>Basic Local Alignment Search Tool</i>
BOLD	<i>Barcode of Life Data Systems</i>
By hi	<i>Bythaelurus hispidus</i>
C	<i>Cytosine</i>
Ce at	<i>Centrophorus atromarginatus</i>
Ce gr	<i>Centrophorus granulosus</i>
Ce si	<i>Cephaloscyllium silasi</i>
Ce sq	<i>Centrophorus squamosus</i>
Ce ze	<i>Centrophorus cf. zeehaani</i>
Ch in	<i>Chelidoperca investigatoris</i>
Ch ma	<i>Chelidoperca maculicauda</i>
Ch oc	<i>Chelidoperca occipitalis</i>
Co ja	<i>Cookeolus japonicus</i>
COI	<i>Cytochrome-c-oxidase subunit I</i>
Cu sp	<i>Cubiceps sp</i>
Cu wh	<i>Cubiceps whiteleggii</i>
Cyt b	<i>Cytochrome b</i>
De pr	<i>Deania profundorum</i>
Di ga	<i>Diaphus garmani</i>
Di jo	<i>Dipturus johannisdavisi</i>
Di spA	<i>Dipturus sp. A</i>
Di spB	<i>Dipturus sp. B</i>
Di th	<i>Diaphus thiollierei</i>
Di wa	<i>Diaphus watasei</i>
Dia spA	<i>Diaphus sp. A</i>
Dn	<i>Dorsonasal</i>
DNA	<i>Deoxyribonucleic acid</i>

Ec br	<i>Echinorhinus brucus</i>
EMBL	European Molecular Biology Laboratory
Et pu	<i>Etmopterus pusillus</i>
FAO	Food and Agriculture Organization
G	Guanine
Ha qu	<i>Halaaelurus quagga</i>
He gr	<i>Hexanchus griseus</i>
He pe	<i>Heptranchias perlo</i>
H-strand	Heavy strand
Hy oc	<i>Hyporthodus octofasciatus</i>
Ia spA	<i>Iago</i> sp. A
Ia spB	<i>Iago</i> sp. B
Is ox	<i>Isurus oxyrinchus</i>
Is pa	<i>Isurus paucus</i>
IT IS	Integrated Taxonomic Information System
IUCN	International Union for Conservation of Nature
K2P	Kimura-2-Parameter
Kbp	Kilo base pairs
Le fl	<i>Lepidocybium flavobrunneum</i>
Li ra	<i>Liopropoma randalli</i>
L-strand	Light strand
Min	Minute
mt genome	Mitochondrial genome
mtDNA	Mitochondrial deoxyribonucleic acid
My sp/Yct ne	<i>Myctophum spinosum</i>
My spA	<i>Myctophum</i> sp. A
NBFGR	National Bureau of Fish Genetic Resources
NCBI	National Centre for Biotechnology Information
ND4	NADH dehydrogenase subunit 4
ND5	NADH dehydrogenase subunit 5
Ne or	<i>Neopinnula orientalis</i>
Ne pi	<i>Neoharriotta pinnata</i>
NJ	Neighbour Joining algorithm
Od pe	<i>Odontanthias perumali</i>
Ok po	<i>Okamejei powelli</i>
Op	Opercular
Pa as	<i>Parascolopsis aspinosa</i>
Pa bo	<i>Parascolopsis boesemani</i>
Pa er	<i>Parascolopsis eriomma</i>
PCR	Polymerase Chain Reaction
PCR-RFLP	PCR Restriction Fragment Length Polymorphism
Pi	Parsimony Informative
PLO	Suprapectoral organ

PO	<i>Thoracic</i>
Pr bl	<i>Priacanthus blochii</i>
Pr ha	<i>Priacanthus hamrur</i>
Pr pr	<i>Priacanthus prolixus</i>
Pr pro	<i>Promethichthys prometheus</i>
Pr re	<i>Pristigenys refulgens</i>
Pr sa	<i>Priacanthus sagittarius</i>
Ps ar	<i>Psenes arafurensis</i>
Ps cy	<i>Psenes cyanophrys</i>
Pt vi	<i>Pteroplatytrygon violacea</i>
PVO	<i>Subpectoral organ</i>
RAPD	<i>Random Amplified Polymorphic DNA</i>
Re be	<i>Rexea bengalensis</i>
Rh va	<i>Rhinobatos variegatus</i>
rRNA	<i>Ribosomal Ribonucleic acid</i>
S	<i>Singleton</i>
Sa bo	<i>Sacura boulengeri</i>
Sa lo	<i>Saurida longimanus</i>
Sa mi	<i>Saurida micropectoralis</i>
Sa spA	<i>Saurida sp. A</i>
Sa spB	<i>Saurida sp. B</i>
Sa tu	<i>Saurida tumbil</i>
Sa un	<i>Saurida undosquamis</i>
SAO	<i>Supralateral</i>
Sec	<i>Seconds</i>
Si	<i>Transition</i>
SNP	<i>Single Nucleotide Polymorphism</i>
So	<i>Suborbital</i>
Sq spA	<i>Squalus sp. A</i>
Suo	<i>Supraorbital</i>
Sv	<i>Transversion</i>
T	<i>Thymine</i>
Ta	<i>Annealing Temperature</i>
Tm	<i>Melting Temperature</i>
To spA	<i>Torpedo sp. A</i>
tRNA	<i>Transfer ribonucleic acid</i>
UPC	<i>Universal Product Code</i>
V	<i>Variable/Polymorphic</i>
VLO	<i>Supraventral</i>
Vn	<i>Ventronasal</i>
VO	<i>Ventral organs</i>

**Chapter 1**

***General Introduction***

## Chapter 1

### General Introduction

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Deep-sea fishes are one of the very interesting groups of animals that live in the darkness that is below the epipelagic or photic zone of the ocean. The deep-sea ecosystem is the largest habitat on Earth, covering  $300 \times 10^6 \text{ km}^2$  that comprise about 63% of the earth's surface, and is the main reservoir of global biodiversity (Smith *et al.*, 2008). However, the deep-sea is the least productive part of the oceans, although some high biomass concentrations of fishes are found on the topographic features like seamounts, mid-oceanic ridges and continental slopes (Norse *et al.*, 2012). Deep-sea fishes can be placed into mesopelagic, bathypelagic and benthopelagic categories, depending upon their depth preferences. Mesopelagic and bathypelagic species are true pelagic fishes, generally of small size even at their adult stage and unlikely to be exploited on a commercial scale (Valinassab *et al.*, 2007). Lantern fishes (Myctophidae) and cyclothonids (Gonostomatidae) are the common mesopelagic fishes that live below the photic zone extending to 1000 m depth and they, along with bathypelagic fishes that live below 1000 m, are highly adapted to live in an environment where food is scarce. The deep-sea fishes from mesopelagic and bathypelagic depths have many unique and interesting adaptations for living in the extreme deep-sea environment.

The production of coastal fishery resources has reached a plateau and this has generated increased interest in the harvest of deep-sea and oceanic fishery resources. Mesopelagic fishes, found at depths between 100 and 1,000 m, are among the most abundant marine organisms that are least studied and underutilized by mankind (Valinassab *et al.*, 2007). The most common among the mesopelagic fishes are the lanternfishes of the family Myctophidae and it represents the most abundant families of deep-sea fishes, comprising at least 20% of the oceanic ichthyofauna (McGinnis 1982). *Benthoosema pterotum* is the most abundant species in the western and the eastern Arabian Sea and is also the largest single species stock of fish in the world (GLOBEC, 1993). However, the deep-sea fisheries are especially vulnerable because they are considered to have high longevity, slow growth, late maturity and low fecundity, meaning they cannot repopulate quickly if they are overfished. These

characteristics of stocks lead to rapid depletion by fishing and recovery can be slow (Morato *et al.*, 2006). In India, since 1980s, the coastal fisheries targeting pelagic and demersal fishery resources have been over exploited, and the commercial fishing fleets have moved further into deeper waters, resulting in the discovery of new commercial deep-sea fishery resources like shrimps and sharks, which are being exploited now (Akhilesh *et al.*, 2011; Shanis *et al.*, 2014). In India, only few studies have been conducted on the deep-sea fishery, mainly from Kerala, Karnataka and Tamil Nadu coast (Thirumilu and Rajan, 2003; Radhika, 2004). Since the baseline information such as taxonomy, distribution and biology of these resources is scanty, making fishery management plans is a very difficult task. Species identity of all deep-water fishes should be properly confirmed to provide baseline data and a better understanding of the potential consequences of large scale exploitation on deep-sea fish resources before initiating its exploitation.

The Indian Ocean is well known for its large number of marine fish species. However, the Indian Ocean area is one of the least studied and more taxonomic research is needed, especially on the diversity of deep-sea ridges, seamounts and deep-sea areas (Eschmeyer *et al.*, 2010). Early works on biodiversity and taxonomy of deep-sea fishes in the Indian Ocean region were conducted by Lt. Col. A. W. Alcock, Sir James Hornell and F. M. Gravely during the late 19<sup>th</sup> century and early 20<sup>th</sup> century. In addition to that, the John Murray expedition (1933-1934) surveyed 212 stations in the Indian Ocean (Arabian Sea) at depths of 27-4793 m (Weitkamp and Sullivan, 1939). After these major works, the taxonomy of the bathypelagic fishes from the continental slope of the southwest coast of India was conducted by Tholasilingham *et al.* (1964) and Jones and Kumaran (1964, 1965) resulting in many new records of deep-sea fishes. Studies on the taxonomy of deep-sea fishes from Indian EEZ have resulted in some distribution extension records and a few species new to science (Akhilesh *et al.*, 2012, 2013; Bineesh *et al.*, 2010, 2013, 2014).

The identification of deep-sea fishes and elasmobranchs is traditionally based on morphological, meristic and anatomical characters. However, the amazing diversity in size, shape and their morphological plasticity makes the fish and their developmental stages difficult to identify using morphological features alone (Victor *et al.*, 2009). The DNA based identification method has been developed and proven as a powerful tool for fish identification, including all stages of their life (Zhang *et*

*al.*, 2004). Eighteen species described by Alcock are now synonymised or with uncertain status. Similarly, more than 10 species described by Lloyd have also been synonymised with other closely related species. However, some recent taxonomic works have resulted in resurrection of some of these synonymised species to valid species (e.g. *Chaunax apus*, *Lophius triradiatus*). These examples show the need for taxonomic revisions of deep-sea families from the Indian waters, supported with wide geographical comparisons and molecular approaches.

Species identification is very critical to the design of fisheries and conservation management plans, which ideally should be implemented on a species-by-species basis (FAO, 1997). However, the field identification of closely related shark species such as carcharhinid sharks and centrophorid sharks is difficult (Last and Stevens, 2009). The important issue that contributes to the taxonomy of deep-sea chondrichthyans is a high degree of morphological similarity between sibling species. Moreover, many of the holotype or syntypes are either non-existent, cannot be located, incomplete or in poor condition. Chondrichthyans (chimaeras, sharks, rays and skates) are widely distributed in all the world's oceans, but are most diverse in the tropical and subtropical Indo-Pacific Ocean (Bonfil, 2002). According to the International Union for the Conservation of Nature (IUCN), the deep-sea chondrichthyans are those sharks, rays and holocephalans whose distributions are mostly confined to depths below 200 m (Kyne and Simpfendorfer, 2007). Nearly half (48.7%) of the global chondrichthyan fauna are inhabitants of deep-sea ecosystems (Kyne and Simpfendorfer, 2007). Deep-sea chondrichthyans are exploited in few targeted fishing activities but the major share is contributed by bycatch from commercial deep-sea shrimp fishery. Overfishing and bycatch have reduced many populations of these apex predators around the world's oceans (Dulvy *et al.*, 2014).

In Indian waters, many deep-sea species of chondrichthyans have been described during 1890-1970 including *Scyllium hispidum* Alcock, 1891, *Raja powelli* Alcock, 1898, *Centrophorus rossi* Alcock, 1898, *Scyllium quagga* Alcock, 1899, *Raja johannisdavisi* Alcock, 1899, *Apristurus investigatoris* Misra, 1962, *Proscyllium alcocki* Misra, 1950 and *Scyliorhinus silasi* Talwar, 1974. Type materials of all of these species are in poor condition and taking precise morphometric data is almost impossible from these materials. This makes the correct



identification of deep-sea chondrichthyans very difficult. However, many recent taxonomic studies on elasmobranchs conducted on a global scale, supported with molecular approaches, have resulted in changes in the species status (White *et al.*, 2007; Ebert *et al.*, 2010).

Deep-sea skates are an extremely diverse group of fishes, characterized by high morphological conservatism (McEachran and Dunn, 1998). Although many of the species under the families Centrophoridae and Rajidae have been described more than a century ago, the species diversity and their taxonomic resolution is not fully understood (Naylor *et al.*, 2012; Verissimo *et al.*, 2014). The high level of species diversity coupled with morphological and ecological conservatism makes the species identification very difficult in the family Rajidae. There are 11 species of gulper sharks reported from Indian waters with three of questionable status (Akhilesh *et al.*, 2014). Similarly, *Centrophorus* is another most taxonomically complex and confusing group among the elasmobranchs in the Indian waters. There are eight species listed from Indian waters and three of them need confirmation (Akhilesh *et al.*, 2014). In Indian waters, targeted deep-sea shark fishery was reported from Andaman waters and southwest coast of India (off Kollam and Kochi) since 1984 (Mustaffa, 1986; Akhilesh *et al.*, 2011). Along with the targeted catch, deep-sea shrimp bycatch also contribute heavily to the shark fishery (Mathew, 1991). Despite their commercial importance and harvesting, lack of catch and effort data, poor taxonomic resolution of species and misidentifications makes the assessment, sustainable utilization and management of deep-sea sharks extremely difficult.

Species separation in many deep-sea fish families has been problematic since their original description and taxonomic issues remain continues (Verissimo *et al.*, 2014). Species identification using traditional methods of identification based on morphological traits may result in misidentification due to overlapping meristic characters and high phenotypic plasticity. Museum representation is one prerequisite for good taxonomic studies and specimens should be available for future comparisons when required. There are many taxonomic issues contributing to the confusion in the alpha taxonomy of many families of deep-sea fishes such as Chlorophthalmidae, Synodontidae, Myctophidae, Ophidiidae, Lophiidae, Centrophoridae, Rajidae etc. In this situation, morphological methods alone will not be enough to resolve the taxonomic resolution and to find out undiscovered species

in the unexplored deep-sea habitat. There is an urgent need to undertake detailed re-evaluation of deep-sea fishes, including both morphological and molecular assessments for correct species identification. In these situations, alternative tools like DNA based techniques could help to resolve taxonomic issues and support the discovery of new species to the science.

DNA techniques have recently entered the realm of taxonomic studies. The application of molecular tools can provide valuable information for species identification and complement the traditional taxonomic data and validation of the systematic position of any living organism. Accurate identification of fishes is very important, especially in the case of morphologically similar species, for fisheries management, biodiversity, and population studies. Identification of fishes using molecular markers allows rapid and accurate assessment of the diversity of species and the validation of systematic positions and has other applications like forensic identification (Hebert *et al.*, 2003a). Global initiatives, such as the Barcode of Life Database ([www.barcodinglife.org](http://www.barcodinglife.org)) and the Fish Barcode of Life ([www.fishbol.org](http://www.fishbol.org)), DNA based identification systems, are based on a relatively small fragment of the mitochondrial COI gene. The DNA Barcoding technique uses the sequence of a region of the mitochondrial Cytochrome c oxidase subunit I gene for rapid, reliable and accurate species identification of animals (Hebert *et al.*, 2003b), including all their life history stages. The DNA barcoding technique now represents the largest effort to catalogue biodiversity using mitochondrial markers. Despite the broad benefits that molecular taxonomy techniques can bring to a diverse range of biological disciplines, a number of shortcomings still exist (Collins and Cruickshank, 2013). However, the analysis involving DNA sequencing and quantitative morphometric and meristic comparisons has added a new dimension to taxonomic research (Vogler and Monaghan, 2007).

Mitochondrial DNA has been widely used to understand the molecular relationships among individuals, populations and species (Cantatore *et al.*, 1994). The mtDNA sequence variations in the fast evolving regions such as D-loop, ATPase 8/6 can be exceedingly useful for identifying and managing fish stocks (Billington *et al.*, 1992). The ATPase sequences have been successfully used for a variety of purposes such as species identification, egg and larva confirmation and food product authentication. Other sequences of the mtDNA, Cyt b and ND2 genes

are used for species and family-level analysis (Johns and Avise, 1998; Kartavtsev and Lee, 2006; Naylor *et al.*, 2012). 16S rRNA subunits are often used to resolve taxonomic questions and support morphological identifications. The 16S rRNA and COI genes have been useful in resolving taxonomic ambiguities, resurrecting species, and identifying market mislabelling in fishes.

### **Objectives**

In India, most of the coastal fishery resources are fully exploited or over exploited and fishing has been extended to deeper areas and targeted fishery for deep-sea shrimps and chondrichthyans have been initiated and some deep-sea fishes occurring as bycatch are also being consumed along the coast. The available information on the accurate diversity of deep-sea fish fauna from Indian EEZ is very limited. Considering the importance of deep sea fishery resources and their management, the present study was undertaken with the following objectives.

1. To investigate the taxonomic diversity of deep-sea fishes found along the southern coast of India and prepare a database.
2. To generate mitochondrial 16S rRNA and COI sequence signatures of the deep-sea fishes.
3. To analyse the genetic divergence within and between species to resolve taxonomic ambiguity.
4. To describe any new species encountered during the study.

**Chapter 2**

***Review of literature***

## Chapter- 2

### Review of literature

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Fishes constitute slightly more than half of the recognised living vertebrates across the world (Nelson, 2006). They make a vital contribution, as animal protein, to the survival and health of a significant portion of the global population. The number of valid fish species recorded so far is more than 32000, with the addition, at an average, of 100-150/year (Eschmeyer *et al.*, 2010; Fricke and Eschmeyer, 2010). The increase in the number of fish species in the recent years can be attributed to more explorations and expeditions in new areas and at greater depths, application of molecular taxonomy and understanding the importance of biodiversity and its cataloguing. Marine fishes are those which spend at least some stage of their life cycle in the sea. Marine fishes live in very diverse habitats such as coral reefs, deep coral areas, seamounts, islands and deep-continental slope areas. The number of valid marine fish species (16,764), is almost equal to that of freshwater fish (15,170) (Eschmeyer *et al.*, 2010).

Fish endemism is reported widely in the marine fish fauna of the Mediterranean Sea, the Red Sea and the Mascarene Islands (Eschmeyer *et al.*, 2010). Mora *et al.*, (2008) estimated that global marine fish inventory is about 79% complete, or 21% still remain to be discovered. However, Eschmeyer *et al.*, (2010) commented that species from some habitats are under-represented in their data and concluded that two habitats, the deep-reef and deep-slope areas, where new marine taxa are mostly found, are poorly sampled and studied so far. The diverse ocean and coastal habitats harbour a wide range of fish biodiversity. The total number of recorded marine fish species is less than that of terrestrial habitats. It is because of the fact that marine diversity has not been fully understood due to logistic constraints in exploration, collection and identification of specimens. India has a rich natural heritage and nurtures a unique biodiversity, placing it among the 12 most biodiverse countries. Out of the nearly 32,000 fish species, 2,553 are known from Indian waters (NBFGR, 2013).

## 2.1 Deep-sea fishes

The deep-sea fishes are those living at depths greater than 200 m and they are categorized into mesopelagic, benthopelagic and bathypelagic fishes. Deep-sea fishes have developed different adaptations for biological and life history parameters or characters to manage with unique environmental conditions found in the deep-sea habitat. These fishes are characterised by special adaptations such as extremely large eyes, the presence of bioluminescence, strong sense of smell, body composition, and expandable stomachs for their survival in the extreme environmental conditions. Most of the deep-sea fisheries have low productivity and therefore only able to sustain very low exploitation rates because of many factors, including exceptional longevity, delayed maturity, slow growth, low specific fecundity, low natural mortality rates, intermittent recruitment of successful year classes and spawning that may not occur every year (Roff, 1984; Pankhurst and Conroy, 1987; Conroy and Pankhurst, 1989; Koslow, 1989). These extreme life-history characteristics have intense implications for conservation and management of deep-sea fishery resources. With the expansion of global fisheries, many deepwater habitats such as seamounts, banks, deep coral areas with the great aggregations of benthopelagic fishes have been discovered for commercial exploitation. The major dominant species found in these habitats are orange roughy, oreosomatids, Patagonian toothfish, and pelagic armorhead fishes (Boehlert and Sasaki, 1988; Koslow, 1996). The Atlantic and Pacific Oceans are well studied, compared to the Indian Ocean, with respect to the taxonomy and biology of deep-sea fishes. In the Indian Ocean, most of the works are limited to the Arabian Gulf, Madagascar, South Africa, Somalia, Mozambique on the western side and South and West of Australia in the East (Atkinson, 1995; Clark, 1995; Haedrich *et al.*, 2001).

## 2.2 Deep-sea fishes of India

The major expeditions by R.I.M.S. *Investigator* in the Indian Ocean and adjacent seas during the period 1884-1914 and 1921-1926, which surveyed 711 stations, with the results published as *A Descriptive Catalogue of the Indian deep-sea fishes in the Indian museum*, are the major work on deep-sea fishes of India. Alcock's publications (1889, 1898 and 1899) gave a detailed account of deep-sea fishes of the

Indian seas, describing several new fishes. The *Valdivia* expedition (1898-1899) covered 12 stations in the Bay of Bengal area and sampled at depths of 296-2500 m. The John Murray expedition carried out during the years 1933-1934 surveyed 212 stations in the Indian Ocean at depths of 11-5106 m. The trawler *Golden Crown* (1908-1909) used in commercial fishing made many trips and the collections were complementary to those made by the R.I.M.S. *Investigator* in shallow waters. The International Indian Ocean Expedition (1959-1963, 1962, 1963 and 1964) explored the Indian Ocean including adjacent seas.

India is the fifth largest fishing nation of the world with an Exclusive Economic Zone (EEZ) of 2.02 million sq. km. Out of the current fishery resource potential of 4.41 million tonnes of annual harvestable resources from the Indian EEZ, the available 2.2 million tonnes from the inshore area is almost fully exploited, leaving scope for further exploitation by utilizing offshore and deep-sea zones (Vivekanandan *et al.*, 2005). Deep-sea fishery resources of India have been studied by various researchers; mainly on taxonomy, distribution, abundance and fishery (James and Pillai, 1990; Zacharia *et al.*, 1991). Many deepwater species have been reported from Indian waters during the 1960s and 1970s (Jones and Kumaran, 1964; 1965; Tholasilingtam *et al.*, 1964; Silas and Regunathan, 1974). However, there have been few studies on the deep-sea resources beyond 250 m depth (Venu and Kurup, 2002; Jayaprakash *et al.*, 2006; Sajeevan *et al.*, 2009). The exploratory survey by FORV *Sagar Sampada* has brought out many rare deep-sea fishes collected beyond 200 m depth. The major studies based on these collections include Sivakami *et al.*, (1998); Kurup *et al.*, (2005) and Jayaprakash *et al.*, (2006).

The study on the abundance of the deep-sea fishes has been carried out by many fisheries scientists, as providing a potential underexploited resource for a variety of human uses and also studies on the distribution and life history traits have been carried out (Kurup *et al.*, 2006). Philip (1994) studied the fishes of the family Priacanthidae from the Indian waters and reported five species. Exploratory survey conducted along the Indian EEZ have revealed higher concentrations of priacanthids along the west coast than the east coast (James and Pillai, 1990; Sivakami, 1990). Fishery Survey of India also carried out research on deep-sea fishes from Indian EEZ

(Joseph, 1984; Philip *et al.*, 1984; Oommen, 1985). A checklist of fishes of 87 families and 242 species from the Indian EEZ based on the collections of *FORV Sagar Sampada* was compiled by Balachandran and Nazar (1990). Manjebrayakath *et al.*, (2009) reported 126 species belonging to 29 families from the Indian EEZ based on the exploratory surveys conducted by *FORV Sagar Sampada*. Recently, many studies on the taxonomy of deep-sea fishes from the southern coast of India have been published, including the redescription of *Glyptophidium oceanium* from the west coast (Kurup *et al.*, 2009), deep-sea cusk eel *Bassozetus robustus* (Cubelio *et al.*, 2009a), *Dicrolene nigricaudis* (Cubelio *et al.*, 2009b) and rare batfish species *Halicmetus ruber* (Benjamin *et al.*, 2013).

Abdussamad *et al.*, (2011) commented that snake mackerels of the family Gempylidae formed a regular fishery along the Tuticorin coast and are exploited by deep-sea trawlers operating beyond 200 m depth zone. Based on the deep-sea shrimp trawl bycatch, several interesting deep-sea fishes were reported from Tuticorin (Kannan *et al.*, 2013a, b; Kannan *et al.*, 2014). The need for exploitation of deep-sea fishes is gradually gaining importance in the recent years as the production from the present fishing grounds alone would not be able to meet the future nutritional demand (Vivekanandan, 2006). So it's essential to understand the exploited species and other resources.

### **2.3 Deep-sea chondrichthyans diversity**

India has a long history of elasmobranch fishery and is one of the leading chondrichthyan fishing nations with an estimated landing of 52,602 tonnes (sharks 44.6%, rays 51.5% and skates 3.9%) in 2012 accounting for 1.3% of the total marine fish landings in the country (CMFRI, 2013). The deep-sea chondrichthyans caught mainly as bycatch are utilized for extracting oil for squalene, and meat is filleted, salted and dried. The important families contributing the deep-sea fishery include Centrophoridae, Rhinochimaeridae, Echinorhinidae, Squalidae and Hexanchidae. Targeted shark fishery by mechanized vessels has developed in Thoothoor of Tamil Nadu and Andaman waters for catching deep-sea sharks (Mustaffa, 1986; Vivekanandan, 2001). Deep-sea shark fishery was established in Kochi and the catches



are mainly caught as bycatch from shrimp fishery from the southwest coast of India (Akhilesh *et al.*, 2011; Akhilesh *et al.*, 2013).

Pioneering deep-sea chondrichthyan research in Indian waters was conducted by Alcock (1899) based on the materials collected by the survey of HMS *Investigator*. Based on these collections, Alcock described several new species of elasmobranchs from Indian waters. The diversity of deep-sea chondrichthyan species along the coast are poorly known, and are known from very few scattered studies. However the diversity is considered to be higher than thought earlier (Silas *et al.*, 1969; Nair and Lal Mohan, 1973; Akhilesh *et al.*, 2010, 2014; Ramachandran *et al.*, 2014). Despite the rich deep-sea elasmobranch diversity, only one new species of shark *Mustelus manglorensis* have been described from Indian waters in the past one decade (Cubelio *et al.*, 2011).

#### **2.4 Molecular techniques in fish taxonomy**

The basic knowledge of diversity through species discovery and description is mostly complete for many families of fishes, but important gaps still remain (Eschmeyer *et al.*, 2010). Ichthyologists use traditional morphological methods to study distinctiveness and relationships among fishes. In morphological studies, morphometric and meristic data are used. Cryptic species have been described in a variety of habitats such as rocky reefs, coral reefs, mesopelagic environment, the Antarctic, and in invading organisms (Bernardi and Goswami, 1997; Bucciarelli *et al.*, 2002). Kon *et al.*, (2007) used DNA sequences to identify cryptic species present in the gobioid fish *Schindleria*. The open ocean, which was long thought as an area of large panmictic populations, has recently become the focus of genetic research that reveals significant genetic discontinuities (Unal and Bucklin, 2010; Zahuranec *et al.*, 2012). In recent years, molecular taxonomic studies have begun to prove their worth, especially when compared with morphology (Ebert *et al.*, 2010; Akhilesh *et al.*, 2012; Iwatsuki *et al.*, 2013; Allen *et al.*, 2013). Despite the broad benefits that molecular taxonomy techniques can bring to a diverse range of biological disciplines, a number of shortcomings still exist (Collins and Cruickshank, 2013). However, the molecular tools and quantitative morphometric comparisons added a new dimension to taxonomic research (Vogler and Monaghan, 2007).

Several decades ago, electrophoresis of proteins by starch gel was first used to identify species (Manwell and Baker, 1963), and then single gene sequence analysis of ribosomal DNA was being used for higher level evolutionary studies (Woese and Fox, 1977). The traditional molecular techniques for species identification of fish depend on detecting protein variations with starch gel electrophoresis (Wang *et al.*, 1984), and liquid chromatography (Osman *et al.*, 1987). The discovery of PCR had a major impact on the research on eukaryotic genomes and various molecular markers were developed for fish genetics. Later, a number of different methods have been designed by various researchers for species identification. The important methods are species-specific PCR (Liu, 2004), PCR restriction fragment length polymorphism (PCR-RFLP) (Brunner *et al.*, 2002), multiplex PCR (Kengne *et al.*, 2001) and Random Amplified Polymorphic DNA (RAPD) (Lakra *et al.*, 2007).

## 2.5 Mitochondrial DNA (mt DNA)

The mitochondrial genome is a small and double stranded circular DNA molecule. It is haploid i.e. each mitochondrion contains only one type of mt DNA, which is cytoplasmically inherited, thus making it predominantly maternally transmitted. Molecular methods have emerged in the form of DNA barcodes (Hebert *et al.*, 2003a; Lane *et al.*, 2007, Lakra *et al.*, 2009) and widely employed in phylogenetic studies because it evolves much more rapidly than nuclear DNA, resulting in the accumulation of differences between closely related species (Timm *et al.*, 2008; Lakra *et al.*, 2011; Zahuranec *et al.*, 2012). Among the DNA markers targeted, it appeared that most studied are mitochondrial genes. The short sequences of 16S rRNA, cytochrome c oxidase I (COI) and NADH2 are extensively used to identify species with a high level of accuracy (Hebert *et al.*, 2003b; Ward *et al.*, 2005; Ward *et al.*, 2008a; Lakra *et al.*, 2011; Naylor *et al.*, 2012; Bineesh *et al.*, 2014).

Mitochondrial DNA has been widely used to understand the molecular relationships among individuals, populations and species (Cantatore *et al.*, 1994). Because of its small size, high abundance in the cell, maternal inheritance and evolutionary rate, it has become useful in evolutionary studies (Curole and Kocher, 1999). But the full history of species is not always reflected due to the maternal

inheritance (Ballard and Whitlock, 2004). Mitochondrial DNA sequence data have been widely used for inferring phylogenetic relationships and species identity in decapod crustaceans (Harrison and Crespi, 1999; Schubart *et al.*, 2001) and in fishes (Miya *et al.*, 2003; Chakraborty and Iwatsuki, 2006; Lakra *et al.*, 2008). Many regions of the mitochondrial DNA have been used for various purposes such as population studies (Ovenden *et al.*, 1993; Zhu *et al.*, 1994) and phylogenetic relationships (Johns and Avise, 1998; Hebert *et al.*, 2004b; Kartavtsev and Lee, 2006). The mtDNA sequence variations in the fast evolving regions such as D-loop, ATPase 8/6 can be exceedingly useful for identifying and managing fish stocks (Grewe and Hebert, 1988; Billington *et al.*, 1992). Other sequences among the protein-coding genes of the mtDNA, Cyt b and COI genes are used for species and family-level analysis (Johns and Avise, 1998; Hebert *et al.*, 2004b; Kartavtsev and Lee, 2006). The ATPase sequences have been used successfully for a variety of purposes such as species identification (Dalmasso *et al.*, 2006; Reid and Wilson, 2006) and egg and larva confirmation (Fox *et al.*, 2005).

Some markers like control region sequence analysis are used to evaluate regional endemism by examining the genetic structure of widespread species (Drew *et al.*, 2008). Some of the freshwater fishes of the genus *Anguilla* having unique geographic distribution have attracted more molecular phylogenetic studies (Tagliavini *et al.*, 1995, 1996; Aoyama *et al.*, 1996, 2001; Tsukamoto and Aoyama, 1998; Aoyama and Tsukamoto, 1997; Lehmann *et al.*, 2000; Lin *et al.*, 2001; Minegishi, 2005). The use of complete mitochondrial genome sequences for taxonomic relationship and phylogeny in fishes has been reported (Miya *et al.*, 2003; Minegishi, 2005; Peng *et al.*, 2006; Miya *et al.*, 2007) which have successfully resolved some controversial phylogenetic relationships (Inoue *et al.*, 2001). With the advent and application of molecular phylogeny, significant progress have been observed in resolving taxonomic ambiguities in the various families, particularly combining data from both molecular biology and traditional biology (Hedges and Poling, 1999; Giribet *et al.*, 2001; Saitoh *et al.*, 2006; Li *et al.*, 2009). Molecular phylogeny of freshwater fishes of the family Cyprinidae was investigated using the mitochondrial cytochrome b sequences by Colli *et al.*, (2009).

### 2.5.1 16S ribosomal RNA (16S rRNA)

The mitochondrial 16S rRNA gene has been widely used to explore the phylogenetic relationships of fishes (Quenouille *et al.*, 2004; Almada *et al.*, 2009), shrimps (Baeza *et al.*, 2009) and cuttlefishes (Anderson *et al.*, 2010). The most recent and extensive phylogenetic study of Pleuronectiformes using sequences of 12S and 16S rRNA mitochondrial genes was done by Azevedo *et al.* (2008). Douady *et al.*, (2003) used mitochondrial 16S rRNA along with 12S and tRNA valine genes from over 20 elasmobranch species to show that batoids are separated from sharks, and that sharks are monophyletic with *Squatina* and pristiophoriforms being squalomorphs.

16S rRNA subunits are often used to resolve the taxonomic questions and support the morphological identifications. The taxonomic classification of members of the genus *Trichiurus* of the family Trichiuridae is confusing, due to the similar morphology and colouration. A rapid, reliable and simple method based on PCR-RFLP was developed to accurately identify the three closely related species of hairtail based on 16S rRNA gene (Chakraborty *et al.*, 2005). Lakra *et al.* (2009) has used the partial sequences of 16S rRNA and COI genes for species identification and phylogenetic relationships of the seven species of sciaenids from Indian waters. Cui *et al.*, (2010) resolved the taxonomic ambiguities in the five species of genus *Pampus* using mitochondrial 16S rRNA and COI genes in combination with morphological characteristics.

Di Finizio *et al.*, (2007) identified species of the family Gadidae by sequencing and PCR-RFLP analysis of 12S and 16S rRNA gene fragments. Similarly, Chakraborty *et al.*, (2007) has developed PCR-RFLP analysis for species identification of hairtail fish fillets from supermarkets in Japan. This research developed a rapid and reliable, simple and inexpensive method for identification of the hairtail species composition in commercial fillets. Many other researchers extensively used 16S rRNA gene marker to develop RFLP based identification of fishes and fish products in rockfish (Klossa-Kilia *et al.*, 2002; Trotta *et al.*, 2005; Li *et al.*, 2006), and species identification and phylogeny (Watanabe *et al.*, 2004; Greig *et al.*, 2005; Karaïskou *et al.*, 2005).

## 2.5.2 Cytochrome c oxidase subunit I (COI)

DNA barcoding uses the sequence of a region of the mitochondrial Cytochrome c oxidase subunit I (COI) gene for rapid and accurate species identification of animals (Hebert *et al.*, 2003a), including all their life history stages. Specific DNA sequences act as unrepeatable signatures and, therefore, constitute a unique DNA barcode for each species. Hebert *et al.*, (2004a, b) showed that the COI gene can discriminate between closely related species across diverse animal phyla. This barcoding system relies on the observation that COI sequence divergence between most congeneric species is generally greater than 2% (Hebert *et al.*, 2003b), whereas intraspecific variation is lower than 1% (Avice, 2000). Barcode efficiency can be further improved by the simultaneous use of two genes showing different evolutionary rates and genomic positions. This approach has been very successful in discriminating marine and freshwater fish species (Hajibabaei *et al.*, 2005; Ward *et al.*, 2005; Hubert *et al.*, 2008; Ward *et al.*, 2009).

## 2.5.3 DNA barcoding technique

Molecular methods in the form of DNA barcodes have been used to differentiate species and identify cryptic species (Hebert *et al.*, 2003b; Lane *et al.*, 2007; Lakra *et al.*, 2009; Cerutti-Pereyra *et al.*, 2012). Mitochondrial DNA has been extensively used in fish phylogenetics, since mitochondrial 16S rRNA gene and the protein coding cytochrome c oxidase subunit I (COI) gene are highly conserved compared to nuclear DNA, resulting in the accumulation of differences between species (Santos *et al.*, 2003; Vinson *et al.*, 2004; Timm *et al.*, 2008). The 16S rRNA and COI genes have been useful in resolving taxonomic ambiguities, resurrecting species, and identifying market mislabelling in fishes (Ward *et al.*, 2005; Iglesias *et al.*, 2010; Iwatsuki, 2013; Iwatsuki *et al.*, 2012; Lee *et al.*, 2013; Keskin and Atar, 2012).

Works on DNA barcoding of Indian fishes have been quite limited. Lakra *et al.*, (2009) has analyzed partial sequences of 16S rRNA and COI genes for species identification and phylogenetic relationships among the commercially important Indian species of sciaenids. Persis *et al.*, (2009) sequenced and proved the utility of the COI gene for carangids identification from Kakinada coast. In a more comprehensive study, Lakra *et al.*, (2011) barcoded 115 species of commercially important marine fishes

collected from east and west coast of India. Recently, many successful nationwide studies on freshwater fishes (Lakra *et al.*, 2010; Benziger *et al.*, 2011; Pandey *et al.*, 2012; Bhattacharjee *et al.*, 2012; Malakar *et al.*, 2012; Laskar *et al.*, 2013; Khare *et al.*, 2014; Chakraborty and Ghosh, 2014a; Khedkar *et al.*, 2014), catfishes (Chakraborty and Ghosh, 2014b), estuarine fishes (Krishna *et al.*, 2012; Viswambharan *et al.*, 2013), marine fishes (Khan *et al.*, 2010; John *et al.*, 2011; Lakra *et al.*, 2013; Rahman *et al.*, 2013; Basheer *et al.*, 2014), sharks and rays (Pavan-Kumar *et al.*, 2014; Pavan-Kumar *et al.*, 2015) have been undertaken using this method.

DNA-based identification of sharks was achieved by sequencing the mitochondrial DNA, including parts of the cytochrome b and threonine tRNA genes, for eleven species of carcharhiniform sharks (Heist and Gold, 1999). Greig *et al.*, (2005) sequenced 35 species of North Atlantic sharks. Ward *et al.*, (2005) strongly validated the efficacy of COI barcodes for identifying chondrichthyans by sequencing 61 species of sharks and rays from Australian waters. Spies *et al.*, (2006) showed the utility of DNA barcodes as a robust method for discriminating 15 skate species of North Pacific Ocean and Bering Sea. Ward *et al.*, (2008b) barcoded 210 species of sharks and rays from Australian waters, showing the utility of the barcode approach for helping to resolve taxonomic issues and for new species discovery. Holmes *et al.*, (2009) used DNA barcoding to identify shark and ray species from dried fins from northern Australian waters, showing that such data can be used by enforcement authorities to manage chondrichthyans species. Santander-Neto *et al.*, (2011) successfully identified a shark carcass by DNA barcoding.

## **2.6 Deep-sea fish identification**

Deep-sea fishes remain among the least explored vertebrate groups. They have an important place in marine ecosystem with high ecological and economic values. Accurate identification is required as a basis for scientific studies (Bely and Weisblat, 2006; Bortolus, 2008). Taxonomic ambiguity exists in many families with poor qualities of type series, bad conditions of trawled samples, sex differences, lack of specialized taxonomist etc. making the identification of deep-sea fishes very difficult. Along with above, the presence of cryptic species in the marine environment is confirmed very

recently, making the management of fishery resources a difficult task (Kon *et al.*, 2007; Steinke *et al.*, 2009; Zemlak, *et al.*, 2009; Zahuranec *et al.*, 2012). Several decades ago, electrophoresis of proteins by starch gel was first used to identify species (Manwell and Baker, 1963), and then single gene sequence analysis of ribosomal DNA was being used for higher level evolutionary studies (Woese and Fox, 1977). The mitochondrial DNA methods were used extensively for molecular systematics during the late 1970s and 1980s (Avice, 1994). Hebert *et al.*, (2003a) proposed that a single gene sequence would be sufficient to differentiate all animal species, and proposed the use of the mitochondrial DNA gene cytochrome C oxidase subunit I (COI) as a global bioidentification system for animals. DNA barcoding based identification has been used for wide range of animal species from sponges to mammals, but has recently taken a new dimension through many large scale projects (Ratnasingham and Hebert, 2007). Ward *et al.*, (2005) barcoded many families of deepwater chondrichthyans successfully and discussed the importance of keeping voucher specimens for future examinations. Ward *et al.*, (2008a) assessed the intraspecies barcode variability for 15 species of marine fishes sampled from Northern (Atlantic and Mediterranean) and Southern (Australasian) Hemisphere waters. The results show thirteen species with no significant evidence of spatial genetic differentiation and two species with cryptic speciation. The deep water sharks are taken as bycatch in commercial fisheries catches and misidentifications are common for many deepwater species. The COI sequence has been developed for 210 species of sharks and rays from 36 families, permitting the discrimination of 99.0% of these species from Australia (Ward *et al.*, 2008b). There are morphology based identification problems among various genera of elasmobranchii, *Centrophorus*, *Centroscymnus*, *Apristurus*, *Squalus*, *Dipturus* etc. Many successful attempts have been made to test the suitability of the DNA barcode approach to discriminate the chondrichthyan species for fisheries management and conservation (De Astarloa *et al.*, 2008; Moura *et al.*, 2008; White *et al.*, 2013).

Ward *et al.*, (2007) used DNA barcoding to confirm the existence of eleven newly described *Squalus* species and verified that barcoding could be used in species identification in groups that are morphologically difficult to distinguish by non-experts. Further research on these groups of sharks led to the resurrection and redescription of

*Squalus suckleyi* from the North Pacific by combining the use of meristic, morphological and molecular data which revealed that *Squalus suckleyi* is clearly distinct from the widespread *Squalus acanthias* (Ebert *et al.*, 2010). Mesopelagic fishes are another important group of fishes with much taxonomic confusion. Fishes of the order Myctophiformes (Teleostei; Scopelomorpha) comprise over half of all deep-sea biomass, and are a critical component of the marine ecosystems worldwide. The identification and phylogenetic studies for the family myctophidae have been carried out using COI as one of the markers (Yamaguchi *et al.*, 2000; Zahuranec *et al.*, 2012; Denton, 2014). Recently, more comprehensive studies have been conducted in the Antarctic Ocean to know the accurate number of species present (Dettai *et al.*, 2011). This large scale project underlines the need for further taxonomic work in Antarctic actinopterygians.

## 2.7 New species descriptions

New fish species descriptions vary widely over time due to many reasons such as the advent of SCUBA, use of ichthyocides, new explorations, use of molecular techniques etc. New explorations coupled with new techniques significantly contribute the study of deep-sea and mid water species (Eschmeyer, 2010). Recently, different molecular markers have been used to support the morphological based descriptions of many fish species. De Astarloa *et al.*, (2008) described a new deepwater skate species, *Dipturus argentinensis* with independent morphological and mitochondrial COI support. A new species of goatfish, *Upeneus heemstra* was described from the Western Indian Ocean and SE India based on DNA barcoding and quantitative morphological screening by Uiblein and Gouws, (2014). Allen *et al.*, (2013) had described two new species of snappers by combining morphological data with a comparison of the mitochondrial cytochrome c oxidase subunit 1 (CO1) genetic marker. Mohapatra *et al.*, (2013) had used COI genetic divergence to support the morphological description of new species of *Hapalogenys bengalensis*. Molecular markers such as COI, 16S rRNA and NADH2 support the redescrptions and resurrections of many fish species. Naylor *et al.*, (2012) used NADH2 sequences for the DNA based approach for the identification of shark and ray species that lead to the rediscovery and the discovery of many



chondrichthyan species globally. The status of the North Pacific *Squalus suckleyi* was revealed by combining the use of meristic, morphological and molecular data (Ebert *et al.*, 2010). White *et al.*, (2013) revised the genus *Centrophorus* with redescription of *Centrophorus granulosus* using the support data of NADH2 marker.

## 2.8 Trade, mislabelling, diet and forensic uses

Detection of improper labelling of raw and processed seafood, including fishes and crustaceans, gets greater attention for reducing commercial fraud and enhancing food safety. The labelling process for seafood products becomes complicated because most of the identification characters are absent. Therefore, application of molecular tools like DNA barcoding could help in the easy and reliable identification of processed seafood products. Vartak *et al.*, (2015) developed molecular reference COI barcodes for 11 species of edible crab from India and the study demonstrated mislabelling of restaurant samples. Several works have been carried out to verify the reliability and to evaluate the amount of commercial fraud in surimi productions and other market substitution using DNA barcoding technique (Pepe *et al.*, 2007; Wong and Hanner, 2008; Barbuto *et al.*, 2010; Keskin and Atar, 2012).

The identification of fish species in processed seafood has become a very important issue now days. Mislabelling of fish products defrauds consumers and also adversely affect estimates of stock size if it influences the reporting of catch data that are used in fisheries management. Bottero *et al.*, (2003) has designed universal primer for the detection of animal tissues in feedstuff. The mislabelling of a fish product may be unintentional if, for example, species that are morphologically similar are caught together, such as in many tropical or coral reef fisheries (Marko *et al.*, 2004; Ardura *et al.*, 2010; Iglésias *et al.*, 2010). Similarly, mislabelling may not be accidental, such as where product substitutions are from species that do not occur in the same ocean and mixing with low value species (Barbuto *et al.*, 2010; Von der Heyden *et al.*, 2010; Miller *et al.*, 2010). Maretto *et al.*, (2010) developed a SNPs based analysis in a highly conserved 16s rRNA gene region of four different species belonging to the order Gadiformes (*Gadus macrocephalus*, *Gadus morhua*, *Melanogrammus aeglefinus* and *Molva molva*) to discriminate the four different species with a single analysis. Carrera *et*

*al.*, (1999) had used the polymerase chain reaction amplification of selected regions of the mitochondrial 16S rRNA gene, followed by restriction site analysis of amplified DNA fragments to differentiate smoked and raw samples of Atlantic salmon from rainbow trout. In a more comprehensive study, Helyar *et al.*, (2014) published a study encompassing a large-scale assessment of UK retailers using species specific real-time PCR probes and mitochondrial COI gene, indicating a potentially significant incidence of incorrect product designation.

Aoyama *et al.*, (2001) used a molecular approach with the 16S rRNA gene to identify the eggs of the Japanese eel *Anguilla japonica* with respect to the determination of the spawning site. Akimoto *et al.*, (2002) amplified the mitochondrial 16S rRNA gene using total DNA as a template from muscle tissues of alfonso and related fish species of alfonso, *Beryx splendens*, *Beryx mollis* and *Beryx decadactylus*. They identified pelagic eggs, which were collected off Izu as *B. splendens* from the nucleotide sequence of the mitochondrial 16S rRNA gene showing the effectiveness for larval identification. As technology advanced, even formaldehyde fixed samples have been identified using various molecular markers. Perez *et al.*, (2005) genetically identified hake and megrim eggs that were fixed in formaldehyde solutions.

Trophic relationships among the species within an ecosystem is a key part of many ecological studies. The identification of dietary component from animal guts is a difficult task and most of the studies report generic level identification due to the absence of important species diagnostic characteristics which are lost during the process of prey digestion. In some cases the hard parts such as otoliths or skeletal parts which are resistant to digestion can be identified to genus or species level only by experts (Smith *et al.*, 2005; Casper *et al.*, 2007). The sequence analysis of the 16S rRNA gene has been used in the ecological studies. The DNA of prey present in animal scats provides a valuable source of information for dietary studies. Many researchers used 16S rRNA gene for developing molecular methods for genetic identification of prey items from various species (Rosel and Kocher, 2002; Purcell *et al.*, 2004). Smith *et al.*, (2005) developed a DNA sequencing technique to identify partially digested prey items taken from the gut content of seven species of large pelagic fishes. In similar ways, Parsons *et al.*, (2005) used the mitochondrial 16S rRNA and cytochrome b genes based

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DNA identification of salmonid prey species in pinniped faecal samples and to distinguish between sea trout *Salmo trutta* and Atlantic salmon *Salmo salar*.

The sequence analysis of the 16S rRNA gene lacked the ability to resolve relationships in some marine taxa such as sparids and percoid fishes (Orrell and Carpenter, 2004). However, Lakra *et al.*, (2009) reported high nucleotide divergence among the sciaenid species in the Indian waters using 16S rRNA gene sequences and Iwatsuki, (2013) also shows similar results in *Acanthopagrus latus* complex, indicating the effectiveness of 16S rRNA gene sequence for species discovery and accurate identification of fish species. de los Angeles *et al.*, (2005) used the inter-specific variation of the mitochondrial r16S gene among silversides for species identification.

Despite a large number of aquatic animal species being regulated by laws, illegal trade still persists throughout the world through the transport of dried, powdered or processed products and juvenile species trafficking. The customs authorities cannot identify the parts of protected species unless they use high-tech novel technology using molecular methods. The enforcement authority could use DNA barcoding for accurate species identification of illegally traded marine fish stocks and for regulating and monitoring trade when morphological keys are not present (Baker *et al.*, 2000; Shivji *et al.*, 2002; Barbuto *et al.*, 2010; Iglésias *et al.*, 2010; Asis *et al.*, 2014).

The accurate identification of fish eggs and larvae to the species level are very important fundamentals of ecological monitoring, environmental impact assessment, fishery compensation, fisheries resource management and in the setup of marine protected areas (Moura *et al.*, 2008; Valdez-Moreno *et al.*, 2010). The identification of larval fishes is a very difficult task due to insufficient morphological identification characters. The experiments and other research conclude that DNA barcoding remains one of the best methods to confirm species identification in larval fishes (Shao *et al.*, 2002; Victor *et al.*, 2009; Matarese *et al.*, 2011; Ko *et al.*, 2013), larval shrimps (Barber and Boyce, 2006), and marine invertebrate larva (Heimeier *et al.*, 2010). However, identifying gastropod spawn from DNA barcodes was unsuccessful, emphasizing the need to develop specific DNA barcoding projects for taxonomically challenging groups such as molluscs (Puillandre *et al.*, 2009). Recently, the application of DNA barcoding

to larval fish identification has turned out to be a popular scientific research area and many successful studies have been undertaken using this method (Pegg *et al.*, 2006; Webb *et al.*, 2006; Paine *et al.*, 2008; Hubert *et al.*, 2010; Kim *et al.*, 2010; Baldwin *et al.*, 2011; Ko *et al.*, 2013). DNA barcoding is widely used in identification studies for genetic identification of prey items from various species (Rosel and Kocher, 2002; Purcell *et al.*, 2004). Recently, this method has been used for fish prey identification (Rosel and Kocher, 2002; Smith *et al.*, 2005).

To overcome the issue of taxonomic ambiguities largely prevailing in deep water fisheries, the present study aimed at species specific molecular signatures using mitochondrial COI and 16S rRNA genes. The aim of the study is not only resolving ambiguities in proper identification for conservation and management of the less studied deep-sea fishes distributed at the depth ranges of 200–1000 metres in the sea but also the important task of identifying species new to science.

**Chapter 3**

***Taxonomy of selected deep-sea fishes***

## Chapter 3

### Taxonomy of selected deep-sea fishes

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Fishes constitute more than half of the vertebrates (Eschmeyer, 2010) and show an amazing diversity in shapes, size, colour and distributions. The practise of fish taxonomy is a prerequisite in studies of natural history, ecology, fishery management and conservation, egg and larval identification, authentication of food products and illegal trade monitoring (Teletchea, 2009; Victor *et al.*, 2009). The increasing international concern about protecting the biodiversity and species gives more efforts in fish taxonomy with increased explorations in many countries including India. Redescriptions of poorly described species are very important for taxonomical practises.

The Arabian Sea with its unique ecological features such as position between two land masses, presence of islands, features like oxygen minimum zone (OMZ), circulation pattern, currents, influence of monsoon and high saline water intrusion from Persian Gulf and Red Sea, etc. supports a very diverse ichthyofauna. In the last decade, a few number of new species and records of marine fishes along the Indian coast have been observed. The taxonomy of deep sea fishes in India is indebted to the outstanding work of Lt. Col. A. W. Alcock, *C.I.E., F.R.S.* on the samples collected during the voyage of the Indian marine survey steamer, HMS *Investigator* and his contributions are included in his publications during 1889-1907. The recent studies on deep sea fish taxonomy from the Indian EEZ include the documentation and redescription of *Glyptophidium oceanium* from the west coast (Kurup *et al.*, 2009), *Bassozetus robustus* (Cubelio *et al.*, 2009a) and *Dicrolene nigricaudis* (Cubelio *et al.*, 2009b). Akhilesh *et al.*, (2012, 2010) studied deep-sea chondrichthyan fauna and recorded many additional species from the deep-waters of south west coast of India. This chapter provides detailed morphological descriptions of poorly described species, resurrection of synonymised species and new distributional records of deep-sea species that came across during the present study.

### 3.1 Redescriptions

The serranid fish genus *Chelidoperca*, proposed by Boulenger (1895) for *Centropristis hirundinaceus* Valenciennes, 1831, comprises seven species; *Chelidoperca hirundinacea* (Valenciennes, 1831), *C. pleurospilus* (Günther, 1880), *C. lecromi* Fourmanoir, 1982, *C. investigatoris* (Alcock, 1890), *C. margaritifera* Weber, 1913, *C. occipitalis* Kotthaus, 1973 and *C. maculicauda* Bineesh and Akhilesh, 2013 (Bineesh *et al.*, 2013).

Members of the *Chelidoperca* are usually found on the continental shelf and slope with muddy bottoms in the Indo-West Pacific (Nelson, 2006; Bineesh *et al.*, 2013). Three species of *Chelidoperca*, namely *C. investigatoris*, *C. occipitalis* and *C. maculicauda* are known from the Arabian Sea (Baranes and Golani, 1993; Manilo and Bogorodsky, 2003; Jayaprakash *et al.*, 2006; Sajeevan *et al.*, 2009; Bineesh *et al.*, 2013). However, *C. investigatoris* and *C. maculicauda* are the only valid species of the genus known from the Indian Exclusive Economic Zone (Bineesh *et al.*, 2013). This study provides a new report of the *Chelidoperca occipitalis* from southern India and provides a redescription of *Chelidoperca occipitalis* and *C. investigatoris* based on recently collected materials from the southwest coast of India.

Specimens of *C. investigatoris* and *C. occipitalis* were collected from commercial deep sea shrimp trawl bycatch landings operated in the Arabian Sea, off Kollam during 2009-2010. These were landed at Sakthikulangara Fisheries Harbour, Kollam (Quilon), Kerala. Species were identified following Alcock, (1890), Kotthaus, (1973), Senou, (2002) and Park *et al.*, (2007). Morphometric measurements of formalin (5%) preserved specimens were taken following Hubbs and Lagler., (1964). The specimens are deposited at Central Marine Fisheries Research Institute (CMFRI), Kochi, Kerala and Zoological Survey of India (ZSI), Kolkata India.

#### 3.1.1 *Chelidoperca investigatoris* (Alcock, 1890)

Indian perchlet

(Figure 3.1, 3.2; Table 3.1, 3.3)

*Chelidoperca investigatoris* (Alcock, 1890) (Syntypes: ZSI; off Chennai). Jayaprakash *et al.*, (2006) (list, India). Sajeevan *et al.*, (2009) (list, India). Bineesh *et al.*, (2013).

**Diagnosis.** A species of *Chelidoperca* with dorsal-fin rays X, 10, fourth spine largest (3.14-3.37 in HL) longer than 3<sup>rd</sup> spine; body depth 26.23-27.82% SL; head length 41.95-48.71% SL; orbit length 8.85-11.06 in SL; 2.5 scales above lateral line to dorsal-fin origin; serrae on margin of preopercle 25-37; head and body bright pinkish in colour. A broad bright yellow band passes from the tip of the snout through the eye to the caudal fin (very clear in the snout region). Narrow pale red borderline on anal fin.

**Description.** (Meristic and morphometric data are given in Table 3.1 and 3.3)

Dorsal fin rays X, 10; pectoral fin rays 15; pelvic fin rays I, 5; anal fin rays III, 6; caudal fin rays 7; branchiostegal rays 7; lateral-line scales 42; Gillrakers 3+4 - 8+4; Vertebrae 23. Body moderately elongate, cylindrical; depth 3.69-3.81 in SL, 1.55-1.73 in HL; head moderately compressed, head length 41.95-48.71% SL, 2.05-2.38 in SL; snout short, pointed, snout length 4.55-5.44 in HL; orbit moderately large, 9.05-11.32 in SL, larger than interorbital width and snout length; interorbital space flat, scaly, scales reaching upto the orbit; interorbital 10.54-15.79 in HL; opercle with 2 flattened spines, lower one prominent; preopercle rounded, finely serrated with 25-37 serrae; caudal peduncle depth 10.77-11.83% SL. Mouth large, terminal, oblique, lower jaw projecting in front of upper jaw; jaws strong, maxilla reaches the vertical through the posterior border of the orbit, teeth in villiform bands in premaxilla and palatines and in a small patch on the vomer; small canines in the mandible and at the maxillary symphysis; upper jaw length in 2.26-2.52 in HL; tongue long slender and spatulate.

Scales ctenoid; maxilla, snout devoid of scales; maxilla truncate posteriorly, with rounded corners; 2.5 scale rows between the 1<sup>st</sup> dorsal fin spine and lateral line. Dorsal fin continuous, deeply notched, originating behind the level of opercle. First dorsal spine small, 7.67-10.16 in HL; 4<sup>th</sup> dorsal spine largest, 2.92-3.02 in HL; dorsal rays branched from half of their length, additional branches at tips; predorsal length 37.40-40.27% SL. Pelvic fins inserted beneath, origin in front of pectoral fins, tips not reaching to anal-fin origin, pelvic fin length 1.91-2.30 in HL, 18.21-25.21%



SL. Pectoral fins long, reaching to level of anal-fin origin, 22.37-27.83% SL. Anal-fin origin below the level of first dorsal-fin soft ray; preanal fin length longer than HL, 64.40-70.12% SL; anal rays branched like dorsal rays. Caudal fin emarginate, with upper lobe longer than lower lobe.

**Coloration:** Head and body pinkish in colour, belly and throat white. A broad bright yellow band passes from the tip of the snout through the eye to the caudal fin. Bright yellow markings on the cheeks, opercles, dorsal, ventral and anal fins. Narrow pale red borderline on anal fin. Colour in formalin- Pale with four incomplete cross bands of grey.

**Distribution.** Known from the deeper waters off Chennai, Tuticorin (Bay of Bengal), Mangalore, Gujarat and Kerala Coast (Arabian Sea) at depths ranging from 180-340 m. The specimens reported herein were collected off Kollam at 220-340 meters depths.

**Remarks.** *Chelidoperca investigatoris* is a common bycatch of deep-sea shrimp trawl fishery off Kollam (Kerala coast) where it is sold in the domestic market.



**Figure. 3.1.** *Chelidoperca investigatoris*. A. ZSI 12821, 103 mm SL, male; B. ZSI 12820, 107 mm SL, female



**Figure. 3.2.** *Chelidoperca investigatoris* GB.31.139.16.2, 125 mm SL

### 3.1.2 *Chelidoperca occipitalis* Kotthaus, 1973

Arabian perchlet

(Figure 3.3, 3.4; Table 3.2, 3.3)

*Chelidoperca occipitalis* Kotthaus, 1973 (Holotype: ZMH 5136; Socotra Islands, Arabian Sea). Manilo and Bogorodsky, (2003) (list, Arabian Sea).

**Diagnosis.** Dorsal-fin rays X, 10, fourth spine largest (2.4-2.9 in HL) slightly longer than 3<sup>rd</sup> spine, all soft rays branched; anal fin rays III, 6; pectoral fin rays 15; Lateral-line scales 44. Gill rakers 3+5-8+4.

Body depth 3.93-4.15 in SL; head length 2.24-2.36 in SL; orbit length 9.40-10.97 in SL; 3 scales above lateral line to dorsal origin; circum-peduncle scales 14. Serrae on margin of preopercle 28-38. Body pinkish in colour with a dark band along body, to caudal. Yellow spots on dorsal, caudal and anal fins.

**Description.** Selected meristic and morphometric data are given in Table 3.2 and 3.3. Data of the holotype of *Chelidoperca occipitalis* were taken from the specimen of the original description.

Dorsal-fin rays X, 10, single, deeply notched, fourth spine largest (2.62-3.54 in HL), slightly longer than 3<sup>rd</sup> spine; anal fin rays III, 6; pectoral fin rays 15; lateral-line scales 44. Gill rakers 3+5-8+4. Vertebrae 23. Branchiostegal rays 6.

Body moderately elongate, cylindrical; Body depth 3.93-4.15 in SL, depth 1.66-1.82 in HL; head moderately compressed, head length 2.24-2.36 in SL; snout short, pointed, snout length 4.00-4.72 in HL; orbit moderately large, larger than

interorbital width and snout length, orbit length 9.40-10.97 in SL; inter orbital space flat, scaly; inter orbital 10.31-14.59 in HL. Opercle with two flattened spines; lower one prominent. Preopercle rounded, finely serrated (28-39). Mouth large, terminal, oblique, lower jaw projecting in front of upper jaw; upper lip not swollen at symphysis; maxilla not reaching the rear edge of orbit in vertical; upper jaw length 2.33-2.46 in HL; jaws with a band of minute slender canine teeth, the band widening anteriorly, a gap in the middle of symphysis, vomerine teeth absent. Tongue long slender and tip knob like.

Scales ctenoid; maxilla, snout devoid of scales; maxilla truncate posteriorly, with rounded corners. No scales in front of orbit. Three scale rows between first dorsal-fin spine and lateral line; single dorsal fin, deeply notched, dorsal fin originating behind opercle in vertical. First dorsal spine small, 7.13-12.35 in HL; fourth dorsal spine largest, (2.62-3.54 in HL); posterior dorsal rays longest. Dorsal rays branched from half of their length, additional branches at the tips. Pelvic fins inserted beneath in front of pectoral fins, not reaching to anal origin, 1.83-1.90 in HL. Pectoral fins long, reaching anal fin origin in the vertical, 1.65-1.93 in HL. Anal fin origin below dorsal ray origin in vertical. Anal rays branched like dorsal rays. Caudal peduncle depth 10.9-11.4% SL, 3.60-3.93 in HL. Circum-peduncle scales 13-14. Caudal fin truncate; upper rays elongated in few specimens.

**Coloration:** Body pinkish-orange in colour with a prominent dark stripe running along the body, from opercular spine to base of caudal fin. Ventral portion of trunk pale with 8-9 white bands on side. Yellow spots on dorsal, caudal and anal fins, those on dorsal, caudal rays prominent. Colour in formalin pale with prominent black stripe along the middle of the trunk and caudal fins with pale spots.

**Distribution.** This species is widely distributed in the Indian Ocean from Socotra Islands, Pakistan (M. Khan pers.comm.) and Veraval (M. Srinath pers.comm.) up to Kerala coast of southern India. The specimens reported herein were collected off Kollam at 180-320 meters depth.

**Remarks.** *Chelidoperca occipitalis* is common in collections made off Kollam and is frequently caught along with *Plesionika* spp. in the bycatch of deep-sea shrimp trawl fishery. It is sold in domestic markets.

**Discussion:** *Chelidoperca investigatoris* was described by Alcock (1890) based on the collection of RIMS *Investigator* from Madras coast (Station 96, depth 98-103 fathoms), but in subsequent publications of Alcock (1899) the type area for same materials are given as off Ganjam coast (Orissa), Bay of Bengal, which was followed in many publications including Bineesh *et al.*, 2013. Herein we correct that Madras is the type locality, due to landing of *Chelidoperca investigatoris* in deep-sea trawls is confirmed.



**Figure. 3.3.** Holotype of *Chelidoperca occipitalis*, ZMH 5136, 114 mm SL, collected from southwest of Socotra Islands, Arabian Sea. A- ventral view, B- dorsal view



**Figure. 3.4.** *Chelidoperca occipitalis* GB.31.139.16.1, 12.5 mm SL from Kollam, southwest coast of India.

*Chelidoperca investigatoris* is the third known species of the genus from Indian waters, but after Alcock (1890), the report of *C. investigatoris* was limited to listing in deep-sea fishery expeditions along the south west coast of India (Jayaprakash *et al.*, 2006; Sajeevan *et al.*, 2009) but no additional details, figures,

descriptions were provided, though the genera had taxonomic confusions. *Chelidoperca investigatoris* differ from other *Chelidoperca* species by the bright pink colour of head and body and broad bright yellow band. The interorbital space of this species is covered with a scaled band. The overlapping colour pattern (dark blotches on the body) of *C. occipitalis* closely resembles *C. pleurospilus* (Günther, 1880), but on a closer examination shows variation in colour from the description by Park *et al.*, (2007). *Chelidoperca occipitalis* has interorbital 10.3-14.6 in HL vs <10.95 in *C. pleurospilus* (Akazaki, 1972). Earlier and original descriptions of both *C. occipitalis* and *C. investigatoris* were with limited character descriptions, which were not enough to diagnose to a species level and no colour plates were available.

### **Comparative materials**

***Chelidoperca investigatoris*:** Syntype, ZSI 12820, 107.5 mm SL, syntype, ZSI 12821, 103.4 mm SL, Off the Madras coast, Tamil Nadu, India, 180-187 m depth, R.I.M.S. *Investigator*. NBFGR CHN 3012-3024, 13 specimens, 127.5-177.9 mm TL, off Kollam, Kerala coast, India, south-eastern Arabian Sea (09°05' N, 75°52'E), 180-280 m depth, collected by K. K. Bineesh and K. V. Akhilesh, 08 February 2009.

***Chelidoperca occipitalis*:** Holotype, ZMH 5136, 114 mm SL, Off Socotra Islands, Arabian Sea, 190-290 m depth. CMFRI GB 31.139.16.1.1-3, 3 specimens, 135-153mm TL, NBFGR CHN 3001- 3011, 11 specimens, 135-163 mm TL, off Kollam, India, Kerala coast, southeastern Arabian Sea (09°20' N, 75°51' E), 180-320 m depth, collected by K.K. Bineesh and K.V. Akhilesh, 22 April 2009.

**Table 3.1.** Morphometric data of *Chelidoperca investigatoris*. Measurements expressed in % of SL

Measurements (% SL)	Syntype ZSI 12820	Syntype ZSI 12821	NBFGR CHN 3015	Range (n=12)	Standard deviation
Standard length (mm)	107.5	103.4	139	101.7-138.8	0.0
Body depth	27.2	27.8	27.1	26.2-27.8	0.5
Head length	47.7	48.7	42.5	41.9-48.7	2.7
Post orbital length	27.6	28.5	25.4	24.9-28.5	1.3
Snout length	9.9	10.7	8.3	7.7-10.7	1
Eye diameter	10.4	11.1	8.8	8.9-11.1	0.9
Upper jaw length	19.7	19.3	18.3	18.3-19.7	0.6
Interorbital width	3.1	3.1	4	3.4-4.1	0.4
Predorsal length	38.6	39.9	37.4	37.4-40.3	1.1
Prepectoral length	43.3	42.6	41.5	39.4-43.3	1.3
Prepelvic length	39.9	37.2	36.1	34.1-39.9	1.8
Preanal length	70.1	66.5	66.2	64.4-70.1	1.8
Pectoral fin length	27.3	27.8	24.3	22.4-27.8	1.9
Pelvic fin length	25.1	25.2	20.8	18.2-25.2	2.5
Length of first anal fin ray	-	-	12.3	11.6- 13.1	0.6
Caudal fin length	-	-	28.3	25.2-28.4	1.4
Caudal peduncle depth	10.8	11	11.7	10.8-11.8	0.5
Anal fin length	-	-	29.9	27.1-30.1	1.2
Anal fin base length	14	14.8	16.6	14.0-16.8	1.1
Dorsal fin length	-	-	63.2	58.5-63.3	2.3
Dorsal fin base length	42.9	45.5	49.9	47.9-49.9	2.5
Caudal peduncle length	22.8	21.3	19.9	19.9-23.9	1.5
First dorsal spine length	3.49*	2.8*	4.8	4.5-5.9	1
Second dorsal spine length	6.58*	6.5*	8.6	8.2-9.7	1.2
Third dorsal spine length	9.7*	9.2*	10.5	10.5-12.9	1.3
Fourth dorsal spine length	-	-	12.6	12.6-14.4	0.8
Last dorsal spine length	-	-	9.9	9.0-9.9	0.4

\* damaged/broken

**Table 3.2.** Morphometric data of *Chelidoperca occipitalis*. Measurements expressed in % of SL

	<b>Holotype ZMH 5136</b>	<b>GB 31.139.16.1</b>	<b>Range (n=14)</b>	<b>Standard deviation</b>
Measurements (% SL)				
Total length (mm)	139.1	160	135-160	-
Standard length (mm)	114	128	110-128	-
Body depth	24.8	25.4	23.5-25.5	0.7
Head length	41.8	43.6	41.4-44.7	1
Post orbital length	22.4	24.2	22.4-24.9	0.6
Snout length	9.9	10.9	8.9-10.9	0.7
Eye diameter	10.6	10.6	9.1-10.6	0.4
Upper jaw length	17.9	18.5	17.2-18.7	0.5
Interorbital width	3.4	4.2	2.9-4.3	0.4
Predorsal length	38.2	38.3	37.2-38.8	0.5
Prepectoral length	40.5	41.1	38.6-41.1	0.9
Prepelvic length	36.4	36.4	34.4-40.2	2.1
Preanal length	64.1	63.6	61.4-64.9	1.3
Pectoral fin length	24.5	25.4	21.6-25.7	1.4
Pelvic fin length	23.5	23.9	22.3-23.9	0.7
Length of first anal fin ray	12.5	11.9	11.6-13.6	0.7
Caudal fin length	22.3	26.7	21.5-26.7	1.9
Caudal peduncle depth	11.2	12	10.9-12.1	0.4
Anal fin length	30.5	31.4	29.8-32.9	1
Anal fin base length	17.7	17.5	15.9-18.2	0.8
Dorsal fin base length	49.5	50.9	46.3-52.4	2
Caudal peduncle length	19.1	20.8	17.1-21.5	1.6
First dorsal spine length	5.3	5	3.6-5.9	0.9
Second dorsal spine length	9.1	9	7.1-9.9	1
Third dorsal spine length	13	12.4	11.9-14.3	0.9
Fourth dorsal fin spine length	14.3	14.8	12.7-15.9	1.2
Last spine length	10.2	9.4	9.4-10.2	0.4

**Table 3.3.** Frequency distributions in two species of *Chelidoperca* for total numbers of gillrakers on first gill arch, numbers of tubed lateral-line scales.

<b>Total numbers of gill rakers on first gill arch</b>							
	14	16	17	18	19	20	21
<i>Chelidoperca investigatoris</i>				1	5	6	1
<i>Chelidoperca occipitalis</i>					7	5	3
<b>Numbers of tubed lateral-line scales</b>							
	41	42	43	44			
<i>Chelidoperca investigatoris</i>			5	4			
<i>Chelidoperca occipitalis</i>			7	2			

### 3.1.3 *Chlorophthalmus corniger*

The greeneyes of the family Chlorophthalmidae (Aulopiformes) are known from tropical and subtropical waters worldwide at upper continental slope depths. Eschmeyer and Fong (2013) list 21 nominal species in the family, two of which are referable to the Atlantic genus *Parasudis* and another three that have been

synonymised with the type species of *Chlorophthalmus*, *C. agassizi* Bonaparte, 1840. Of the remaining 16 species of *Chlorophthalmus* currently regarded as valid, 11 have been reported from the Indo-Western and Central Pacific. More than half of these have been identified and listed in publications as occurring in the Indian Ocean.

Bottom trawls operated by FORV *Sagar Sampada* from 1983 to 1991 in the southeastern Arabian Sea have revealed the existence of rich grounds of species of *Chlorophthalmus* that were the most dominant species in the catch, followed by *Cubiceps whiteleggi* (Khan *et al.*, 1996). High abundances of greeneyes were also reported from several exploratory surveys conducted in the southwestern region of the Indian EEZ (Prasad and Nair, 1974; James and Pillai, 1990; Venu and Kurup, 2002). High-density pockets of *Chlorophthalmus bicornis* Norman, 1939 were located at depth ranges of 301–400 m and 201–300 m respectively (Kurup *et al.*, 2005).

Five species of *Chlorophthalmus* have been reported from Indian waters: *Chlorophthalmus bicornis*, *C. corniger*, *C. nigromarginatus*, *C. agassizi* and *C. punctatus* Gilchrist, 1904 (Venu and Kurup, 2002; Jayaprakash *et al.*, 2006; Pillai *et al.*, 2009; Sajeevan *et al.*, 2009). Although *C. agassizi*, *C. punctatus* and *C. nigromarginatus* were listed as occurring in Indian waters, the validity of these occurrences may be questioned as no detailed descriptions of species were given in publications discussing their occurrence.

*Chlorophthalmus corniger* was described by Alcock (1894) from four specimens collected from off Chennai (Tamil Nadu) on the east coast of India during the surveys of HMS *Investigator* at depths of 270–350 m. Norman (1939) described *C. bicornis* on the basis of a single specimen collected in the Gulf of Aden at a depth of 274–366 m during the *John Murray Expedition*. *Chlorophthalmus bicornis* was considered unique in having the “lower jaw terminating in a strongly projecting, transverse horizontal plate, the corners of which are produced to form strong tooth-like processes”. Following the original description of *C. corniger*, no further reports of the species from Indian coasts were published, apart from references to the species listed by authors based on the original descriptions and collection (Kotthaus, 1967; Silas, 1969; Sato and Nakabo, 2002; Manilo and Bogorodsky, 2003; Satapoomin, 2011). The examination of fresh specimens collected off Chennai,



Kollam and Tuticorin, identifiable as *C. corniger* and a detailed examination of the syntype series of *C. corniger* and *C. bicornis* revealed the two species to be identical and *C. corniger* to be the senior synonym of *C. bicornis*.

Specimens of greeneyes were obtained from commercial deep-sea shrimp trawler bycatch landings operated at 200 to 500 m depths off Kollam (Kerala) on the southwest coast, off Tuticorin (Tamil Nadu) on the southeast coast and off Chennai (Tamil Nadu) on the east coast of India in 2009–2012. Identifications to species level were based on Alcock (1894, 1899) and Norman (1939). Methods for counts and measurements follow Nakabo (2002). Measurements were made to the nearest 0.1 mm using dial callipers. Measurements are expressed as percentage of standard length ( $L_S$ ) or head length (HL). Specimens were deposited in the Designated National Repository (DNR), Central Marine Fisheries Research Institute, Cochin, Kerala, India (Accession Numbers: GB.8.6.1.4, GB.8.6.1.4.1, GB.8.6.1.4.2 and GB.8.6.1.4.3).

#### ***Chlorophthalmus corniger* Alcock, 1894**

Figure 3.5-3.7; Table 3.4

Spinyjaw greeneye

***Chlorophthalmus corniger* Alcock, 1894** [off Madras (now Chennai), India]. Kotthaus, 1967 (Arabian Sea); Silas, 1969 (Arabian Sea, India); Satapoomin, 2011 (list, Southwestern Thailand).

*Chlorophthalmus bicornis* Norman, 1939 (Holotype: BMNH 1939.5.24.457); Sato and Nakabo, 2002; Manilo and Bogorodsky, 2003 (list, Arabian Sea fishes).

**Diagnosis.** A species of *Chlorophthalmus* with the following combination of characters: lower jaw terminating in a distinct forwardly projecting horizontal plate with strong, spine-like processes directed forward from the plate's corners; body silvery grey, with numerous minute black spots and traces of broad darker crossbars; base of anterior dorsal fin spines and distal parts of dorsal fins black; adipose fin tiny with numerous black spots; caudal fin black; 3.5 scales above lateral line; three rows of cheek scales; head very large, 34.3–40.1%  $L_S$ ; eye large, 29.8–40.8% HL; pectoral fin long, extending to beyond dorsal fin base, 21.7–26.2%  $L_S$ .

**Description.** (See Table 3.4 for morphometric values). Dorsal fin rays 10–11; Anal fin rays 9–10; Pectoral fin rays 16–17; Pelvic fin rays 9; Gill rakers 5+17–21; pored lateral line scales 47–49. Body elongate and compressed, head and body subcylindrical; head length 34.3–40.1%  $L_S$ , 2.5–2.9 times in  $L_S$ ; head depth 44.2–63.9% HL. Snout short 20.5–28.8% HL. 3.5 scales above lateral line; three rows of cheek scales. Nostrils located midway between tip of snout and anterior margin of orbit. Eye large, 29.8–40.8% HL, elliptical, directed dorsolaterally. Interorbital narrow. Maxilla extending posteriorly to below the front of the pupil. Tip of lower jaw projecting well anteriorly, terminating in a distinctly projecting horizontal plate with strong, spine-like processes directed forward from the plate's corners (Fig. 3.6). Jaws with small recurved conical teeth in a posteriorly tapering band. Vomer and palatine with irregular rows of conical teeth. Tongue narrowing slightly anteriorly, possessing small canine-like teeth, which are smaller than the teeth in the jaws. Scales cycloid, moderately well attached. Anus closer to pelvic fin origin than to anal fin. Pectoral fin long, tip reaching posterior to dorsal fin base. Anal fin base length 8.5–13.7%  $L_S$ . Adipose dorsal fin tiny, length 8.5–21.5% HL, positioned above the base of fourth or fifth anal fin ray. Pectoral fin long, 21.7–26.2%  $L_S$ .

**Colouration.** Body silvery grey with minute black dots and traces of broad darker crossbars. Operculum dark dorsally, silvery ventrally. Dorsal fin pale with prominent black pigmentation distally and at base of anterior dorsal fin spines. Adipose dorsal fin pale with numerous minute black dots. Caudal and pectoral fin black.

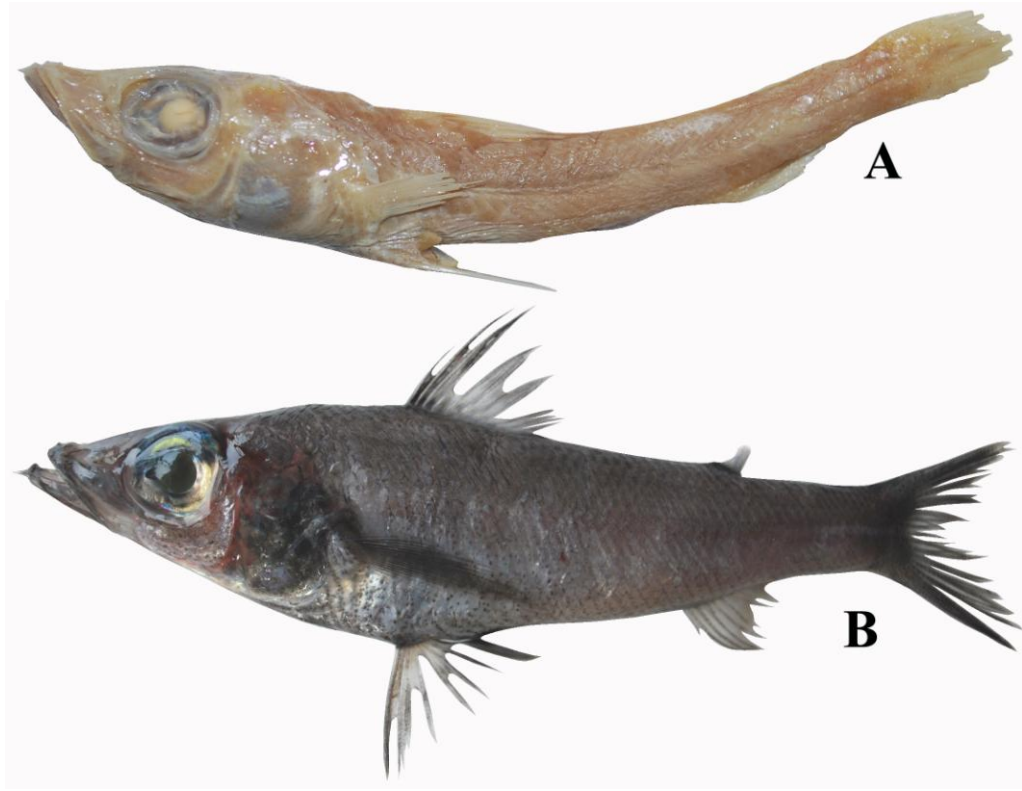
**Distribution.** Known from southern Indonesia (off the south coast of Java) and south-western Thailand to India (off Chennai, Tuticorin, and Kerala), the Gulf of Aden and off the coast of Somalia, at depths between 220–500 m. Records from the Philippines during the *MS 'Vauban'* cruise in 1976 (De la Paz and Interior, 1979), the South China Sea (Randall and Lim, 2000) and elsewhere in the Western Pacific (Paxton and Niem, 1999) are misidentifications.

**Utilization.** The species is primarily consumed fresh, but is also processed for fishmeal.

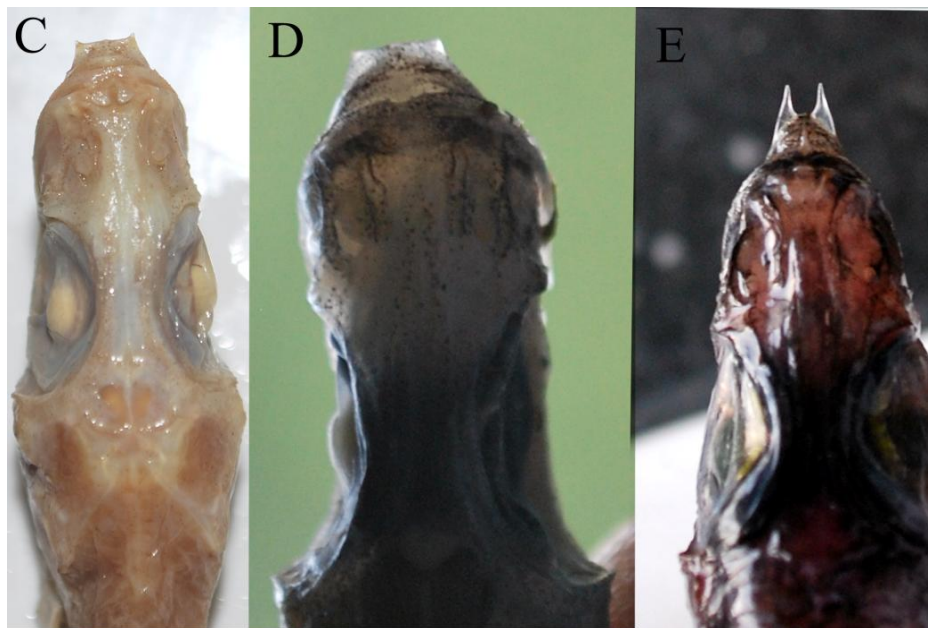
## Remarks

Norman (1939) described *C. bicornis* on the basis of a single specimen collected in the Gulf of Aden at a depth of 274-366 m during the *John Murray Expedition*. He compared it only with *C. agassizi*, without any mention of *C. corniger* which is described from a neighbouring area and also has the same lower jaw processes. The variation in morphometric characters related to growth has been analysed and documented in the present study. The structural variation in the lower jaw of *C. corniger* (i.e., increasing size and stronger lower jaw projection) is attributed to ontogenic changes. In the case of *C. corniger*, a plate like projection without any processes is observed at 55 mm  $L_S$  and smaller sizes (Fig.3.6D), and this projection becomes stronger with two processes as the fish develops (>57 mm  $L_S$ ). The largest specimen (65.9 mm  $L_S$ ) of the four syntypes of *C. corniger*, ZSI 13713, was collected in the Bay of Bengal and is here designated lectotype of the species. The remaining specimens (ZSI 13712: SL 61. mm; ZSI 13714: SL 61.2 mm; ZSI 13715: SL 58.2 mm), are here designated paralectotypes. Alcock's colour descriptions were based on small specimens and stated as silvery grey with numerous broad, ill-defined dusky cross-bands; fins hyaline, the tip of the caudal and the base and tip of the dorsal black; numerous parallel oblique rows (very conspicuous on the thorax and belly) of tiny black specks with a silvery centre, resembling incipient luminous spots. This description matches the smaller specimens except for the presence of dusky cross-bands as seen in the larger specimen (which is selected as lectotype for this reason).

*Chlorophthalmus corniger* is a regular by-catch component of the deep-sea shrimp trawls which target the shrimp species *Plesionika quasigrandis* Chase, 1985, Arabian red shrimp *Aristeus alcocki* Ramadan, 1938, *Metapenaeopsis andamanensis* (Wood-Mason, 1891) and *Heterocarpus* spp. Other dominant species in the bycatch are the pelican flounder *Chascanopsetta lugubris* Alcock, 1894, the Shadow driftfish *Cubiceps whiteleggi*, the perchlets *Chelidoperca investigatoris* (Alcock, 1890) and *Chelidoperca occipitalis* Kotthaus, 1973, the shortfin neoscopelid *Neoscopelus microchir* Matsubara, 1943, the duckbill *Bembrops caudimacula* Steindachner, 1876, Watases lanternfish *Diaphus watasei* Jordan and Starks, 1904, the deep-sea herring *Bathyclupea hoskynii* Alcock, 1891, the hooked tonguesole *Cynoglossus carpenteri* Alcock, 1889, the Indian ruff *Psenopsis cyanea* (Alcock, 1890), the royal



**Figure 3.5.** Lateral views of *Chlorophthalmus corniger* (A). Lectotype ZSI 13713, 65.9 mm  $L_S$  (B). GB.8.6.1.4.3, 120.7 mm  $L_S$ , off Kollam, Kerala (fresh).



**Figure 3.6.** Dorsal view of the head of three specimens of *Chlorophthalmus corniger* showing the structure of the projecting lower jaw plate. (C) Lectotype ZSI 13713, 65.9 mm  $L_S$  (D) 52.8 mm  $L_S$ , off Kollam, Kerala (fresh). (E) GB.8.6.1.4.3, 120.7 mm  $L_S$ , off Kollam, Kerala (fresh).

escolar *Rexea prometheoides* (Bleeker, 1856), Sackfish *Neoepinnula orientalis* (Gilchrist and von Bonde, 1924), Smooth angler *Lophiodes mutilus* (Alcock, 1894) and the sharptooth seabass *Synagrops philippinensis* (Günther, 1880). The family

Chlorophthalmidae dominates the deep-sea bycatch shrimp compositions, with *C. acutifrons* and *C. corniger* being the two most dominant species. The data collected from the shrimp trawls indicate that *C. corniger* is abundant at 280–450 m.



**Figure 3.7** Lateral view of *Chlorophthalmus bicornis* Holotype BMNH 1939.5.24.457.

**Materials examined**

*Chlorophthalmus corniger* syntypes: Lectotype ZSI 13713

Paralectotypes: ZSI 13712, ZSI 13714 and ZSI 13715. CMFRI GB.8.6.1.4, GB.8.6.1.4.1, GB.8.6.1.4.2 and GB.8.6.1.4.3.

*Chlorophthalmus bicornis* Holotype: BMNH 1939.5.24.457, John Murray Expedition; Gulf of Aden, station 177, depth 274–366 m.

**Table 3.4.** Proportional measurements expressed as % of SL and HL of *Chlorophthalmus corniger*. Minimum and maximum values include type specimens listed individually and nine other specimens used for taxonomic studies

Species	<i>C.corniger</i> ZSI 13713 Lectotype	<i>C. bicornis</i> BMNH 1939.5.24.457	<i>C.corniger</i> ZSI 13712 Paralectotype	<i>C. corniger</i> ZSI 13714 Paralectotype	<i>C. corniger</i> ZSI 13715 Paralectotype	Min	Max	SD
Standard length (mm)	65.9	73.6	61.1	61.2	58.2	52.8	122.7	26.7
(% SL)								
Body depth	18.1	20.6	18.5	18	15.5	15.5	25.8	2.8
Body width	NA	10.9	NA	NA	NA	7.6	15.3	2.3
Head length	38.6	36.8	39.6	39.9	38.8	34.3	40.1	1.9
Preanal length	NA	54.2	NA	NA	NA	42	54.2	3.7
Predorsal length	41.3	40.8	42.1	42.5	41.4	39.5	42.5	1.0
Prepectoral length	38.8	37.6	38.8	38.4	39.6	32.8	39.6	2.2
Prepelvic length	46.1	54	45.9	50.1	47.7	39.6	54	3.8
Preanal length	73.8	44.8	76.2	80.9	NA	44.8	80.9	8.9
Dorsal fin base length	13.7	14	12.8	14.5	NA	11.4	15.8	1.5
Pectoral fin length	NA	24.5	NA	NA	NA	21.7	26.2	1.4
Pelvic fin length	NA	22.4	NA	NA	NA	18.5	22.4	2.1
Interpelvic width	NA	7.9	NA	NA	NA	2	8	2.6
Pelvic fin origin to anus	NA	7.8	NA	NA	NA	6.1	9.5	1.1
Anus to anal fin origin	NA	17.3	NA	NA	NA	17.3	25.7	2.6
Anal fin base length	12.6	NA	13.7	11.3	NA	8.5	13.7	1.7
Anal fin height	NA	NA	NA	NA	NA	11.8	18	2.2
Caudal peduncle length	19.3	11.5	17.4	20.5	19.6	11.5	20.5	2.3
Caudal peduncle depth	8.7	9.4	8.4	9.6	8.2	7.2	10.5	1.0
Head depth	NA	16.9	NA	NA	NA	16.9	24.4	2.7
Head width	NA	19	NA	NA	NA	8.5	19	2.9
Snout length	9.9	9.1	10.6	10.1	10.1	7.8	10.8	0.8
Orbit diameter	12.3	15	11.8	12.5	12.8	11	15	1.0
Interorbital width	3.5	3.6	3.5	3.6	3.6	2.5	3.8	0.5
Upper jaw length	14.6	15	15.1	16.7	15.1	12.7	16.7	1.0

Postorbital length	16.3	13.8	16.3	17.7	17.5	12.6	17.7	1.5
Adipose fin length (%HL)	NA	4.5	NA	NA	NA	3.4	8	2.8
Head depth	NA	45.8	NA	NA	NA	44.2	63.9	6.9
Head width	NA	51.7	NA	NA	NA	22.4	51.7	8.3
Snout length	25.6	24.6	26.8	25.4	26.1	20.5	28.8	2.2
Orbit diameter	31.9	40.8	29.8	31.4	32.9	29.8	40.8	2.8
Interorbital width	9.2	9.7	8.8	9	9.3	7.2	9.7	0.9
Upper jaw length	37.9	40.6	38	41.9	39	34.5	41.9	2.0
Postorbital length	42.3	37.6	41.2	44.5	45	33.1	45	3.2
Adipose fin length	NA	12.2	NA	NA	NA	8.5	21.5	4.2

### 3.1.4. *Sphenanthias whiteheadi*

The bandfishes of the family Cepolidae (Perciformes) are known from all tropical and subtropical waters and comprise 22 valid species in 4 genera worldwide (Eschmeyer *et al.*, 2010). Members of the genus *Sphenanthias* can be differentiated from the similar *Owstonia* in having lateral lines separate and not forming loops in front of dorsal fins (Smith Vaniz, 2001; Liao *et al.*, 2009). The genus *Sphenanthias* is represented by only one valid species in the Arabian Sea (Manilo and Bogorodsky, 2003). The *Sphenanthias whiteheadi* was described from four specimens collected from the southwest coast of India, off Kollam at 300 m (Talwar, 1973). *Sphenanthias simoterus* Smith, 1968, *Owstonia weberi* (Gilchrist, 1922), and *Owstonia totomiensis* Tanaka, 1908 are listed as occurring in this area, but the validity of these occurrences are questionable since no detailed description was given in publications discussing their occurrence. After the original description of *S. whiteheadi* there were no reports of this species from the Indian coast. The present study confirms the validity and status of *Sphenanthias whiteheadi* Talwar, 1973 and provides redescription of these species.

Specimens of *Sphenanthias whiteheadi* were collected from the southwest coast of India near Kollam (Kerala) and the southeast coast of India near Tuticorin (Tamilnadu) in December 2009 from commercial deep-sea shrimp trawler bycatch landings operated at 220-350 m depth. Identification of the species was based on Talwar (1973). Measurements were made to the nearest 0.1 mm using digital callipers. All measurements are expressed as percentage of standard length (SL). The specimens of *Sphenanthias whiteheadi* were deposited at Central Marine Fisheries Research Institute, Kochi, Kerala, India (Accession number: GB.31.31.4.1).

#### ***Sphenanthias whiteheadi* Talwar, 1973**

(Figure 3.8, 3.9; Table 3.5)

Indian bandfish

*Sphenanthias whiteheadi* Talwar, 1973 off Quilon, India, about 9 °N, 76 °E, depth 300 meters. Holotype: ZSI F6275/2. Paratypes: ZSI F6276/2, 6277/2, 6278/2 Manilo and Bogorodsky, 2003 (list Arabian Sea, India).



**Diagnosis:** Dorsal fin rays IV, 22-23; Anal fin rays 1, 16; Pectoral fin rays 19-20; Pelvic fin rays I, 5; Lateral Line scales 39-41. Gill rakers 18-21+ 35-39. Vertebrae 28. Pelvic fins long in males reaching on to anal, black pigmentation between membrane of maxilla and premaxilla (Talwar, 1973).

**Description:** Measurements are given in Table 3.5. Values of type specimens in Talwar, 1973 follows in parenthesis.

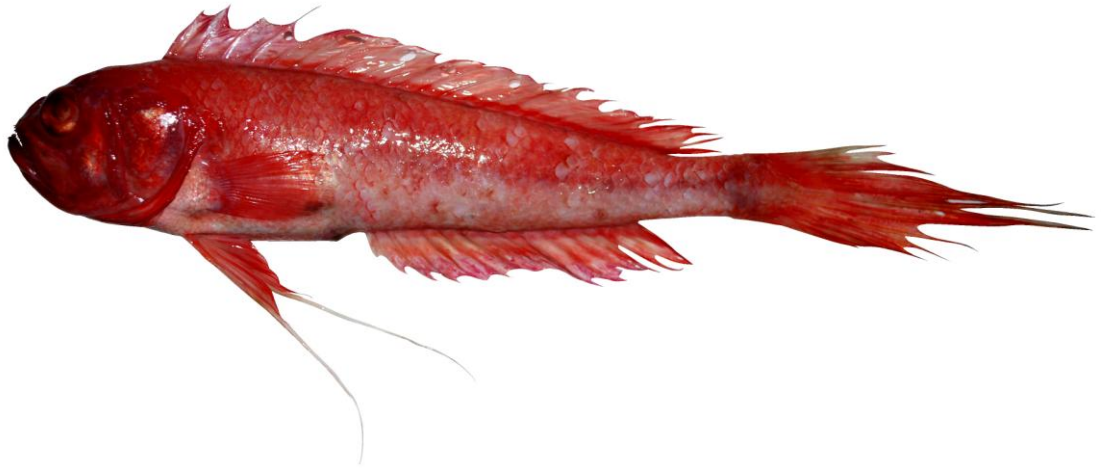
Body compressed, elongated with tapering tails. Males larger than females. Head large, head length 26.1-31.8% SL (25.7-30% SL) 3.1-3.8 in SL (3.3-3.9 in SL), body depth at dorsal origin 22.8-26.5% SL (25.1-30.9% SL), eyes large, eye length (bony orbit) 6.9-8.6% SL (8-9.4% SL), interorbital 5.6-6.1% SL (6-6.9% SL), mouth large, lower jaw projecting, maxilla broad. Lower margin of preopercle serrated with 6-9 spines. Opercle with small scales. Lateral line runs close to dorsal fin base and ends at dorsal fin last rays mostly at 22<sup>nd</sup> and 23<sup>rd</sup> rays. Lateral line scales (pored) 36-38. Teeth in upper jaw uniserial, teeth absent in symphysis of both jaws. Pelvic fins in males elongate (Fig.3.8) 43-46% SL (43.3-55.6% SL) females (Fig.3.9) 24.7-25.2% SL (28.8-35.7% SL). Pelvic fin proximal ray attached to body. Dorsal fin base 66.6-68.5% SL. The first two dorsal fin spines placed closer than other spines. Caudal fin elongate and lanceolate. *Sphenanthias whiteheadi* cannot be mistaken with *S. simoterus* Smith, 1968. *S. simoterus* having peculiar characters; D III, 21; A I, 14; GR 13+ 24, LL 46-48.

**DNA analysis:** A 652 bp of mitochondrial COI region was amplified bidirectionally for one specimen.

**Utilization:** The species is primarily used for consumption.

Common name: Indian bandfish.

**Remarks:** The specimens of *Sphenanthias whiteheadi* were collected off the coast of Tuticorin in the Gulf of Mannar at a depth of 220–350 m by deep-sea shrimp trawler. The holotype and three paratypes were collected at Kollam, north of Tuticorin, along the southeast coast of India. This species may prove to be more widespread along the Indian coast.



**Figure 3.8.** *Sphenanthias whiteheadi* male



**Figure 3.9.** *Sphenanthias whiteheadi* female

**Table.3.5.** Morphometric measurements of *Sphenanthias whiteheadi* from southwest and southeast coast of India (in % SL) (n=8)

Measurements	Male- GB.31.31.4.1	Male- PFD/B/11	Male- PFD/B/12	Female- PFD/B/13	Female- PFD/B/14	Female- PFD/B/15	Range
Total length mm	405	378	230	218	222	340	218-405
Standard length mm	255	243	140	150	148	240	140-255
Body depth( dorsal origin)	22.8	26.5	28.5	27.7	27.3	26.0	23-29
Body depth (anal origin)	20.0	21.6	23.1	24.4	23.7	22.6	20-24
Head length	26.1	28.2	31.8	31.7	31.8	28.3	26-32
Eye diameter	6.9	8.6	11.3	11.6	11.6	8.0	6.1-11
Snout length	4.7	5.1	4.6	4.7	5.6	5.0	4.5-5.6
Interorbital	5.6	6.1	6.0	6.8	6.8	6.5	5.5-6.8
Post orbital	15.6	16.6	17.7	14.1	14.2	16.0	14-17
Maxilla width	4.9	5.4	6.2	6.5	6.6	5.6	4.8-6.6
Pectoral fin length	15.4	17.3	20.6	20.4	21.6	17.9	15-21
Pectoral fin base length	6.5	7.0	7.4	7.4	7.5	6.7	6.4-7.5
Pelvic spine length	9.9	9.7	14.4	11.4	11.6	10.1	9.6-15
Pelvic fin length	43.1	46.1	47.8	24.7	25.2	29.9	24-48
Pelvic fin base length	2.9	3.0	2.5	3.5	3.7	2.9	2.5-4
First dorsal spine length	7.0	5.6	9.2	7.0	7.7	5.2	5-9
2nd dorsal spine length	8.0	6.7	11.0	8.5	8.4	6.5	6.5-11
3rd dorsal spine length	9.0	7.7	12.8	14.0	13.3	7.0	6.9-14
4th dorsal spine length	8.0	8.3	14.7	12.7	12.8	8.5	8-15
Dorsal fin base length	68.4	68.5	66.6	66.9	68.1	64.5	64-68
Anal spine length	4.0	8.0	5.6	5.9	6.0	5.1	3.9-8
Anal fin base length	37.4	36.4	37.0	33.2	33.2	35.6	33-37
Predorsal length	20.0	22.6	24.5	23.2	23.9	21.8	20-24
Pre anal length	47.7	47.7	51.9	50.3	48.8	51.0	47-52
Caudal peduncle width	8.7	8.5	8.0	8.7	9.0	8.4	8-9
Caudal peduncle length	14.0	14.0	14.0	12.0	12.8	15.8	12-16

### 3.1.5. *Rhinobatos variegatus*

The family Rhinobatidae (Chondrichthys: Rajiformes) comprises rays popularly known as guitar fishes or shovelnose rays. The family currently includes 4 recognized genera and 46 valid species, of which *Rhinobatos* is the largest genus with a total of 36 species (Last *et al.*, 2004). Since Norman's revision of the family Rhinobatidae (Norman, 1926), 12 species of Rhinobatids were described, mostly from Pacific and Indian Oceans. Nine species of Rhinobatids were reported from the Arabian Sea. The species reported from Arabian Sea are *Rhinobatos punctinifer*, *R. obtusus*, *R. annandalei*, *R. granulatus*, *R. halavi*, *R. salalah*, *R. lionotus*, *R. variegatus* and *R. thoiniana*.

Nair and Lal Mohan, (1973) described *R. variegatus* based on a single 645 mm TL female specimen collected from off Mandapam, Gulf of Mannar at depth of 200-250 fathom. After the original description no other information was available on the species and holotype is the only previous known specimen. The holotype specimen (Accession No: CMFRI. F. 176) has been deposited at Museum of Central Marine Fisheries Research Institute (CMFRI) Mandapam; but this specimen appears to have been lost. (N. Ramamurthy, pers. comm.). In the present study, *Rhinobatos variegatus* was redescribed based on specimens from southern coasts of India.

*Rhinobatos variegatus* specimens were collected during weekly observations of fish landing centers along the southern coast of India, from Sakthikulangara Fisheries Harbour (SKF), Kollam, Kerala and Tuticorin Fisheries Harbour (TFH), Tuticorin, Tamilnadu (type locality). Species identification follows Nair and Lal Mohan, (1973). Morphometric measurements of formalin (10%) preserved specimens were taken following Last *et al.*, (2004). The newly collected specimens of *R. variegatus* were measured and expressed as percentage of total length (Table 3.7).

The members of genus *Rhinobatos* Linck, 1790 is separated from other Rhinobatids by the following characters; spiracles with a pair of narrow folds on their posterior margins; Nostrils diagonal, width much greater than internarial space; anterior nasal flaps of nostrils not expanded posteriorly and fused without diagonal

and transverse bands and without an angular marking behind the eyes. The genus *Rhinobatos* is the largest genus of the family Rhinobatidae with 36 species. Seven species occur in India, i.e. *R. granulatus*, *R. annandalei*, *R. obtusus*, *R. lionotus*, *R. thouniana* and *R. variegatus*.

***Rhinobatos variegatus* Nair and Lal Mohan, 1973**

Stripenose Guitarfish

(Figure 3.10; Table 3.6)

Stripenose Guitarfish

*Rhinobatos variegatus* Nair and Lal Mohan, 1973. On a new deep sea skate, *Rhinobatos variegatus*, with notes on the deep sea sharks *Halaelurus hispidus*, *Eridacnis radcliffei* and *Eugaleus omanensis* from Gulf of Mannar. *Senckenbergiana Biol.*, 54 (1/3): 71-80.

Holotype. CMFRI. F. 176. 645mm TL, adult female, Gulf of Mannar, Off Mandapam, depth 200-250 fathoms (specimen lost).

**Materials examined.** GA.1.7.5.6, adult male, 610 mm TL, Off Tuticorin, Bay of Bengal, 20 m depth, collected by K. K. Bineesh on 13 March, 2009 from demersal trawls landed at Tuticorin Fisheries Harbour.

**Other materials-** 9 specimens: CMFRI/PFD/M/6.2, gravid female, 690 mm TL, Off Quilon, Arabian Sea. CMFRI/PFD/M/6.3, gravid female, 620 mm TL, off Quilon (9°04'N 74°32'E), Arabian Sea, 15 m depth, 11 April 2009 Sakthikulangara Harbour. CMFRI/PFD/M/6.4, male, 583 mm TL, off Tuticorin, Bay of Bengal, 14 m depth. CMFRI/PFD/M/6.5, female, 543 mm TL, off Quilon (9°04'N 74°32'E), Arabian Sea, 15 m depth.

**Diagnosis:** A medium sized *Rhinobatos* with the following combination of characters; disc wedge shaped, dorsal surface edges yellowish, with variegated markings. Snout with lateral grey bands dorsally. Disc generally smooth, except small/rudimentary tubercle on dorsal median line and anterior to orbit and near spiracle in larger specimens, but not prominent. Shorter snout, snout length 2.7-3 times interspiracular distance. Orbit medium sized, orbit diameter 1.1-1.2 times internarial distance. Distance between first gill slit 1.3-1.4 times distance between fifth gill slit. Distance between fifth gill slit 2.8-3.1 in ventral head length. Anterior

nasal flaps well developed. Interdorsal 2.7-3 in first dorsal base. Two cutaneous folds in spiracle, outer one larger than inner.



**Figure 3.10** *Rhinobatos variegatus* dorsal view (GA.1.7.5.6, male, 610 mm TL)

**Description.** Proportional measurements in percentage of total length (TL) are given (Table 3.6). Disc wedge shaped, disc width 28.9-31.2% TL; 3.2-3.5 in TL; 1.3 times as wide as long, disc length (DL) 37.7 -40.4% TL; disc anterior margin straight with short concavity near snout tip, apices nearly rounded. Head short, head length (ventral) 24.2-25% TL; moderately elongate bluntly pointed snout, snout length (pre socket) 2.3-2.5 in mouth width, 12.6-13.7% TL, 4-4.2 in TL; snout length (horizontal) 12.2-13.7% TL, snout length 2.7-3 in interspiracular length; preoral length 5.6-5.8 times internarial distance, 2.7-2.9 times mouth width. Medium sized orbit, orbit length 3-3.2% TL; orbit 4-4.3 in snout. Inter orbital space slightly concave.

Length of first gill slit 2.3-2.8 in nostril length. Distance between first gill slit 1.3-1.4 times distance between fifth gill slit, 2-2.3 in ventral head length. Distance between fifth gill slit 2.8-3.1 in ventral head length. Inter dorsal space 2.7-2.9 in first dorsal base. Pre cloacal length 2.5-2.7 in TL, 1-1.03 in DL. Spiracles moderately large with two folds in the posterior margin length 2.3-3.1 times eye length, opening sub-rhomboidal in shape. Inner most folds shorter than outer one, 0.3-0.4% TL. Distance between bases of folds 0.2-0.4% TL. Nostril moderately large, oblique, nasal flaps well developed. Nostril length 1.1-1.3 in internarial distance. Internarial distance 4.2-4.7 times distance between first gill slits, 3-3.4 times distance between fifth gill slits. Mouth width 2-2.1 times internarial distance.

Dorsal fins medium-sized, first dorsal fin taller than second; first dorsal fin height 1.4-1.7 times base length; length of first dorsal 1-1.2 in its height. First dorsal base length 2.3-2.5 times inner margin length. Interdorsal space moderate, Inter

dorsal space first 2.7-3 in first dorsal base; second dorsal-fin length 1.2-1.5 times its height, base length 2.3-2.7 times inner margin length. First dorsal fin well behind pelvic-fin insertion. Pelvic fins medium sized, elongate, 5.6-6.5 in TL, 1.8-2.2 in base length; short based, base length 0.7-1.2 in inner margin length. Tail broad, depressed; flat ventrally and rounded dorsally. Body depth at pelvic fin insertions 10.7-12% TL; Body width at insertions of pelvic fins 1 -1.1 times width at first dorsal fin origin. Lateral tail fold long and well developed, length 37.4-40.5% TL, originating from near posterior inner margin of pelvic, extending to ventral caudal origin and broadest from dorsal fins. Small caudalfin. Dorsal caudal margin 11.5-13.2% TL.

**Live Coloration:** Body bright yellowish brown dorsally, dense cover of faint/pale blotches along the body. Illustrations shows the lining of dorsal side, pectoral margins. Snout tip plain with three brownish red broad lateral bands and one medially. Pectoral and pelvic fins with light purple blue variegated markings. Ventral surface uniformly pale, ventral snout tip of juveniles/pups, with a prominent black spot, which may be absent in larger specimens.

**Distribution and habitat:** Western Indian Ocean: India. Possibly endemic to the southern coasts of India. *Rhinobatos variegatus* is so far known only off Tuticorin. Present study extends its distributional range to west coast of India, where it is common in landings as bycatch in demersal trawlers operating at very shallow waters, where the operation depth ranges from 10 to 40 m.

**Etymology:** Species name is based on the variegated markings of the pectoral and pelvic margins

**Common name:** Stripenose Guitarfish

**Size:** *Rhinobatos variegatus* is an ovoviviparous species with litters of up to 6, but averaging 1-4. Litter size ranged from 177-196.8 mm TL and 63-68.26 mm. Pregnant females were observed from 580 mm TL. Maximum size observed is 650 mm for males and 750 mm for females.

**Remarks:** This is the first report of *Rhinobatos variegatus* from the west coast of India and second report after the original description by Nair and Lal Mohan (1973) which was based on a single female specimen collected from Gulf of Mannar, off

Tuticorin. *Rhinobatos schlegelii* Norman, 1926 is another species similar to *R. variegatus* in colour pattern with the nostril width shorter than distance from nostril to lateral margin of disc which is similar for *R. variegatus* with nostril width (2.9-3.2% TL). But can be differentiated in its unique colour pattern and morphometrics. Nostril length 1.1-1.2 times in internarial distance and pre orbital distance 2.7-3 times inter spiracular distance. Preoral length 2.7-2.9 in mouth width and for *Rhinobatos schlegelii* it is  $3\frac{1}{3}$  times mouth width. Eye length 4.4 in snout length but in *Rhinobatos schlegelii* it's nearly 5 times. *Rhinobatos schlegelii* is described from Japan and considered as a NW pacific endemic, with nominal reports from China Taiwan and Korea (Compagno and Ishihara, 2009). *Rhinobatos leucospilus* Norman, 1926 is another species closely similar to *R. variegatus*, but can be differentiated from the latter in the presence of symmetrical bluish grey spots on the snout. In *Rhinobatos leucospilus* the snout length is shorter, snout 2-2.5 in interspiracular distance.



**Table 3.6.** Proportional dimensions as % of total length and means and ranges of measured specimens of *Rhinobatos variegatus*

	GA.1.7.5.6,	CMFRI/PFD/ M/6.3	CMFRI/PFD/ M/6.2	CMFRI/PFD/ M/6.5	CMFRI/PFD/ M/6.4	min	max	Avg.	SD
Total length, TL (mm)	610.0	620.0	690.0	543.0	583.0	543.0	690.0	609.0	62.5
Disc width-maximum)	28.6	30.2	30.3	31.2	28.9	28.9	31.2	30.2	0.9
Disc length	37.3	39.3	39.8	40.4	37.7	37.7	40.4	39.3	1.2
Head length-dorsal)	24.4	24.0	24.1	24.8	24.4	24.0	24.8	24.3	0.4
Head length-ventral)	24.0	24.2	24.3	25.0	24.5	24.2	25.0	24.5	0.4
Snout length-presocket)	12.9	12.7	12.6	13.7	13.4	12.6	13.7	13.1	0.6
Snout length horizontal	12.9	12.7	12.2	13.7	13.1	12.2	13.7	12.9	0.6
Orbit diameter	2.2	2.3	2.4	2.2	2.3	2.2	2.4	2.3	0.1
Spiracle width	1.9	2.0	1.8	2.1	2.0	1.8	2.1	2.0	0.1
Spiracle length	1.0	1.3	1.3	1.3	1.0	1.0	1.3	1.2	0.1
Small fold length	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.0
large fold length	0.5	0.5	0.6	0.5	0.6	0.5	0.6	0.5	0.1
Distance between bases of folds	0.4	0.4	0.4	0.4	0.2	0.2	0.4	0.3	0.1
Orbit and spiracle length	4.4	4.4	4.4	4.1	4.3	4.1	4.4	4.3	0.2
Interorbital width	3.2	3.2	2.9	3.1	2.9	2.9	3.2	3.0	0.1
Interspiracular width	4.7	4.6	4.7	4.7	4.5	4.5	4.7	4.6	0.1
Snout to max width	29.0	31.8	33.3	33.3	31.2	31.2	33.3	32.4	1.1
Preoral length	14.9	14.8	14.5	15.4	15.4	14.5	15.4	15.0	0.4
Mouth width	5.4	5.5	5.4	5.7	5.3	5.3	5.7	5.5	0.2
Prenarial distance	12.0	12.0	11.9	12.6	12.4	11.9	12.6	12.2	0.3
Nostril length	3.1	3.0	2.9	2.9	3.2	2.9	3.2	3.0	0.2
Anterior aperture-width)	1.1	1.0	1.1	1.0	1.1	1.0	1.1	1.0	0.0
Anterior nasal flap-base length	2.5	2.5	2.5	2.7	2.6	2.5	2.7	2.6	0.1
Anterior nasal flap base-width	1.6	1.5	1.5	1.8	1.5	1.5	1.8	1.6	0.1
Posteriolateral nasal flap- total length	2.4	2.4	2.4	2.3	2.3	2.3	2.4	2.3	0.1
Posteriolateral nasal flap- width	0.6	0.6	0.6	0.7	0.5	0.5	0.7	0.6	0.1

Posterior nasal flap- base length	1.4	1.5	1.3	1.6	1.3	1.3	1.6	1.4	0.1
Posterior nasal flap- width	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0
Distance across anterior nasal apertures	8.4	8.2	8.0	8.1	8.4	8.0	8.4	8.2	0.2
Internarial distance-minimum	2.7	2.6	2.5	2.7	2.6	2.5	2.7	2.6	0.1
Distance between anterior nasal flaps	1.6	1.3	1.1	1.3	1.3	1.1	1.3	1.2	0.1
Distance from nostril to disc margin	3.3	3.9	3.7	4.1	3.4	3.4	4.1	3.7	0.3
First gill opening	1.1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.0
Third gill opening	1.4	1.5	1.5	1.4	1.3	1.3	1.5	1.4	0.1
Fifth gill opening	1.0	1.0	1.2	0.9	1.0	0.9	1.2	1.0	0.1
Distance between first gill openings	11.4	11.6	12.0	11.8	11.0	11.0	12.0	11.6	0.4
Distance between third gill openings	8.0	8.2	8.4	8.8	8.0	8.0	8.8	8.4	0.4
Distance between fifth gill openings	8.0	8.0	8.6	8.9	8.0	8.0	8.9	8.4	0.5
Pelvic fin- length	15.3	15.6	17.7	16.5	16.3	15.6	17.7	16.5	0.9
Pelvic fin- anterior margin length	8.9	8.8	9.2	10.1	8.7	8.7	10.1	9.2	0.6
Pelvic fin- width	4.6	4.8	3.9	4.6	4.1	3.9	4.8	4.3	0.4
Pelvic fin-base length	8.0	8.1	10.1	8.2	7.5	7.5	10.1	8.5	1.1
Pelvic fin- inner margin length	9.1	7.2	7.0	6.4	8.9	6.4	8.9	7.4	1.1
First dorsal fin- length	6.3	6.4	6.2	6.0	6.2	6.0	6.4	6.2	0.2
First dorsal fin- anterior margin length	9.3	9.3	9.2	8.4	9.0	8.4	9.3	9.0	0.4
First dorsal fin- height	7.4	7.2	6.9	6.0	7.2	6.0	7.2	6.9	0.6
First dorsal fin- base length	4.5	4.5	4.4	4.3	4.4	4.3	4.5	4.4	0.1
First dorsal fin- inner margin length	1.8	2.0	1.9	1.8	1.9	1.8	2.0	1.9	0.1
First dorsal posterior margin	6.6	7.0	6.3	5.6	7.2	5.6	7.2	6.5	0.8
Second dorsal fin- length	6.7	6.9	6.3	6.2	6.6	6.2	6.9	6.5	0.3
Second dorsal fin- anterior margin length	9.4	9.1	9.3	8.2	9.3	8.2	9.3	9.0	0.5
Second dorsal fin- height	6.1	6.9	5.8	6.4	6.0	5.8	6.9	6.3	0.5
Second dorsal fin- base length	5.0	5.0	4.7	4.4	4.7	4.4	5.0	4.7	0.3
Second dorsal fin- inner margin length	2.0	1.9	1.7	1.9	1.8	1.7	1.9	1.8	0.1
Second dorsal posterior margin	6.0	6.4	5.8	5.5	6.9	5.5	6.9	6.2	0.6
Caudal fin- dorsal margin	12.5	13.1	12.2	11.5	12.8	11.5	13.1	12.4	0.7

Caudal fin- preventral margin	6.9	7.5	6.7	8.3	7.4	6.7	8.3	7.5	0.7
Snout to first dorsal fin origin	5.7	58.9	59.4	58.6	56.7	56.7	59.4	58.4	1.2
Snout to second dorsal fin origin	7.5	75.5	76.1	75.9	74.4	74.4	76.1	75.5	0.7
Snout to upper caudal fin origin	86.9	87.1	87.7	88.0	87.1	87.1	88.0	87.5	0.5
Snout to lower caudal fin origin	87.5	88.4	88.7	89.3	88.3	88.3	89.3	88.7	0.5
Snout to pelvic fin origin	34.1	35.8	35.5	36.9	34.4	34.4	36.9	35.6	1.0
Snout to anterior vent	36.8	39.1	39.6	39.2	37.0	37.0	39.6	38.7	1.2
Pelvic fin insertion to dorsal fin origin	16.1	14.6	14.0	13.0	15.5	13.0	15.5	14.3	1.1
Interdorsal distance	13.4	12.1	12.9	12.2	12.7	12.1	12.9	12.5	0.4
Caudal peduncle length-dorsal	7.3	6.2	7.6	7.8	7.7	6.2	7.8	7.3	0.8
Body width- pectoral fin insertion	12.4	13.9	13.5	13.3	11.9	11.9	13.9	13.1	0.9
Body width- pelvic insertion	10.1	10.0	10.4	9.9	10.5	9.9	10.5	10.2	0.3
Disc width- anterior orbit	16.4	16.8	17.3	17.5	16.5	16.5	17.5	17.0	0.5
Disc width- anterior orbit	16.3	16.6	16.6	17.1	16.3	16.3	17.1	16.7	0.3
Body width- first dorsal fin origin	10.8	10.3	10.8	9.9	10.5	9.9	10.8	10.4	0.4
Body width- second dorsal fin origin	6.0	5.4	5.8	5.1	5.6	5.1	5.8	5.5	0.3
Lateral tail fold length	39.7	37.4	38.6	40.5	39.8				
Clasper length inner	12.6	0.0	0.0	0.0	11.9	0.0	11.9	3.0	5.9
Clasper length outer	6.6	0.0	0.0	0.0	6.4	0.0	6.4	1.6	3.2
Clasper width at insertion	1.4	0.0	0.0	0.0	1.3	0.0	1.3	0.3	0.7

## 3.2 Resurrections

### 3.2.1 *Odontanthias perumali*

The subfamily Anthiinae of the family Serranidae consists of 24 genera. They are brightly coloured fishes and a taxonomically confusing group. These fishes are very rare in collection because of their occurrence in deep reef habitat. The fishes of genus *Odontanthias* Bleeker, 1873 are known from the Indo-Pacific region and consist of fourteen species (Randall and Heemstra, 2006). *Holanthias perumali* Talwar, 1976 described from the southwest coast of India was considered as a junior synonym of *Odontanthias rhodopeplus* (Gunther, 1872) (Randall and Heemstra, 2006). According to the major review work on the group Randall and Heemstra, (2006), *Holanthias* Gunther, 1868 was considered as an Atlantic genera (except *Odontanthias hensleyi* Anderson and Graciela, 2012) and all the species of *Holanthias* in Indian Ocean has been subsequently moved to *Odontanthias* Bleeker, 1873 except those in *Meganthias* Randall and Heemstra, 2006. The present study by comparisons of the morphology, coloration and COI mitochondrial DNA and examination of holotype of *O. perumali* Talwar, 1976, revealed that it is a valid species different from *O. rhodopeplus* under which it was earlier synonymised. This study resurrects and redescribes *Odontanthias perumali* based on fresh materials from southwest coast of India.

Specimens of *Odontanthias perumali* were collected during May 2008 to December 2012 at Cochin Fisheries Harbour, Kerala, southwest coast of India. Morphometric and meristic counts have been made as per the method prescribed by Hubbs and Lagler (1964) with some changes as in Heemstra and Randall, (1979) and Randall and Heemstra, (2006).

#### ***Odontanthias perumali* (Talwar, 1976)**

(Figure 3.11; Table 3.8)

**Diagnosis:** Dorsal rays X 13; anal rays III, 7; pectoral rays 17-18; lateral- line scales 30 – 33; Gill rakers 12-14 + 27-30. Third dorsal spine elongated and with a black tip. Dorsal rays very long. Caudal lunate.

**Description:** (Morphometric data provided in table 3.7). Dorsal rays X, 10; pectoral rays 17, pelvic rays I,5, anal rays III, 7; Lateral -line scales 30 – 33; gill rakers on first arch upper limb 13-14 and lower limb with 28-30.

Body oblong and moderately compressed. Greatest body depth 37.8-40.12% SL. Head large; head length 38.3- 42% in SL. Preorbital region, in front of nostrils naked, rest of head scaly. Eye diameter 8.2-10.8% in SL. Inter orbital distance 10.5-10.8% in SL. Length of caudal -peduncle 17.5-17.9% of SL (Table 3.8) Mouth large and oblique, lower jaw projecting beyond upper jaw when mouth closed. Upper jaw length 17-18.5% in SL. Maxillary broadly expanded posteriorly. Nostrils close together in front of eye. Posterior one larger ovoid in shape. Upper jaw with a band of outer large teeth and inner band of villiform teeth. Anterior most three teeth of outer band near symphysis enlarged and curved inward. Lower jaw with a pair of canine anteriorly on each side of symphysis and two canines on sides. Vomer and palatines with teeth. Large oval patch of teeth on tongue. Opercle with flat spines. Finely serrated preopercle. Serrae at the angle of preopercle enlarged 2-7 large spines at angles of specimens examined.

Dorsal fin unnotched and continuous. Inserted little ahead, above upper end of gill opening. Second dorsal spine base above starting of lateral-line. Third dorsal spine largest with a black pennate tip, its length 13.9-33.5% of SL. Dorsal rays are very long. Two to five dorsal rays large and filamentous. Anal fin originating below the base of second and third dorsal ray. Length of first anal spine 5.8- 6.5% SL. Pelvic fin inserted slightly anterior to lower base of pectoral fin and reaches well beyond anal fin spines. Caudal lunate, upper lobe longer.

Scales moderately large, ctenoid. 8-9 lateral line scales on caudal peduncle. 6-8 in a series from origin of dorsal to lateral line and 17 (19) from origin of anal to lateral line. 1 ½ - 2 1/2 scales above lateral line at the base of Dorsal fin spine. Lateral line forming an angle below last dorsal soft ray. Scales on the base of dorsal soft rays and caudal rays. Comparative morphometrics of the currently studied specimen with that in earlier published works has been presented in Table 3.7.



**Figure 3.11.** *Odontanthias perumali* 162 mm SL

**Colour:** Body varyingly coloured. Rose coloured scales on the sides below lateral line. Upper part above the lateral line with dark-light olive green in colour. Predorsal to interorbital space a narrow stripe of rosy scales. Pectoral- fin base with orange and yellow scales. Dorsal, pelvic and anal fins white to light pink colour. A yellow band from upper lip and from front of snout passing below eye ending below the base of pectoral fin. Upper jaw, Lower jaw, thorax and abdomen are lavender to white in colour. Towards the caudal-peduncle scales with white spot. Base of the caudal fin with a narrow band of green color. A prominent white band in the caudal fin in between dark reddish brown bands. Distal end of caudal fin yellowish. In one specimen caudal fin is black in colour. Fins lavender, dorsal rays with very dark brown to orange spots. Third dorsal spine with a black tip.

**Distribution:** Indian coast from Karnataka to Chennai, on deep reefs.

Materials examined: ZSI F 6566/2, ZSI F 6567/2, four specimens SL 188, 194, 162 and 151 mm.

**Table 3.7.** Comparative morphometric of *Odontanthias perumali* in % of SL

Measurements	ZSI F	ZSI	Min	Max	Mean	SD
	6567/1	F6567/2				
Total length (mm)	136.53	137.67	129.14	138.66	134.7	4.07
Standard length (mm)	167	146	151	194	173	*
Head length	39.52	42.47	38.3	41.98	40	1.8
Pre orbital length	7.78	7.19	6.62	7.73	7.01	0.49
Post orbital length	21.26	22.6	22.7	25.83	24.52	1.38
Inter orbital length	9.88	10.27	10.49	10.82	10.64	0.14
Orbit length	10.18	10.96	8.25	10.77	9.5	1.09
Greatest body depth	37.72	38.36	37.77	40.12	38.91	0.98
Pectoral fin length	25.75	25.34	24.23	25.93	24.95	0.76
Pectoral fin base length	*	*	7.28	7.73	7.47	0.19
Pelvic fin base	*	*	4.64	5.15	4.88	0.22
Pelvic fin length	*	*	25.83	32.99	27.86	3.44
Pelvic fin spine length	*	*	11.17	14.2	13	1.29
Dorsal fin length	*	*	33.33	46.36	39.93	5.78
Dorsal fin base length	*	*	60.64	64.2	62.4	1.52
First dorsal fin spine length	*	*	4.64	5.56	5.07	0.43
Second dorsal fin spine length	*	*	8.51	9.27	8.8	0.33
Third dorsal spine length	*	*	13.91	33.51	20.28	8.93
Fourth dorsal spine length	*	*	11.34	13.58	12.47	1.11
First dorsal soft ray length	*	*	17.02	21.19	18.6	1.83
Second dorsal soft ray length	*	*	31.13	43.3	35.94	5.59
Third dorsal soft ray length	*	*	29.79	43.81	36.37	6.8
Fourth dorsal soft ray length	*	*	20.99	38.66	27.39	7.85
Fifth dorsal soft ray length	*	*	12.77	23.12	18.08	4.8
Anal fin base length	*	*	18.02	19.26	18.71	0.6
Anal fin length	*	*	16.7	23.71	19.07	3.15
First anal spine length	*	*	5.82	6.49	6.02	0.32
Second anal spine length	*	*	11.07	13.58	12.4	1.28
Third anal spine length	*	*	12.48	14.7	13.35	1.05
First anal ray length	*	*	13.62	17.79	15.69	1.71
Second anal ray length	*	*	14.92	20.5	17.2	2.49
Fifth anal ray length	*	*	13.58	22.16	16.76	3.74
Caudal fin length	*	*	30.46	38.14	33.46	3.33
Caudal fin base length	*	*	12.89	14.2	13.57	0.59
Upper maxilla length	17.37	18.49	17.01	18.52	17.61	0.73
Lower jaw length	*	*	11.34	13.58	12.73	0.99
Dorsal to snout length	*	*	29.26	32.72	30.71	1.45
Caudal peduncle lower	*	*	17.53	17.9	17.65	0.17
Caudal peduncle length upper	*	*	12.37	15.89	14.34	1.49
Caudal peduncle depth	*	*	10.82	11.73	11.38	0.4
Lateral line scales	33	33	30	31	30	*
Gill rakers upper	12	12	13	14		*
Gill rakers lower	29	29	28	30		*

### 3.3 New distributional records

#### 3.3.1 *Scombrobrax heterolepis*

*Scombrobrax heterolepis* Roule, 1921 (Scombrobracidae) is monotypic member of suborder Scombrobracoidei (Nelson, 2006). The longfin escolar, *Scombrobrax heterolepis*, also known as the black mackerel, is a widespread but uncommon deepsea fish known from tropical and subtropical areas of the Pacific, Indian and Atlantic Oceans and not known from the eastern Pacific and south-eastern Atlantic. Inhabiting continental shelves and slopes at depths between 130 and 1374 m (Higgins *et al.*, 1970; Costa *et al.*, 2007). Found in stomachs of tunas and billfishes and this species in turn preyed on fishes, cephalopods and crustaceans (Parin, 1990). Fecundity was estimated about 220/250,000 in both ovaries (Carvalho-Filho *et al.*, 2010) and the spawning probably occur throughout the species range and the year (Potthoff *et al.*, 1980). To date no specimens were ever reported from Indian waters and this study confirms the presence of *Scombrobrax heterolepis* in Indian waters.

On 16 October 2010 a single specimen of *Scombrobrax heterolepis*, 188.5 mm SL was collected from a commercial deep-sea shrimp trawler operated in the Arabian Sea, off the south-western coast of India (Fig 3.12). The specimen was deposited at Central Marine Fisheries Research Institute, Kochi, India under the Accession Number GB.43.6.16.10. The morphometric and Meristic characters of the specimen agree well with data from the previous studies (McEachran and Fechhelm, 2005; Carvalho-Filho *et al.*, 2010) (Table 3.9). This is the first record from the west coast of India and confirms the widespread distribution of this species.

#### ***Scombrobrax heterolepis* Roule, 1921**

(Figure 3.12; Table 3.8)

Longfin escolar

**Diagnosis:** Dorsal fin spines and rays XII+I, 14; Anal fin spines and rays III, 16; Pectoral fin rays 19; Lateral line scales 47; Lower limb of first gill arch with 5 well-developed gill rakers, about 10 clusters of minute spines on upper limb. Body moderately elongate and compressed; head large, 34.6% SL, the interorbital region flat, 8.5% SL, very large eye, 10.3% SL and its diameter almost as long as the conical snout, 3.5 in HL, terminal large mouth, the upper jaw protractile, the lower



projecting slightly beyond the upper; teeth in upper jaw in a row of small to moderate, compressed canines, with three very large, stout canines; teeth of lower jaw larger without large canine; Two large nasal openings each side of snout. Two dorsal fins, base of first dorsal fin about twice in base of second dorsal fin; origin of first dorsal fin slightly posterior to pectoral-fin base. Caudal fin forked and moderately small. Very long pectoral fins, 31.9% SL, reaching anal-fin origin in vertical. Pelvic fins originating below origin of pectoral fins; Lateral line running closely to dorsal base, ending slightly before end of second dorsal fin. The morphometric characteristics of the present specimen match with the representatives described by previous studies except anal finbase length, which was lesser (16.2% SL in the present study) compared to 19-22% of SL reported by Carvalho-Filho *et al.*, 2010.

**Colour:** Body uniformly dark brown without distinct markings, fins darker;



**Figure 3.12.** *Scombrolabrax heterolepis*, 188.5 mm SL, Arabian Sea, off the south-western coast of India

### 3.3.2. *Diaphus garmani*

Lanternfish of the family Myctophidae are found in all Oceans of the world, with 32 genera and with at least 240 species (Nelson, 2006). An important characteristic of myctophids is the presence of luminescent organs called photophores present along their ventral body surface and head. The different patterns of photophores have been used, along with meristic data in species identification. These fishes have species-specific diel vertical migration patterns (Watanabe, *et al.*, 1999). Myctophids, like many other mesopelagic fishes are an important constituent of the diet of commercially important oceanic fishes and marine mammals (Jackson *et al.*, 1998).

Investigations on the myctophid fauna of the western and northern Arabian Sea were carried out by R/V Dr. Fridtjof Nansen during 1975-1981 and 1983-84

(Gunnar *et al.*, 1999). Studies on distribution and abundance of Myctophidae in the EEZ of India were carried out by *FORV Sagar Sampada* during 1985-1986 (Raman and James, 1990). About 55 species of Myctophids are known from the Arabian Sea including its southern part of the Indian Ocean (Nafpaktitis, 1978). Karuppasamy *et al.*, (2007) reported 27 species of myctophids from Indian EEZ. Somvanshi *et al.*, (2009) reported 5 species of myctophids from south west coast of India.

**Table 3.8.** *Scombrobrax heterolepis*. Proportional measurements in % of SL

	Present study	McEachran and Fechhelm*, 2005	Carvalho-Filho <i>et al.</i> , 2010
Number of specimens	1	No mentioned	12
Total length	117.2	-	-
Standard length	100.0	-	-
Head length	34.6	34-35	30-34
Body depth	25.5	24-25	22-24
Body width	12.4	-	-
Snout length	9.9	09-11	09-10
Eye diameter	10.3	09-10	08-11
Interorbital width	8.5	-	-
Upper jaw length	15.5	16-17	13-15
Lower jaw length	11.7	-	-
First dorsal finbase length	34.8	-	-
Second dorsal finbase length	15.4	-	15-18
Anal finbase length	16.2	-	19-22
Pectoral finbase length	4.6	-	-
Predorsal length	40.2	40-43	38-39
Prepectoral length	34.8	-	-
Preanal length	70.8	-	67-73
Pectoralfin length	31.9	30-36	31-37
Pelvicfin length	8.2*	14-15	10-13
Caudal peduncle length	13.1	-	-
Caudal peduncle width	5.2	-	-
Caudal peduncle depth	9.6	-	-
<b>Counts</b>			
Number of specimens	1		12
Dorsal fin spines and rays	XII+I,14	-	XII+I,14-15
Anal fin spines and rays	III, 16	-	III, 15-17
Pectoral fin rays	19	-	18
Lateral line scales	47	-	47-50
Lower gill rakers	5	-	4-6

\*No original data; data of several authors, including juveniles and larvae

\*Broken

*Diaphus* is the most speciose of the myctophid genera, with 70–75 known species (Nafpaktitis *et al.*, 1995). The members of this genus can be assigned to two distinct groups on the basis of the presence or absence of a suborbital (So) luminous organ and an inner series of broad-based, forward-hooked teeth on the posterior part of the premaxilla (Nafpaktitis, 1978). *Diaphus garmani* is a small diaphid fish attains a maximum length of about 60 mm.

Fishes were captured in April 2009 by a commercial deep-sea shrimp trawler operated in the outer shelf of west coast of India between 8 – 11 °N and 74 – 76 °E. Three specimens of *Diaphus garmani* were captured during shrimp trawl operations that ranged from 250 to 450 m depth. Other catch included considerable quantities of pandalid shrimps *Plesionika* spp., marine hatchetfishes *Polyipnus* spp. and *Neoscopelus microchir*. The specimens of *D. garmani* were deposited at CMFRI, India under the accession number GB.27.1.5.25.

#### ***Diaphus garmani* Gilbert, 1906**

(Figure 3.13; Table 3.9)

Garman's lanternfish

**Diagnosis:** Origin of dorsal fin over base of ventral fin. Origin of anal fin behind end of base of dorsal fin. Base of adipose fin directly over end of base of anal fin. Dn is small directed anterolaterally. PLO distinctly nearer to lateral line than to base of pectoral fin. VLO midway between lateral line and base of ventral fin. SOA on a straight line, SAO<sub>2</sub> slightly behind line through centers of SAO<sub>1</sub> and SAO<sub>3</sub>. SAO<sub>3</sub> at lateral line. AOa<sub>1</sub> abruptly elevated, directly above AOa<sub>2</sub>; last AOa also elevated. Pol in contact with lateral line. AOp<sub>1</sub> over end of base of anal fin. First three Prc evenly spaced, forming a gently arc; Prc<sub>3</sub>-Prc<sub>4</sub> interspace enlarged, with Prc<sub>4</sub> about 1.5 times its diameter below midlateral line. A vertically elongated, roughly rectangular luminous scale at PLO.

Identification followed by Nafpaktitis *et al.*, (1978) and relied on the luminous organs on the head, number of fin rays, gill rakers and morphometric characters. Other morphometric and meristic data for the specimens collected from the south west coast of India are similar to those recorded by Kawaguchi and Shimizu (1978) for fish collected from the Southeast Asian Seas. Photophore nomenclature follows Hulley (1984). Photophores were counted as follows: PVO=2,

PO=4, VO=3, SAO=3, Aoa=7, Aop=4 and Prc=4. Number of fin rays (all soft): dorsal=15, pectoral=12, pelvic=9, anal=15 and caudal=20. Gill rakers on first arch=22 (8+14). Lateral line organs=38. Morphometric measurements of the specimens are presented in Table 3.9.

Among the other records of occurrence of the species include, Eastern Atlantic: Canary Islands, off Senegal. Western Atlantic: west of 30 °W between 36 °N and 10 °S. Indo-West Pacific: off East Africa, Comoro Islands and west coast of Madagascar; off Japan south of 40 °N, southeast Asian seas, Australia and New Zealand Eastern Pacific: near Hawaii, and off Acapulco, Mexico and South China Sea. Western Indian Ocean: between 16 °S and 26 °S and off Sri Lanka. (Nafpaktitis *et al.*, 1978; Dalpadado and Gjosaeter, 1993). The specimens reported here considerably extend the known distribution of this species to the west coast of India.

*Diaphus garmani* is the tenth species of the genus *Diaphus* to be recorded from Indian waters, together with *D. problematicus* Parr, *D. effulgens* Goode and Been, *D. fragilis* Taning, *D. perspicillatus* Ogilby, *D. aliciae* Fowler, *D. lucidus* Goode and Been, *D. phillipsi* Fowler, *D. signatus* Gilbert, *D. watasei* Jordan and Starks (Karuppasamy *et al.*, 2007).



**Figure 3.13.** *Diaphus garmani*, 54 mm SL

**Table 3.9.** Morphometric measurements and meristic of *Diaphus garmani*

Measurements	from off Japan 32 (25.5-53.0 mm SL)	from Sulu Sea 6 (52.0-59.5 mm SL)	South China Sea 1 (40.0 mm SL)	Atlantic 45 (27.5-48.0 mm SL)	Present study 3 (53.4-58.9 mm SL)
Head length	26.5-30.7	26.2-28	27.9	27.6-30.4	27.8-31.3
Head depth	21.6-25.0	22.3-23.4	23.7	21.4-24.2	22.5-24.5
Eye diameter	6.9-8.7	6.9-7.6	7.5	6.9-8.8	7.0-7.4
Upper jaw length	19.4-22.6	19.3-20.3	21.9	20.0-22.3	20.3-24.6
Body depth	20.8-24.1	22.4-23.4	22.9	22.2-24.7	22.4-23.4
Caudal depth	9.2-12.9	10.4-11.6	10.7	10.3-12.2	10.6-11.4
Predorsal length	40.8-44.0	40.7-41.9	42.9	41.1-44.7	40.4-41.4
Preventral length	40.4-44.7	39.1-44.1	42.4	39.8-44.9	39.7-43.7
Prepectoral length	27.4-30.5	26.2-29.2	28.4	27.6-30.3	28.3-30.1
Preanal length	59.3-63.3	59.9-64.6	59.6	59.1-63.1	60.1-61.7
Preadipose length	76.2-80.2	77.2-80.5	79.8	77.8-81.8	79.2-80.7
Upper jawlength/E.D	2.4-3.1	2.6-2.9	2.6	2.4-3.2	2.5-2.8
H.D/ED	3.3-4.1	3.5-4.0	3.3	3.3-4.3	3.2-3.8
Dorsal fin base length	17.7-21.2	17.3-20.4	20.4	18.8-22.4	19.2-20.8
Anal fin base length	17.5-21.3	10.0-20.6	20.2	19.7-22.6	18.8-20.1
Dorsal fin ray	14 (13-15)	15 (14-16)	15	15 (14-16)	15
Anal fin ray	15 (14-17)	15 (14-16)	15	16 (15-17)	15
Pectoral fin ray	12 (11-12)	11-Dec	11	12 (11-12)	12
Gill rakers on first arch	7 (6 or 8, rarely 5) + 1+13-14 (rarely 12) =20-23 (rarely 18)	7+1+14(13)=22(21)	7+1+13=21	7 (6 - 8)+ 5 (4-6very rarely 7) =12(11-13, very rarely 14)	7+1+14=22
AO photophores	6-8+5-6 (rarely 4)=11-13 (rarely 14)	6-7+5-6=11,12	6+6=12		7+4
Lateral line scales	37-38	37-38		38-39	38

### 3.4. Rare deep-sea fishes

#### 3.4.1. *Snyderina guentheri*

The rare deep water scorpion fish *Snyderina guentheri* (Boulenger, 1889) is recorded for the second time after a gap of 35 years from Indian waters based on three specimens collected from bottom trawlers off Kollam, southwest coast of India. Information on *S. guentheri* is desirable since this fish has been rarely noted in ichthyological literature. Talwar (1976) reported the occurrence of *S. guentheri* based on 14 specimens, ranging from 113-163 mm SL, collected on March 1975 off Kollam (8–45 °N and 75 – 50 °E) at a depth of 300 m. The present report of three additional specimens of *S. guentheri* ranging from 116- 172 mm SL, collected on April 2008 off Kollam, southwest coast of India, between 8-11 °N and 74-76 °E, at depth 100-200 m. These three specimens are deposited at CMFRI under the Accession number GB.27.1.5.25.

#### ***Snyderina guentheri* (Boulenger, 1889)**

(Figure 3.14; Table 3.10)

Gunther's waspfish

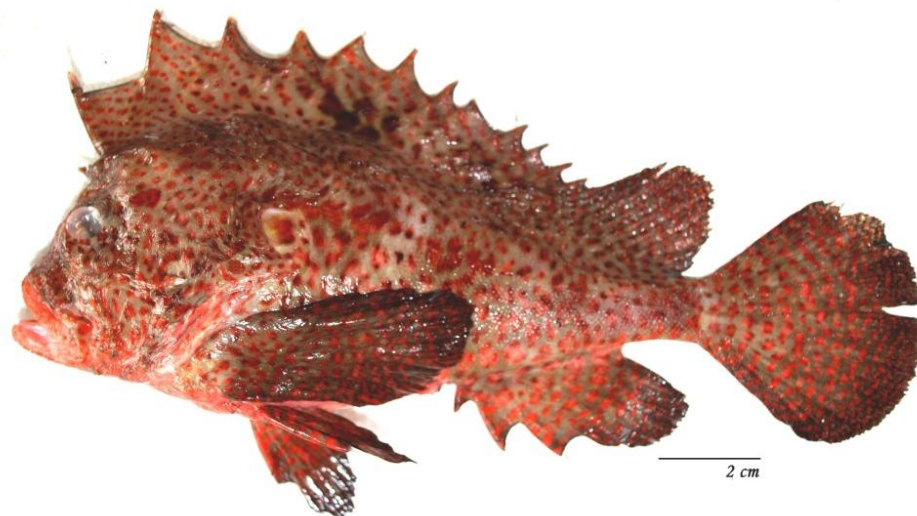
**Description:** D XIII 10; A III 6; P 13; V I 5. Gill rakers on first arch 4 + 9. Lateral line tubules 22. Body robust, compressed, depth of body 39.5%, length of head 43.1% of standard length. Diameter of eye 11.95, interorbital width 22.37, length of snout 27.1 and length of pectoral fin 78.6% of head length. Upper part of head without spiny ridges, its upper profile with a concavity before eyes; preorbital with a short, blunt spine directed forward and a strong, backwardly directed spine equal in length to eye diameter; suborbital ridge without spines; hind border of preopercle with a spine, slightly shorter than preorbital spine. Mouth large, oblique, lower jaw projecting; maxillary extending to vertical from anterior third to half eye diameter. Nostrils subtubular, anterior with a flap. Two bony ridges beginning above posterior nostril, converging on interorbital space and continued as a broad, median ridge to base of first dorsal spine. No barbules.

Teeth in villiform bands in jaws and vomer; palatines edentate. Head naked, body with small non-imbricate scales. Dorsal fin continuous, origin above vertical through anterior third eye diameter; first spine shortest, 0.5 eye diameter, third

longest, about 0.5 length of head; soft rays divided; 3<sup>rd</sup> spine longest; dorsal and anal fins adnate to caudal peduncle by membrane. Pectorals extending beyond origin of anal fin in young specimens; pelvic extending up to origin of anal fin; caudal rounded. The genus *Snyderina* was established for the reception of a new species, *S. yamanokami* from Japan. The proportional measurements and counts of *S. guentheri* is given below (Table 3.10).

**Table 3.10.** Proportional measurements and counts of *Snyderina guentheri*

Measurements (mm)	<i>Snyderina guentheri</i>
Standard length	172
In SL	
Head length	74.2
Body depth	68
Snout length	20.08
Upper jaw length	25.4
Pectoral length	58.35
Pelvic length	44.68
Orbit diameter	8.87
Inter orbital width	16.6
Post orbital length of head	48.16
Predorsal length	28.68
Preanal length	117.4
Caudal peduncle depth	16.4
Dorsal fin rays	XIII, 11
Anal fin rays	III, 6
Pectoral fin rays (left, right)	13
Pelvic fin rays	I, 4
Gill rakers on first arch (left, right)	14, 14
Branchiostegal rays	6
Pores in lateral line (left, right)	23, 24
Vertebrae	26



**Figure 3.14.** *Snyderina guentheri* 164 mm SL

**Chapter 4**

***Molecular identification of deep-sea***

***Chondrichthyans***



## Chapter 4

# Molecular identification of deep-sea Chondrichthyans

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### 4.1. Introduction

Chondrichthyans are considered as one of the major demersal capture fishery resources of Indian Exclusive Economic Zone (EEZ) and being exploited for many decades. The deep-sea chondrichthyan species includes sharks, rays and holocephalans distributed at depth below 200 m (Kyne and Simpfendorfer, 2007). The deep-sea chondrichthyan fishery of Indian EEZ is both targeted and by-catch in the deep-sea shrimp fishery. The major species contributed in the fishery include *Neoharriotta pinnata*, *Echinorhinus brucus*, *Centrophorus granulosus*, *C. atromarginatus*, *C. squamosus* and *Centrophorus cf. zeehaani* (Raje *et al.*, 2007; Mohanraj *et al.*, 2009; Akhilesh *et al.*, 2011). Along with targeted catch and occasional bycatch of deep-sea fishery, several other species of sharks and rays are caught which are discarded to the sea due to the low or no market value. It is well known that slow growth, late maturation, longevity of chondrichthyans in the deep-sea have limited potential to recover from overfishing (Simpfendorfer and Kyne, 2007). However, the baseline data available on taxonomy, distribution, species composition, biology etc are very scanty from Indian seas.

Nearly 35 percent of chondrichthyan species are confined to deep-sea habitat. Knowledge of the deep-sea chondrichthyan fauna is scanty and many species are discovered worldwide regularly. Recently, several species of deep-sea chondrichthyans were described that include *Apristurus breviventralis*, *Squalus nasutus*, *S. crassispinus*, etc. Most of the deep-sea chondrichthyan species from Indian waters were described during 1890-1970s. However, many of them were synonymised or considered to be invalid at present. New species described from Indian waters which still remain valid includes *Pentanchus investigatoris* Misra, 1962, *Cephaloscyllium silasi* (Talwar, 1974), *Rhinobatos variegatus* Nair and Lal Mohan (1973), *Heteronarce prabhui* Talwar, (1981) and *Mustelus mangalorensis* Cubelio, Remya and Kurup, 2011.

Recently, molecular markers have been used to support the morphological description and identification of deep-sea chondrichthyan species. Ward *et al.*, (2008b) used COI sequences for discriminating *Squalus* and *Apristurus* species and also led to the discovery and description of many new species. Naylor *et al.*, (2012) used NADH2 to identify the sharks and rays species diversity globally. In India, despite the rich biodiversity of deep-sea chondrichthyan species, identification of species was based on only morphological characters. There was no molecular study conducted to know the genetic diversity within and between species. Given the historical and current taxonomic ambiguity and inconsistency in the taxonomy of deep-sea chondrichthyan species, this chapter is to provide a comprehensive DNA barcode library for the deep-sea chondrichthyan species from the Indian EEZ and also to investigate their phylogenetic relationships.

## **4.2. Materials and methods**

### **Study area**

The area selected for the present study was southern coast of India, which comprises more than 40% of Indian coastline, adjoining five maritime states, Goa, Karnataka, Kerala, Tamil Nadu and Andhra Pradesh.

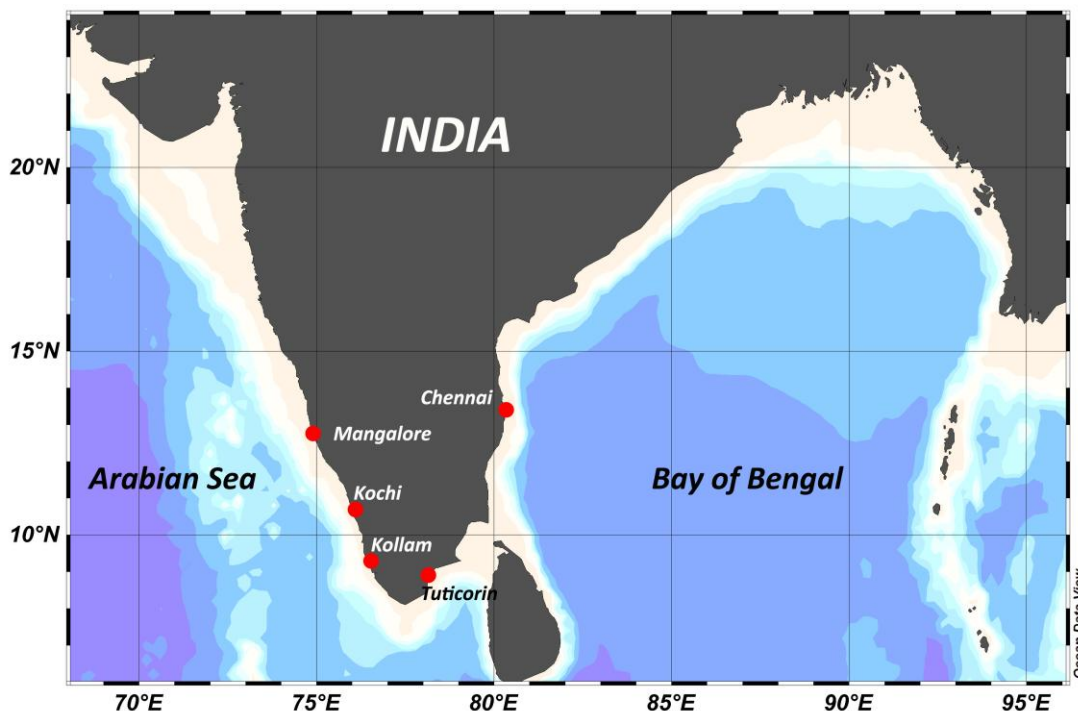
### **Landing centre collections**

Field survey was conducted during 2008–2013 at commercial fish landing centres along the southern coast of India to study the diversity of deep-sea fish fauna in the commercial catch. Fish samples for taxonomic and subsequent molecular analysis were collected from Mangalore Fisheries Harbour, Cochin Fisheries Harbour, Saktikulangara Fishing Harbour, Tuticorin Fishing Harbour and Kashimedu Fishing Harbour (Fig. 4.1).

### **Exploratory survey collections**

The deep-sea fish samples were collected during the research programme of FORV *Sagar Sampada* (CMLRE/MoES) cruise numbers 281, 291 and 322. The first collection was made during cruise number 281 (2010), covered the latitude 8 °24'–11°.13 N and longitude 74 °56'–76 °07' E on the southwest coast of India. There are 37 fishing operations covering eight transect using HSDT-CV and EXPO were conducted at depth ranges 180–1200 m. Representative fish samples was collected at each stations. Second cruise collection (cruise number 291) was covered the latitude

10°35' – 18°50' N and longitude 80°22' – 85°22' E at depth ranges 389–535 m along the east coast of India (Fig. 4.2). Final collection was made during January 2014 (cruise number 322) covered 14 deep-sea stations covered the latitude 18°50' N – 18°58' N and longitude 85°22' – 85°25' E at depth ranges 389–535 m along the west coast of India.



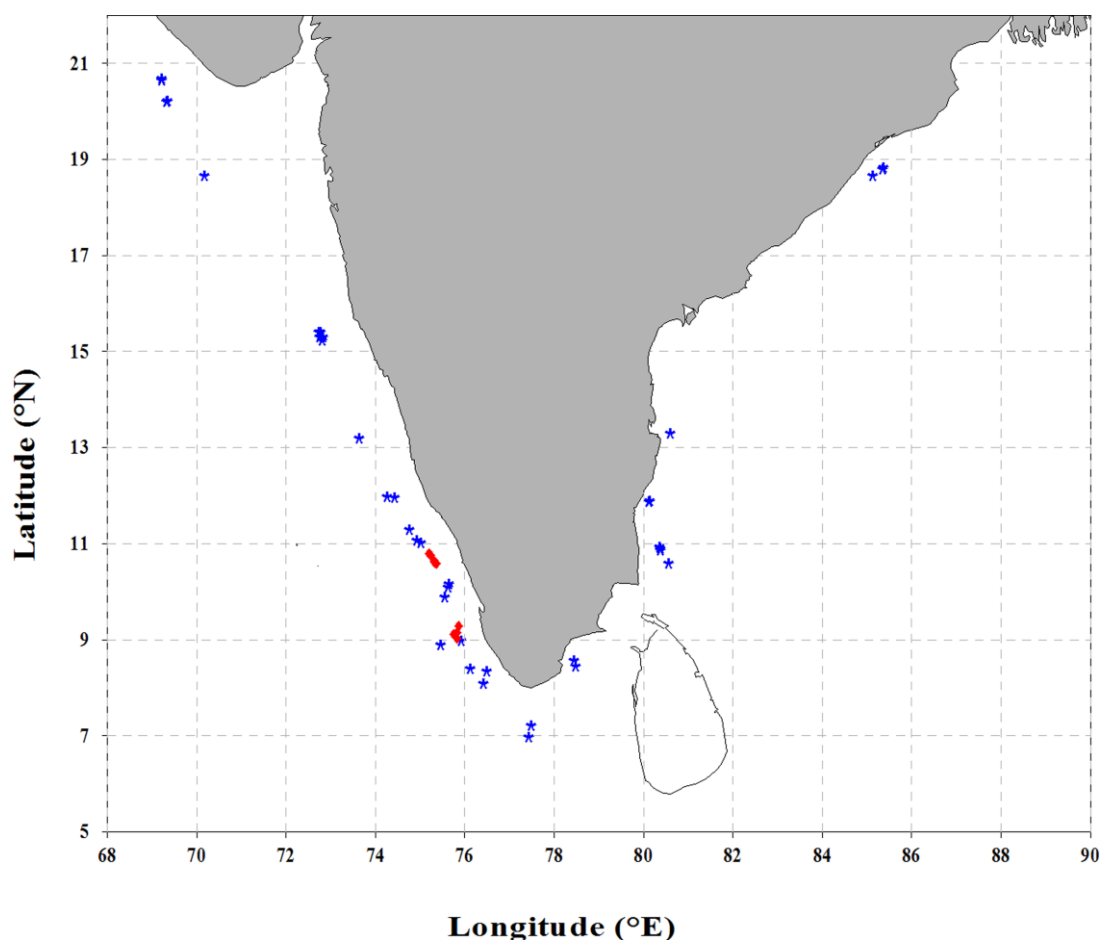
**Fig. 4.1.** Map showing the collection localities of deep-sea fishes

Another major collection was made during the fishery resource survey programme of Fishery Survey of India (FSI) by using the vessel *Matsya Varshini* (OAL 36.2 m) during February 2010 and covered the latitude between 8° & 21°N on the southwest coast of India (Fig. 4.2). Nine fishing operations were conducted using shrimp trawl (head rope: 48 m, codend mesh: 30 mm) at depth ranges 250–550 m.

### 4.3. Tissue collections and cataloguing

After the collections, samples were brought to the lab for further analysis. Each specimen was washed well and all fins were spread and fixed with formalin before taking high quality photographs. Approximately 100 mg of white muscle tissues or gill tissues were collected from each species and preserved in 95% ethanol in properly labelled sterile 2 ml storage vials and kept at 20 °C until further analysis. Species identification was done based on Alcock (1899), Misra (1969), Talwar and Kacker (1984), Compagno (1984), Last and Stevens (1994), Carpenter and Niem (1999) and Compagno *et al.*, (2005). All specimens were photographed and the

vouchers were deposited at CMFRI, Kochi; ZSI, Kolkata; ZSI, Kozhikode and NBFGR, Kochi. Species, family, voucher number and GenBank accession numbers are given in the Appendix I and Appendix II.



**Fig. 4.2.** Map showing the exploratory survey collection localities of deep-sea fishes

#### 4.4. Mitochondrial DNA analysis

##### Genomic DNA isolation

The whole genomic DNA from the samples was isolated by following protocol of Miller *et al.*, (1988) with minor modification. (Appendix V for details on extraction method). DNeasy (Qiagen) kit, following manufacturer's instruction, was used to extract DNA from samples, where the salting out method failed to yield satisfactory results. The quality of DNA isolated was checked through 0.8% agarose gel (Appendix VI for details). The concentration of isolated DNA was diluted to a final concentration of 100 ng/ $\mu$ l after checking with UV spectrophotometer. Two genes of mt DNA, cytochrome C oxidase I (COI) and 16S rRNA were amplified by employing specific universal primers. For COI more than one set of primers (varied

primers) were used depending on the compatibility. Annealing temperatures ( $T_a$ ) were adjusted depending on the melting temperature ( $T_m$ ) of the respective primer used. The details of primer sequences used are listed in Appendix III. PCR conditions for each fragment are detailed in Appendix IV.

Each PCR procedure included a negative control (no DNA template). Verification of successful amplification was assessed by 1.8% agarose gel electrophoresis. After successful PCR amplification of the target fragments, amplified products were purified before the template was sequenced in both directions. The cleaned up PCR products were used as the template for sequencing PCR to increase the amount of product linearly with the number of cycles. Nucleotide sequencing was performed by the dideoxy chain-termination method (Sanger *et al.*, 1977) using ABI Prism Big Dye Terminator v3.1 Cycle Sequencing kit, (Applied Biosystems, USA). Terminators are dideoxynucleotides labelled with different coloured fluorescent dyes that will present different emission spectra on an electrophoresis gel illuminated by laser. Each PCR product was sequenced using both forward and reverse amplification primers. The resulting DNA fragments were cleaned before sending to the sequencing facility.

### **Amplification and sequencing**

The mitochondrial 16S rRNA gene was amplified in a 25  $\mu$ l reactions volume containing 1X assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 1.5 mM  $MgCl_2$  (Genei, Bangalore, India), 5 pmoles of each primer, 200  $\mu$ M of each dNTP (Genei, Bangalore, India), 1.5 U *Taq* DNA polymerase and 20 ng of template DNA. The primer used for the amplification of the partial 16S rRNA gene were 16SAR (5'-CGCCTGTTTATCAAAAACAT-3') and 16SBR (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi *et al.*, 1991). The thermal profile used was 36 repetitions of a three step cycle consisting of denaturation at 94 °C for 1 min, annealing 50 °C for 1 min and extension at 72 °C for 1.5 min including 4 min for initial denaturation at 94 °C and 7 min for final extension at 72 °C.

The partial sequence of COI gene was also amplified using primers Fish F1 (5' – TCA ACC AAC CAC AAA GAC ATT GGC AC - 3') and Fish R1 (5' – TAG ACT TCT GGG TGG CCA AAG AAT CA - 3') (Ward *et al.*, 2005) in 25  $\mu$ l reactions volume containing 1x assay buffer (100 mM Tris, 500 mM KCl, 0.1%

gelatin, pH 9.0) with 1.5 mM MgCl<sub>2</sub> (Genei, Bangalore, India), 5 pmoles of each primer, 200 μM of each dNTP (Genei, Bangalore, India), 1.5 U *Taq* DNA polymerase and 20 ng of template DNA. The thermal condition consisted of initial preheat at 95 °C for 3 min, denaturation 94 °C for 30 s, annealing 50 °C for 30 s, extension 72 °C for 35 s, repeated for 29 cycles, followed by a final extension for 3 min at 72 °C. The PCR products were visualized on 1.5% agarose gels. Samples with intense bands were selected for sequencing. Sequencing reactions used a BigDye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems, Inc). All samples were sequenced bidirectionally using an ABI3730 capillary sequencer following the manufacture's protocol.

### Sequence analysis

The raw DNA sequences were edited and aligned using BioEdit sequence alignment editor version 7.0.5.2 (Hall, 1999). The extent of sequence differences between species was calculated by averaging pair-wise comparisons of sequence differences across all individuals. The sequence divergence values within and between species were calculated using Kimura 2-parameter (K2P) distance model implemented in MEGA 5 (Tamura *et al.*, 2011) software. The number of polymorphic sites and nucleotide diversity (Pi), nucleotide composition and number of transition and transversion between species were determined by DnaSpver 3 (Rozas *et al.*, 2006). Neighbour-joining (NJ) trees of K2P distance were created to provide graphic representation of divergence with 1000 replications.

### 4.5. Results

The sequence generated during this study included 268 individual sequences from 38 species and 28 genera of 20 families used for partial sequence analysis of 16S rRNA and COI genes (Appendix I). A total of 542 sequences were generated, including both forward and reverse sequences. All sequences were compared with NCBI GenBank and BOLD for initial identification confirmation. The partial sequences of mt DNA generated under this study were deposited in the GenBank database (Appendix II).

### 4.5.1. Cytochrome Oxidase subunit I (COI) and DNA barcoding

#### Taxon Diversity

A total of 186 individuals of 38 species of chondrichthyans (two chimaerids, 26 sharks and 10 rays) were collected from the southern coast of India at depth ranges of 200–1000 m. A total of 186 COI sequences for 38 species were generated (Appendix I). Amplified sequence length varied among species and families but consistent within species. The shortest sequence observed was 640 bp in *Benthobatis moresbyi* and longest with 672 bp in *Zameus squamulosus*. Sequences were aligned and multiple alignments resulted in consensus length of 640 sites per taxon. All sequences were compared with NCBI GenBank and BOLD for identification and confirmation. Out of the total 640 sites obtained, 344 (53.75%) were constant, 296 (46.25%) variable and 296 (46.25%) parsimony sites. A total of 65 haplotypes were observed across the taxa. Within each species (n=5), minimum number of haplotypes was one (*Benthobatis moresbyi*, *Eridacnis radcliffei*) and maximum was 4 in *Dipturus* sp. A. The overall mean distance of individuals among chondrichthyans under present study was estimated as 25.7%. The maximum interspecific K2P distance was 35% between *Centrophorus atromarginatus* and *Dipturus* sp. A and minimum was 2.8% divergence between *Centrophorus atromarginatus* and *C. zeehaani*. The minimum intraspecies distance observed was 0.1% in *Deania profundorum* and maximum intraspecies distance observed 0.6% in *Dipturus* sp. A. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 4.1 to 4.9.

Overall nucleotide contents across all samples for chimaeras, rays and sharks were estimated. The average percentage of the different nucleotides were T = 31.6, C = 2.9, A = 25.3, G = 17.1. As expected, the average transitional pairs (si=75) were more frequent than transversional pairs (sv=55) across all the taxa. The average transition to transversion rate (si/sv) estimate was 1.35.

#### Scyliorhinidae

Seven species under four genera belonging to the family Scyliorhinidae under the order Carcharhiniformes were analysed. The species identified are *Cephaloscyllium silasi*, *Halaaelurus quagga*, *Bythaelurus hispidus* and four species of *Apristurus* that are putative new species, here designated as *Apristurus* sp. A,

*Apristurus* sp. B, *Apristurus* sp. C and *Apristurus* sp. D. The amplified sequence length varied from 664 bp in *B. hispidus* to 662 bp in *C. silasi*. Out of the total 640 sites obtained 428 (66.8%) were constant, 212 (33.12%) variable, 184 (28.75%) parsimony sites and 28 (4.37%) singleton sites. The polymorphic sites of selected family are illustrated in Fig. 4.3. A total of 8 haplotypes were observed across the taxa. Within each species (n=5), minimum number of haplotypes was one (*Bythaelurus hispidus*) and maximum was 3 in *C. silasi*. The overall mean distance of individuals among the family was estimated as 17.2%. The maximum interspecific K2P distance was 19.8% between *C. silasi* and *Apristurus* sp. A and minimum was 3.9% divergence between *Apristurus* sp. C and *Apristurus* sp. D. The minimum intraspecies distance observed was 0.0% in *B. hispidus* whereas maximum intraspecies distance observed was 0.4% in *Apristurus* sp. A. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 4.1.

*H. quagga*, described from off the Malabar Coast, and *C. silasi* described from off Kollam (Arabian Sea), both show 0.3% intraspecies variation. The six specimens of *Bythaelurus hispidus* (type locality, Andaman Sea) were collected from a wide geographic area (Chennai, Bay of Bengal and off Kollam (Arabian Sea)) shows zero percentage intraspecific divergence. Family-wise phylogenetic tree was constructed (Fig. 4.4). The NJ tree show that *Bythaelurus hispidus*, *H. quagga* and all species of *Apristurus* occupy one hand of the clade and *C. silasi* separated well distantly.

**Table. 4.1.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Scyliorhinidae based on COI gene

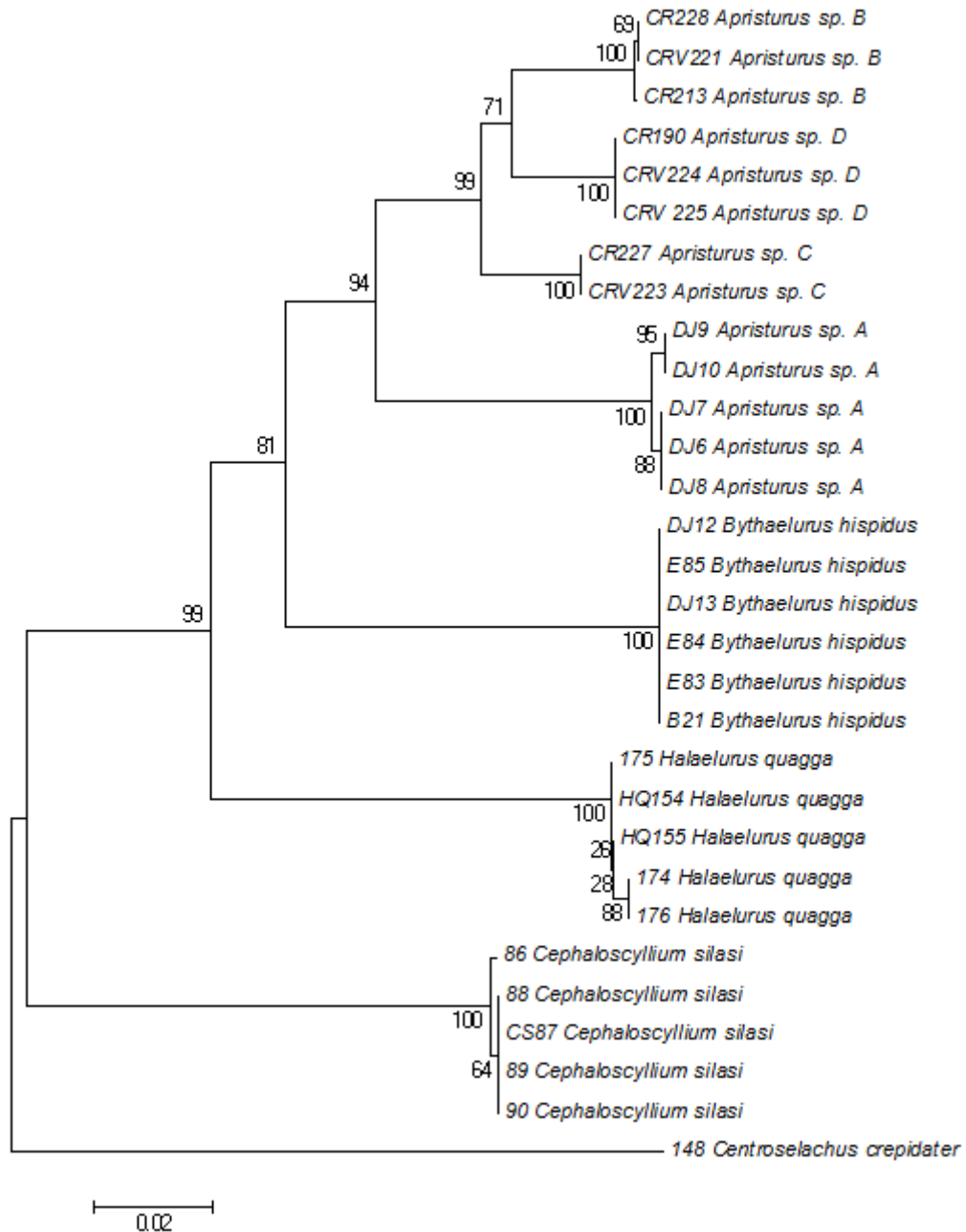
	By hiH1	Ce siH1	Ce siH2	Ce siH3	Ha quH1	Ha quH2	Ap spAH1	Ap spAH2
By hiH1								
Ce siH1	0.250							
Ce siH2	0.255	0.006						
Ce siH3	0.250	0.003	0.009					
Ha quH1	0.206	0.255	0.255	0.255				
Ha quH2	0.211	0.260	0.260	0.255	0.003			
Ap spAH1	0.179	0.269	0.270	0.269	0.182	0.187		
Ap spAH2	0.181	0.264	0.264	0.264	0.179	0.184	0.005	



SCYLIORHINIDAE

		1 2 2 2 2 2 3 3 3 3	3 4 4 4 4 5 5 5 5 5	6 6 6 7 7 7 8 8 8 9	1 1 1 1 1 1 1 1 1 1
		7 3 6 7 8 9 2 3 5 8	9 1 2 4 7 0 2 3 6 9	2 5 8 1 4 7 0 3 6 8	0 1 1 2 2 3 3 4 4 4
By hiH1		A T A C T A C C C C	T A C A T C C C A A	T G T G C T A T T T	G A C C T C T T A C
Ce siH1		C . . A C . T . A T	C T . T . A . T . G	C . A T A C G . . .	A . T T . . C . . T
Ce siH2		C . . A C . T T A T	C T . T . A . T . G	C . A T A C G . . .	A . T T . . C . . T
Ce siH3		C . . A C . T . A T	C T . T . A . T . G	C . A T A C G . . .	A . T T . . C . . T
HA quH1		. G . A . . T . A .	. G T . . A . G G C	A A C T A . G A . .	. . A . C T . C G T
HA quH2		. G . A . . T . A .	. G T . . A . G G C	A A C T A . G A . .	. . A . C T . C G T
Ap spAH1		G . G A . G . . A T	. . T G C T . T G .	. . . . A . G G C C	. C T . . T . . G T
Ap spAH2		G . G A . G . . A T	. . T G C T . T G .	. . C . A . G G . C	A C T . . T . . G T
		1 1 1 1 1 1 1 1 1 1	1 1 1 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 2
		4 5 5 6 6 6 7 7 8 8	8 8 9 0 0 0 0 1 1 1	2 2 2 3 3 4 5 5 5 6	6 6 6 6 7 7 7 8 8 8
		9 2 8 1 4 7 3 6 2 5	8 9 4 0 3 7 9 2 5 8	1 4 7 3 9 8 1 4 7 0	3 4 7 9 2 3 5 1 4 7
By hiH1		A A G T G C C C T T	T T A T A G C G C C	T A A C C T C C C T	C C C T T C A G C G
Ce siH1		. . A . C A A . A A	C C G C . . T A T .	. G G T . A T A A .	T . . C A . . A T A
Ce siH2		. . A . C A A . A A	C C G C . . T A T .	. G G T . A T A A .	T . . C A . . A T A
Ce siH3		. . A . C A A . A A	C C G C . A T A T .	. G G T . A T A A .	T . . C A . . A T A
HA quH1		G T A C A T T T . C	A . G . . . A . T	. . . T T . T A T .	. T . C C . . C A A
HA quH2		G T A C A T T T . C	A . G . . . A . T	. . . T T . T A T .	. T . C C . . C A A
Ap spAH1		. . A . . . A . . .	. . . . G . . . G T	G C . T . C T . . G	T T T A C T G T A A
Ap spAH2		. . A . . . A . . .	. . . . G . . . G T	G C . T . C T . . G	T T T A C T G T A A
		2 2 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 4 4 4
		9 9 0 0 1 1 1 2 2 2	2 3 3 3 3 3 4 4 4 4	4 5 5 5 5 5 6 6 7 7	7 7 8 8 8 9 9 0 0 0
		6 9 2 8 1 4 7 0 3 6	9 0 2 5 6 8 1 2 4 5	7 0 3 6 7 9 2 8 1 2	4 7 3 6 9 2 9 1 4 7
By hiH1		C A A C C G T T C G	C C A C A C C T A G	C C T G C T T C A G	C C C T C A G C A A
Ce siH1		T G . G A A A C . C	. . . T G T T A . .	T T C T G A C T G A	. T T C . . . T . .
Ce siH2		T G . G A A A C . C	. . . T G T T A . .	T T C T G A C T G A	. T T C . . . T . .
Ce siH3		T G . G A A A C . C	. . . T G T T A . .	T T C T G A C T G A	. T T C . . . T . .
HA quH1		A . C A A A A . T A	A T . A G T . . . .	A T . C . A . T . .	T . . . T . A T T .
HA quH2		A . C A A A A . T A	A T . A G T . . . C	A T . C . A . T . .	T . . . T . A T T .
Ap spAH1		A G . . A A C . T C	A T G . G . . . G .	A . . A . A . T . .	T T . . T G A T G T
Ap spAH2		A G . . A A C . T C	A T G . G . . . G .	A . . A . A . T . .	T T . . T G A T G T
		4 4 4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4 5 5	5 5 5 5 5 5 5 5 5 5	5 5 5 5 5 5 5 5 5 5
		1 1 2 3 3 4 4 5 5 6	6 7 8 8 8 8 9 9 0 0	0 0 1 1 1 2 2 2 2 2	2 2 3 3 3 3 4 4 4 5
		0 3 2 1 7 6 9 5 9 4	7 0 2 3 4 5 1 4 3 4	6 9 2 5 8 1 4 5 6 7	8 9 0 3 6 9 2 5 8 1
By hiH1		C A C C C C A C G C	C A C T T G T A T A	T C T A T T T C T T	T C T C A T T A C A
Ce siH1		T . T T T . . T . A	A . . C . A A G C G	A T . T C . . T . A	. . C T T C . . T T
Ce siH2		T . T T T . . T . A	A . . C . A A G C G	A T . T C . . T . A	. . C T T C . . T T
Ce siH3		T . T T T . . T . A	A . . C . A A G C G	A T . T C . . T . A	. . C T T C . . T T
HA quH1		T . T T T T G . A T	T . A C . A . . . .	. T C C C A . . . .	G . C . . G A G . .
HA quH2		T . T T T T G . A T	T . A C . A . . . .	. T C C C A . . . .	G . C . . G A G . .
Ap spAH1		T G T T A T . . . .	T G . C . A . . . .	. . C T . . A . . . .	. . A . T . G . . . .
Ap spAH2		T G T T A T . . . .	T G . C . A . . . .	. . C T . . A . . . .	. . A . T . G . . . .
		5 5 5 5 5 5 5 5 5 5	5 5 5 6 6 6 6 6 6 6	6 6 6 6 6 6 6 6	
		5 6 6 6 6 6 7 7 7 8	8 8 9 0 0 0 1 1 1 2	2 2 2 3 3 3 3 3	
		4 0 1 4 6 9 2 5 8 1	4 7 9 2 5 8 1 4 7 0	3 6 9 2 5 6 8	
By hiH1		T A T C C A C C C C	C C C C A C A G C A	C T T A C C A	
Ce siH1		. G C T A . . A T T	T A . T G A G . T C	T . . . T T .	
Ce siH2		. G C T A . . A T T	T A . T G A G . T C	T . . . G . T .	
Ce siH3		. G C T A . . A T T	T A . T G A G . T C	T . . . T T .	
HA quH1		C . . T A T . T T .	T . T . G . . . . .	. C . G . . G	
HA quH2		C . . T A T . T T .	T . T . G . . . . .	. C . G . . G	
Ap spAH1		. . . . T . . . . T	T . T . G . T A . .	T C C G . . . .	
Ap spAH2		. . . . T . . . . T	T . T . G . T A . .	T C C G . . . .	

Figure 4.3. Alignment of partial DNA sequences of the mitochondrial gene, COI of the family Scyliorhinidae (only variable sites are reported)



**Figure 4.4.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Scyliorhinidae inferred from DNA Sequences of mitochondrial gene COI

### Rajidae

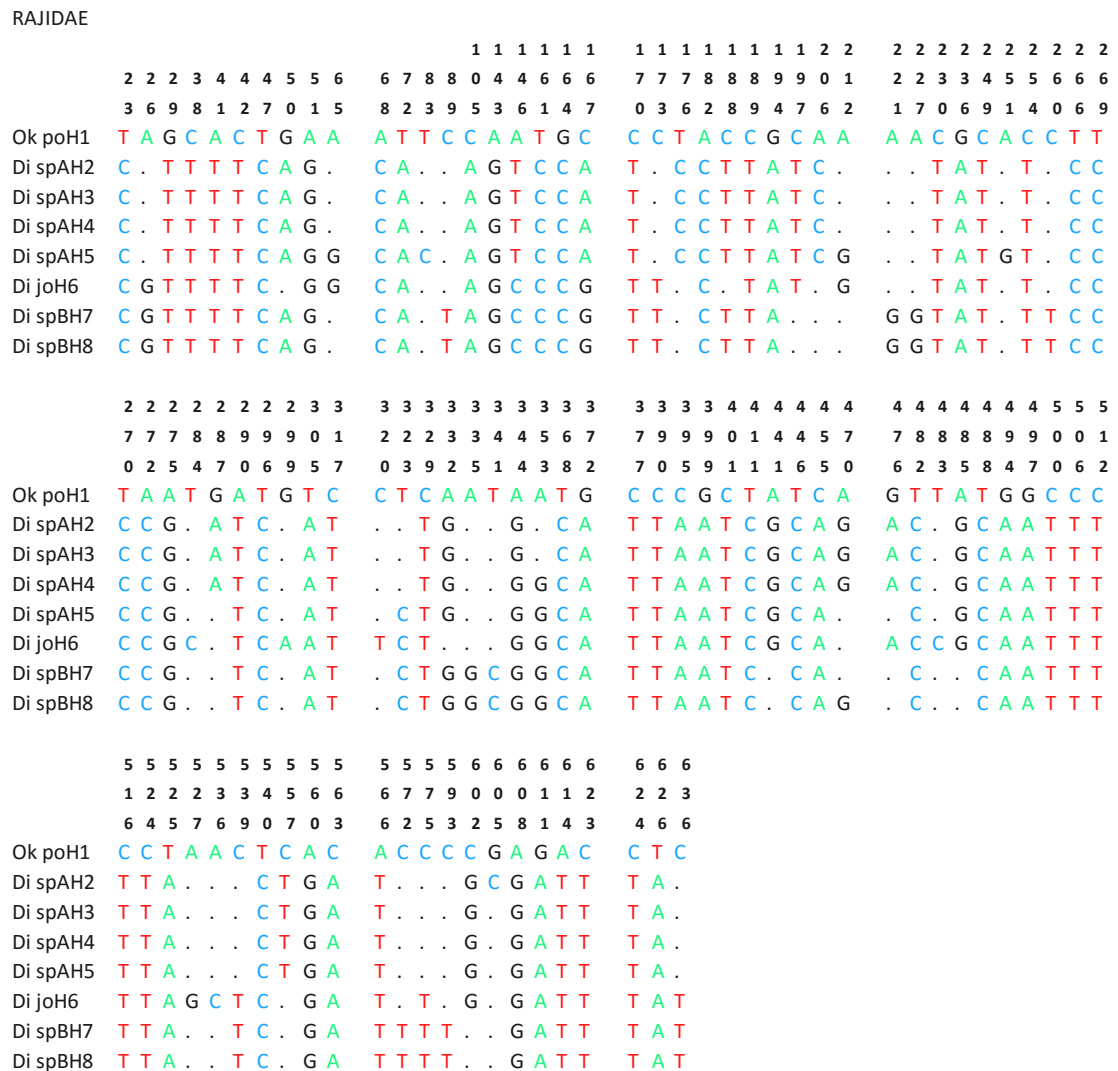
Four species of skate belonging to two genera *Dipturus* and *Okamejei* under the family Rajidae were analysed. *Dipturus* sp. A confirmed as putative new species and another one *Dipturus* sp. B not assigned to any species due to the taxonomic ambiguity exist in the Indian *Dipturus* genus. The amplified sequence length varied from 633 bp in *Okamejei powelli* to 660 bp in *Dipturus* sp. A. Out of the total 640 sites obtained 468 (73.12%) were constant, 172 (26.87%) variable, 104 (16.25%)

parsimony sites and 68 (10.62%) singleton sites. The polymorphic sites of selected family are illustrated in Fig. 4.5. A total of 8 haplotypes were observed across the taxa. Within each species (n=5), minimum number of haplotypes was one (*Okamejei powelli*, *Dipturus* cf. *johannisdavisi*) and maximum was 4 in *Dipturus* sp. A. The overall mean distance of individuals among the family estimated was 4.5%. The maximum interspecific K2P distance was 12% between *Okamejei powelli* and *Dipturus* cf. *johannisdavisi* and minimum was 2.8% divergence between *Dipturus* sp. A and *Dipturus* cf. *johannisdavisi*. The minimum intraspecies distance observed was zero percentage in *O. powelli* whereas maximum intraspecies distance observed was 1.2% in *Dipturus* sp. A. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 4.2.

*Dipturus* sp. A collected from off the Kerala coast, and off Chennai show maximum of 1.3% intraspecies variation warrants more taxonomic studies. The six specimens of *Okamejei powelli* (type locality, Gulf of Martaban, Myanmar) were collected from wide geographic area Chennai, (Bay of Bengal) and off Kollam (Arabian Sea) shows zero intraspecies variation. Family-wise phylogenetic tree was constructed using NJ (Fig. 4.6) method with *Rhinobatos variegatus* as outgroup. *Dipturus* sp. A, *Dipturus* cf. *johannisdavisi* and *Dipturus* sp. B were seen to occupy towards one hand of the clade and *Okamejei powelli* separated well distantly. All these clades were supported by high bootstrap values.

**Table. 4.2.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Rajidae based on COI gene

	Ok poH1	Di spAH2	Di spAH3	Di spAH4	Di spAH5	Di joH6	Di spBH7	Di spBH8
Ok poH1								
Di spAH2	0.142							
Di spAH3	0.140	0.002						
Di spAH4	0.142	0.003	0.002					
Di spAH5	0.146	0.016	0.014	0.013				
Di joH6	0.154	0.042	0.041	0.039	0.036			
Di spBH7	0.149	0.046	0.044	0.042	0.042	0.039		
Di spBH8	0.151	0.044	0.042	0.041	0.044	0.041	0.002	

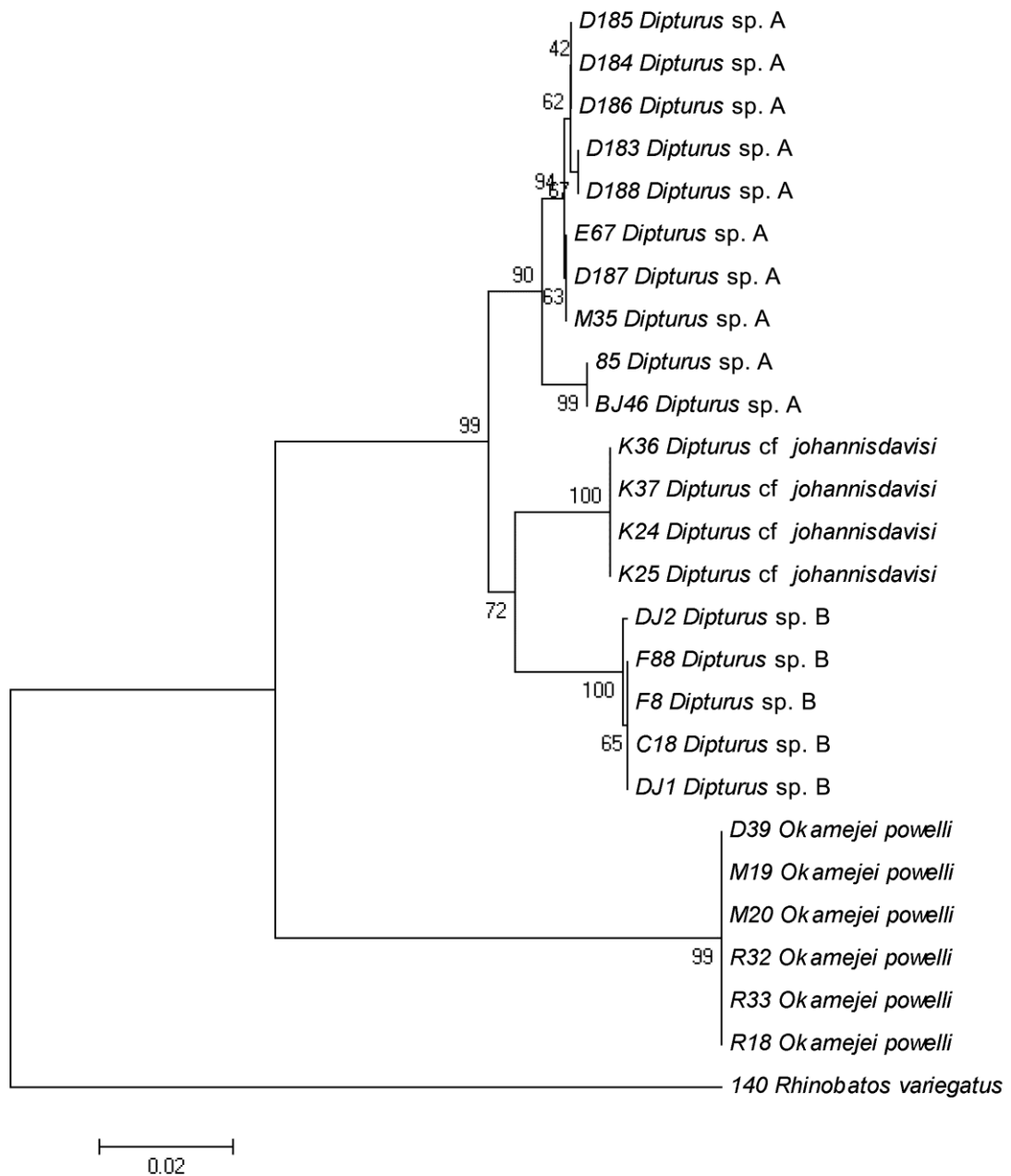


**Fig. 4.5.** Alignment of partial DNA sequences of the mitochondrial gene, COI of the family Rajidae (only variable sites are reported)

### Centrophoridae

The gulper sharks represented by two genera, *Centrophorus* and *Deania*, under the family Centrophoridae were studied. The amplified sequence length varied from 640 bp in *Centrophorus atromarginatus* to 655 bp in *Deania profundorum*. Out of the total 640 sites obtained 520 (81.25%) were constant, 120 (18.75%) variable, 77 (12.03%) parsimony sites and 43 (6.71%) singleton sites. The polymorphic sites of selected family are illustrated in Fig. 4.7. A total of 7 haplotypes were observed across the taxa. Within each species (n=5), minimum number of haplotypes was one (*C. atromarginatus*, *C. granulatus*, *C. squamosus*) and maximum was 2 in *C.*

*zeehaani* and *Deania profundorum*. The overall mean distance of individuals among the family estimated was 5.0%. The maximum interspecific K2P distance was 9.6% between *D. profundorum* and *C. atromarginatus* and minimum was 2.6% divergence between *C. atromarginatus* and *C. zeehaani*. The minimum intraspecies distance observed was 0.2% in *Deania profundorum* whereas maximum intraspecies distance observed was 0.3% in *Centrophorus zeehaani*. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 4.3.



**Figure 4.6.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Rajidae inferred from DNA Sequences of mitochondrial gene COI

CENTROPHORIDAE

	1 1	1 1 1 1 1 1 1 1 1 1	1 1 1 2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 3 3
	3 3 4 6 7 7 7 9 0 1	1 1 2 4 4 5 5 6 6 6	7 7 9 1 2 3 3 4 5 5	5 6 6 8 8 9 9 9 0 0
	2 9 7 8 1 2 9 9 9 0	3 6 5 6 9 2 5 1 4 7	3 6 4 8 1 3 6 9 1 4	7 3 4 1 7 0 6 9 2 5
De prH1	C C T G T A T A T A	C C T T A C A T T G	G C A T A C A T A A	A T C T C C G A C G
De prH2	. . . . .	. . . . .	. . . . .	. . . . .
Ce sqH1	T T . . C . . . . G	T T C . G T . C . A	A . G . . T . . . T	. C T . T T A C T A
Ce ATH1	T T C A A G C G G G	T T . C G T . . . .	A T . . . T G C G T	G . T C T T A . T A
Ce grH1	T T . . A . . . . G	T T . . G T G C C .	A . G C C T . . . C	C C T . T T . C T A
Ce zeH1	T T . . A . . . . G	T T . . G T . . . A	A T . . . T . C . T	. . T C T T A . T A
Ce zeH2	T T . . A . . G . G	T T . . G T . . . A	A T . . . T . C . T	. . T C T T A . T A

	3 3 3 3 3 3 4 4 4	4 4 4 4 4 4 4 5 5	5 5 5 5 5 5 5 5 5	6 6 6 6 6 6 6
	1 2 3 4 5 6 8 0 2 2	3 4 5 6 7 7 8 9 0 1	1 1 2 3 3 5 6 6 7 9	0 0 1 1 1 2 3
	7 6 5 7 6 5 6 4 5 8	4 0 5 1 3 9 5 7 0 2	3 5 1 0 3 7 3 9 9 9	2 5 1 4 6 0 5
De prH1	G C G T G G C C C C	T T C A C C C C T T	G C A C C A C T T T	A A A G A C C
De prH2	. . . . .	. . A . . . . .	. . . . .	. . . . .
Ce sqH1	. T T C A A T . T T	C . A C . G . C C	. . C T . . G . C .	. T . A . . T
Ce ATH1	A . T C A A T T T T	. C . C T G T . C C	. . C T T . G C C .	C G G . . . T
Ce grH1	. T T C A A T . T T	. . A C T G . T C C	. T C T . . G . C C	T . . A . . T
Ce zeH1	A . T C A A T T T T	. C . C T G . . C C	A . C T T . G . C .	C G G . C A T
Ce zeH2	A . T C A A T T T T	. C . C T G . . C C	A . C T T G G . C .	C G G . C A T

**Figure 4.7.** Alignment of partial DNA sequences of the mitochondrial gene, COI of the family Centrophoridae (only variable sites are reported)

**Table 4.3.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Centrophoridae based on COI gene

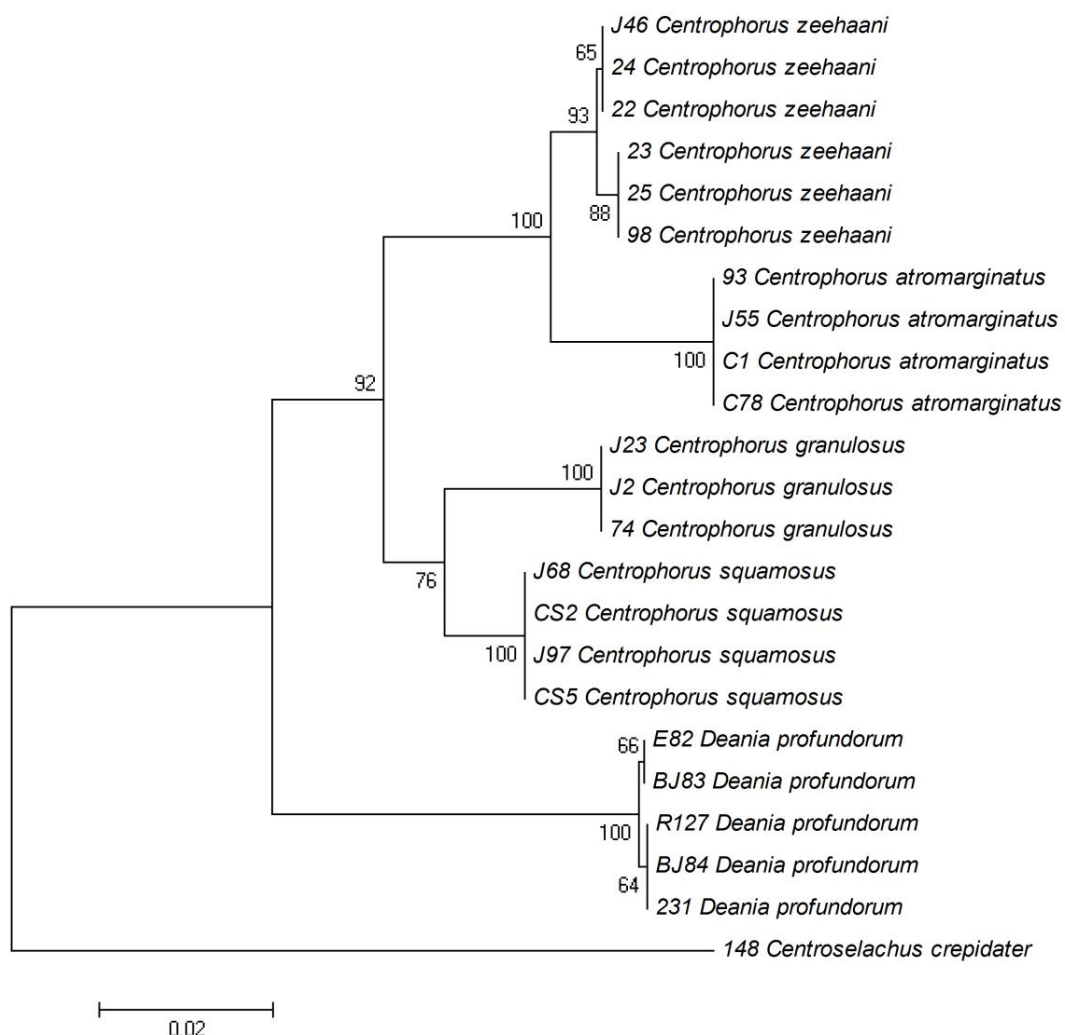
	De prH1	De prH2	Ce sqH1	Ce atH1	Ce grH1	Ce zeH1	Ce zeH2
De prH1							
De prH2	0.002						
Ce sqH1	0.073	0.071					
Ce atH1	0.095	0.096	0.056				
Ce grH1	0.082	0.080	0.027	0.062			
Ce zeH1	0.080	0.082	0.039	0.026	0.051		
Ce zeH2	0.084	0.086	0.042	0.026	0.054	0.003	

Family-wise phylogenetic tree was constructed using NJ (Fig. 4.8) method. All four *Centrophorus* species, *Centrophorus granulosus*, *C. squamosus*, *C. zeehaani* and *C. atromarginatus* were seen to occupy towards one hand of the clade and *D. profundorum* separated well distantly. All these clades were supported by high bootstrap values. *Centroselachus crepidater* was used as out-group.

### Lamnidae

Two mackerel sharks belonging to one genus (*Isurus*) were studied. Out of the total 640 sites obtained 501 were constant, 139 variable, 85 parsimony sites and 54 singleton sites. The analysis revealed the average nucleotide frequencies (%) as T = 28.7, C = 28.4, A = 24.0, and G = 18.9. The average transitional pairs (si=43) were more frequent than transversional pairs (sv=12) with an average ratio of R = 3.6. The

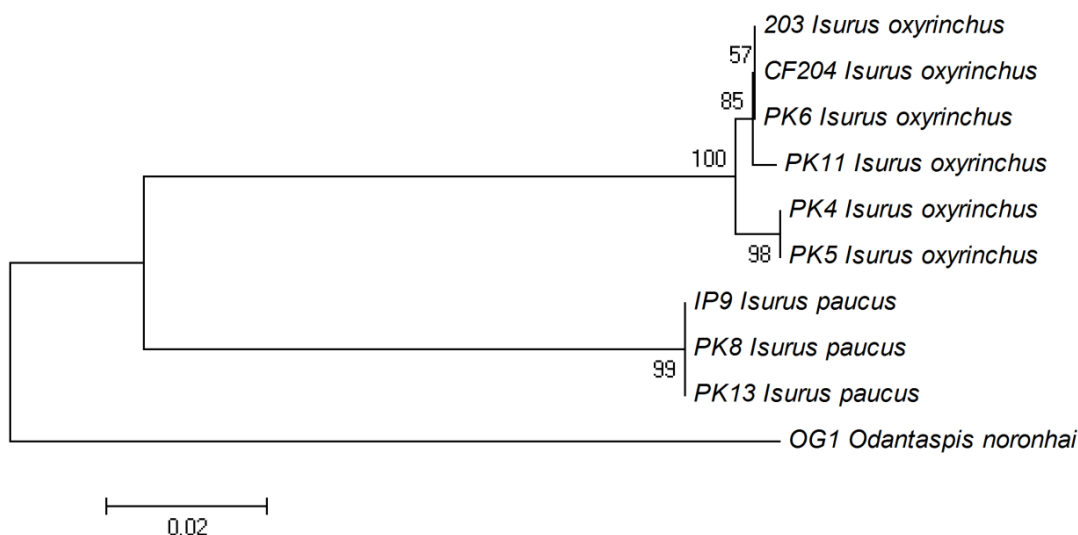
overall mean distance of individuals among the family was estimated as 9.7%. The interspecific pair-wise genetic distance was 14.9%. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 4.4. Two clusters were formed in the NJ tree (Fig. 4.9).



**Figure 4.8.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Centrophoridae inferred from DNA Sequences of mitochondrial gene COI

**Table 4.4.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Lamnidae based on COI gene

	Is oxH1	Is oxH2	Is oxH3	Is paH1
Is oxH1				
Is oxH2	0.003			
Is oxH3	0.008	0.011		
Is paH1	0.145	0.145	0.149	



**Fig. 4.9.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Lamnidae inferred from DNA Sequences of mitochondrial gene COI

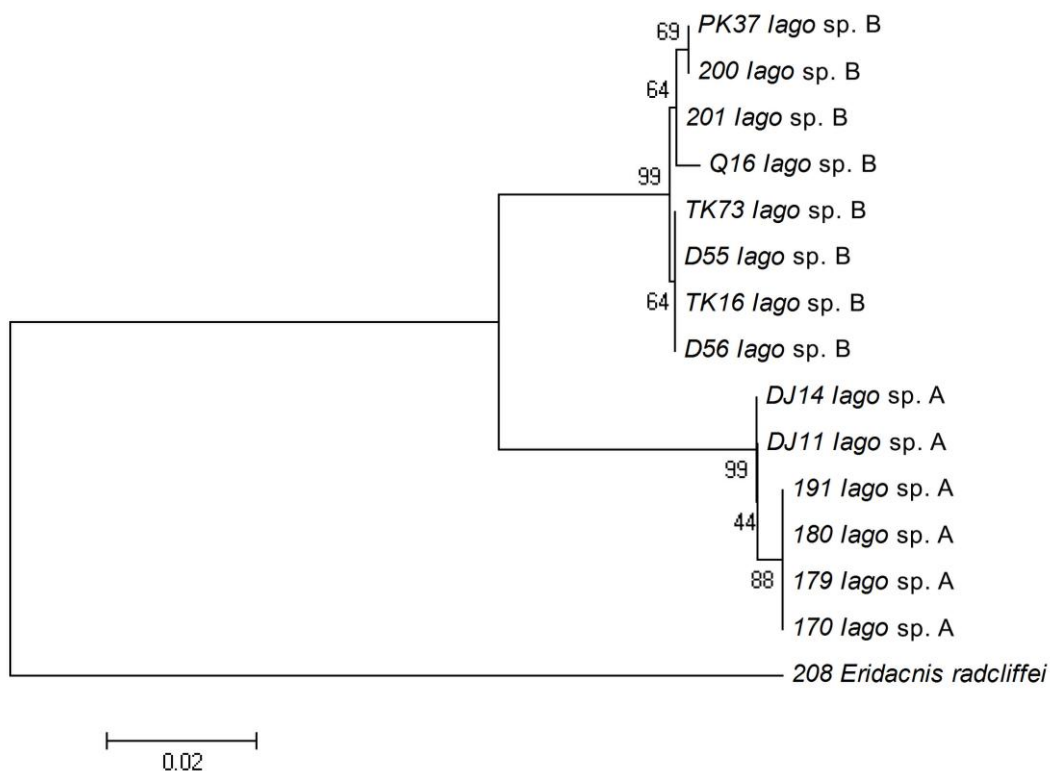
### Triakidae

The houndsharks of the genus, *Iago* belonging to the family Triakidae were characterised for DNA barcodes. The amplified sequence length varied from 640 bp in *Iago* sp. B to 662 bp in *Iago* sp. A. Out of the total 640 sites obtained 599 were constant, 41 variable, 39 parsimony sites and 2 singleton sites. The analysis revealed the average nucleotide frequencies (%) as T = 31.1, C = 26.6, A = 26.4, and G = 15.9. The average transitional pairs (si=18) were more frequent than transversional pairs (sv=3) with an average ratio of R = 6.55. A total of 6 haplotypes were observed and the overall mean distance of individuals among the family was 3.4%. The interspecific pair-wise genetic distance was calculated as 6.5%. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 4.5. The NJ tree showed complete separation of outgroups from the houndsharks by two major clusters (Fig. 4.10). The individuals of both *Iago* revealed two distinct closely related species.

**Table. 4.5.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Triakidae based on COI gene

	Ia spBH1	Ia spBH2	Ia spBH3	Ia spBH4	Ia spAH5	Ia spAH6
Ia spBH1						
Ia spBH2	0.002					
Ia spBH3	0.003	0.002				
Ia spBH4	0.005	0.003	0.005			
Ia spAH5	0.063	0.062	0.063	0.065		
Ia spAH6	0.060	0.058	0.060	0.061	0.003	





**Figure 4.10.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Triakidae inferred from DNA sequences of mitochondrial gene COI

#### 4.5.2. 16S rRNA analysis

Mitochondrial partial sequences of 16S rRNA generated from 20 species were used in the phylogenetic analysis. The amplified sequence lengths varied from species to species and among families but consistent within species. A total of 25 haplotypes were observed across the taxa. The minimum number of haplotypes observed within each species ( $n=5$ ) was one (*Centrophorus atromarginatus*, *C. squamosus*, *Okamejei powelli*) and maximum was observed as two (*Deania profundorum*, *Dipturus* cf. *johannisdavisi*, *Apristurus* sp. A and *Dipturus* sp. A). The shortest sequence was observed in *C. atromarginatus* (525 bp) and the longest sequences in *Dipturus* sp. A (591 bp). The sequences were aligned using BioEdit and the multiple alignments resulted in consensus length of 525 sites including base pairs and gaps. These includes 313 conserved/constant (C), 209 variable/polymorphic (V) and 209 parsimony informative (Pi) sites. The analysis revealed the average nucleotide frequencies (%) as T = 28.3, C = 20.3, A = 32.7, and G = 18.7. The average transitional pairs ( $si=34$ ) were more frequent than transversional pairs ( $sv=27$ ) with an average ratio of  $R = 1.25$ .

The mean pair-wise genetic distance values (K2P) estimated based on 16S rRNA using MEGA Version 5 (Tamura *et al.*, 2011) are given in Table 4.6. The average genetic distance (K2P) of individuals among chondrichthyan species under this study was 12.5%. The minimum K2P distance between species estimated was 1.3% (between *Dipturus* sp. A and *Dipturus* cf. *johannisdavisi*) in the family Rajidae. The maximum intergeneric divergence estimated was 22.5% observed between *N. pinnata* and *P. violacea*. The minimum intraspecific genetic distance (K2P) of 0.2% in *Dipturus* cf. *johannisdavisi* and maximum 1.1% in *Dipturus* sp. A were reported within species among haplotypes. The pair-wise genetic divergence values of the haplotypes (intergeneric and interspecies) within the various deep-sea chondrichthyan families are given in Table 4.6 to 4.9.

### Centrophoridae

Four species of gulper sharks, *Centrophorus atromarginatus*, *C. squamosus*, *C. zeehaani* and *Deania profundorum* were analysed. The amplified sequence length varied from 512 bp in *C. atromarginatus* to 518 bp in *D. profundorum*. Multiple aligned sequences resulted in a total length of 512 bp sites including base pairs and gaps that included 493, 19, 19 and none conserved, variable, parsimony informative and singleton sites, respectively. A total of five haplotypes, one each from *C. atromarginatus*, *C. squamosus* and *C. zeehaani* and two from *D. profundorum* were observed. The analysis revealed the average nucleotide frequencies (%) as T = 28.3, C = 20.7, A = 31.8, and G = 19.3. The average transitional pairs (si = 7) were more frequent than transversional pairs (sv = 1.0) with an average ratio of R = 11.73.

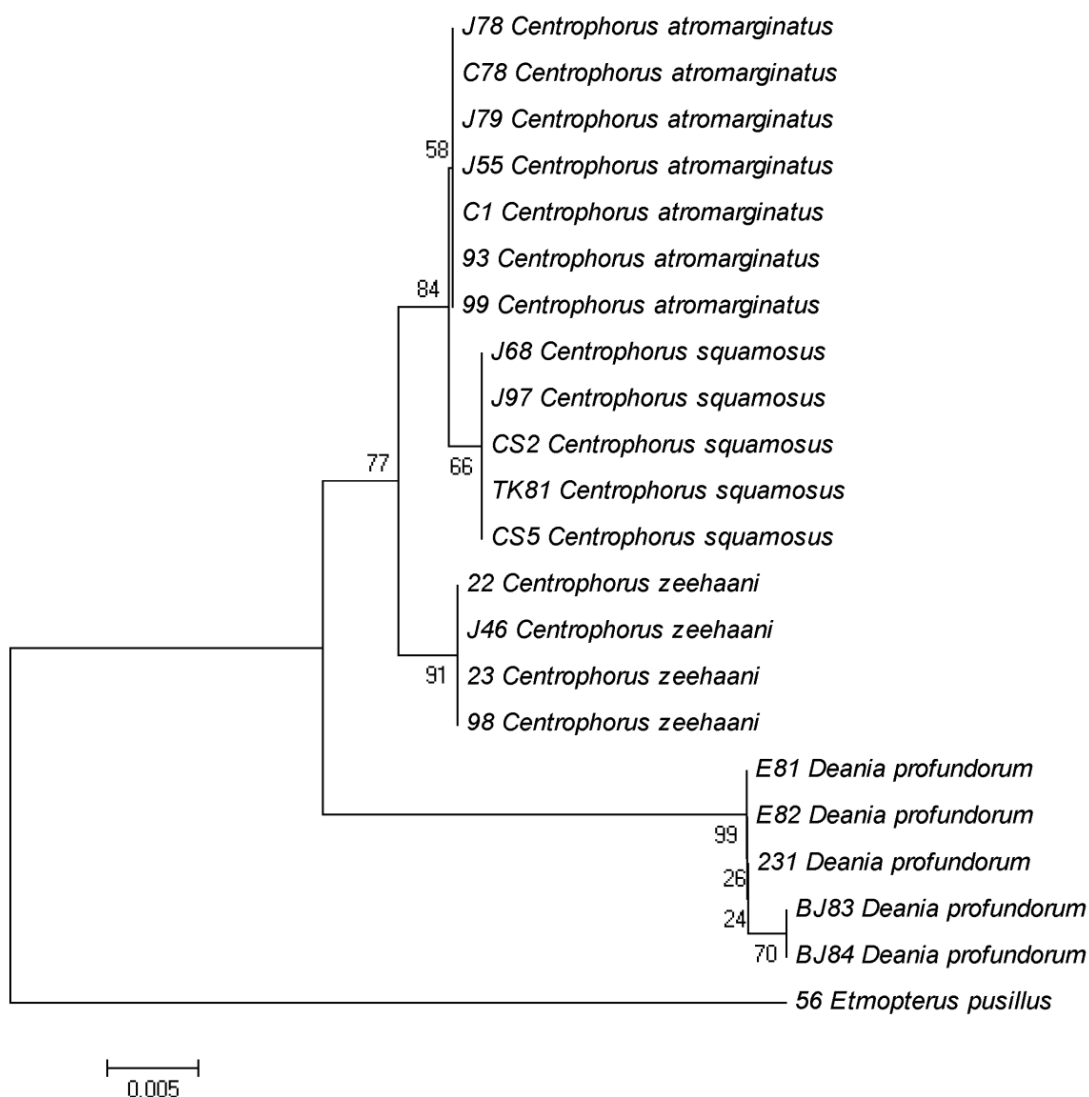
The overall mean distance of individuals among the family was estimated as 0.014 (1.4%). The maximum interspecific K2P distance was 3.5% between *C. squamosus* and *D. profundorum* and minimum was 0.2% divergence between *C. atromarginatus* and *C. squamosus*. The minimum intraspecies distance observed was 0.0% in both *C. zeehaani* and *C. squamosus* while maximum intraspecies distance observed was 0.2% in *D. profundorum*. The pair-wise genetic divergence values of the haplotypes within the family Centrophoridae are given in Table 4.7. Two clades were recognised in the NJ tree (Fig. 4.11). The separation of *C. atromarginatus*, *C. zeehaani* and *C. squamosus* were observed in the first clade. The second clade was occupied by *D. profundorum*, which distinctly separated from the first clade. All the nodes were supported by high bootstrap values ranging from 77 to 84%.

**Table. 4.6.** Mean pair-wise genetic divergence (Kimura 2-parameter) in 16S rRNA sequences under the present study

	Et	pu	Ec	br	Ne	pi	He	gr	Sq	spA	He	pe	Pt	vi	Rh	va	To	spA	Ce	at	De	pr	Ce	ze	Di	spA	Di	spB	Di	jo	Ok	po	Ce	si	Ap	spA	By	hi	Ha	qu			
<i>Etmopterus pusillus</i>																																											
<i>Echinorhinus brucus</i>	0.091																																										
<i>Neoharriotta pinnata</i>	0.192	0.194																																									
<i>Hexanchus griseus</i>	0.108	0.109	0.196																																								
<i>Squalus</i> sp. A	0.057	0.062	0.188	0.071																																							
<i>Heptranchias perlo</i>	0.096	0.094	0.200	0.017	0.060																																						
<i>Pteroplatytrygon violacea</i>	0.172	0.187	0.225	0.173	0.166	0.170																																					
<i>Rhinobatos variegatus</i>	0.126	0.126	0.192	0.114	0.121	0.110	0.108																																				
<i>Torpedo</i> sp. A	0.183	0.187	0.212	0.201	0.177	0.184	0.211	0.131																																			
<i>Centrophorus atromarginatus</i>	0.059	0.050	0.201	0.081	0.022	0.065	0.180	0.123	0.184																																		
<i>Deania profundorum</i>	0.076	0.071	0.213	0.081	0.036	0.072	0.178	0.139	0.206	0.031																																	
<i>Centrophorus zeehani</i>	0.061	0.055	0.201	0.078	0.022	0.065	0.177	0.129	0.186	0.004	0.027																																
<i>Dipturus</i> sp. A	0.134	0.147	0.212	0.133	0.138	0.125	0.158	0.119	0.184	0.140	0.143	0.138																															
<i>Dipturus</i> sp. B	0.133	0.146	0.213	0.134	0.137	0.127	0.160	0.123	0.183	0.139	0.142	0.137	0.021																														
<i>Dipturus</i> cf. <i>johannisdavisi</i>	0.137	0.145	0.212	0.130	0.136	0.123	0.159	0.124	0.182	0.138	0.141	0.136	0.015	0.017																													
<i>Okamejei powelli</i>	0.125	0.127	0.201	0.137	0.129	0.121	0.162	0.116	0.180	0.132	0.132	0.129	0.062	0.064	0.065																												
<i>Cephaloscyllium silasi</i>	0.136	0.107	0.198	0.144	0.099	0.137	0.208	0.165	0.170	0.110	0.120	0.105	0.198	0.189	0.190	0.184																											
<i>Apristurus</i> sp. A	0.145	0.130	0.200	0.136	0.118	0.126	0.204	0.151	0.214	0.118	0.125	0.118	0.185	0.173	0.180	0.172	0.107																										
<i>Bythaelurus hispidus</i>	0.125	0.110	0.194	0.120	0.097	0.110	0.193	0.146	0.205	0.097	0.107	0.100	0.177	0.171	0.175	0.170	0.092	0.042																									
<i>Halaelurus quagga</i>	0.136	0.126	0.194	0.123	0.115	0.110	0.197	0.154	0.219	0.121	0.131	0.118	0.169	0.160	0.164	0.159	0.102	0.078	0.060																								

**Table. 4.7.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Centrophoridae based on 16S rRNA gene

	Ce atH1	Ce sqH1	De prH1	De prH2	Ce zeH1
Ce atH1					
Ce sqH1	0.002				
De prH1	0.031	0.033			
De prH2	0.033	0.035	0.002		
Ce zeH1	0.006	0.008	0.029	0.031	

**Fig. 4.11.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Centrophoridae inferred from DNA Sequences of mitochondrial gene 16S rRNA



*Dipturus* sp. B and *O. powelli* while two each from *Dipturus* sp. A and *Dipturus* cf. *johannisdavisi* were observed. The analysis revealed the average nucleotide frequencies (%) as T = 27.6, C = 21.8, A = 30.9, and G = 19.6. The average transitional pairs (si = 13) were more frequent than transversional pairs (sv = 6.0) with an average ratio of R = 2.32. The overall mean distance of individuals among the family was estimated as 3.4%. The maximum interspecific K2P distance was 7.2% between *Dipturus* cf. *johannisdavisi* and *O. powelli* while minimum was 1.4% divergence between *Dipturus* cf. *johannisdavisi* and *Dipturus* sp. A. The minimum intraspecies distance observed was 0.0% in both *O. powelli* and *Dipturus* sp. B while maximum intraspecies distance observed was 1% in *Dipturus* sp. A. The pair-wise genetic divergence values of the haplotypes within the family Rajidae are given in Table 4.8. Family-wise phylogenetic tree was constructed using NJ (Fig. 4.13) method including related species *Rhinobatos variegatus* as outgroup to test the divergence level. Two clades were recognised. The separations of *Dipturus* cf. *johannisdavisi*, *Dipturus* sp. A and *Dipturus* sp. B were observed in the first clade. The second clade was occupied by *O. powelli*, which distinctly separated from the first clade. All the nodes were supported by high bootstrap values ranging from 100%.

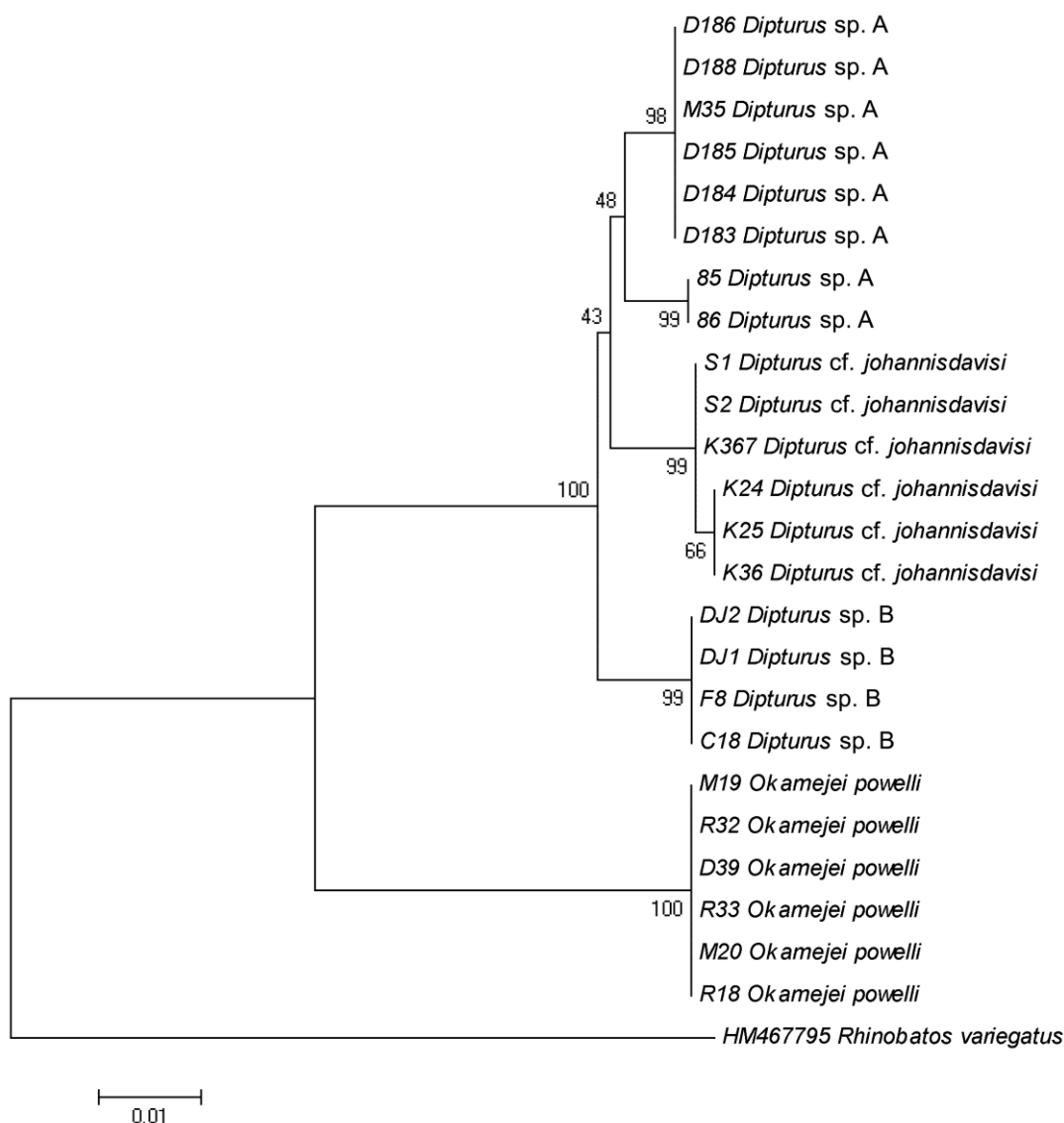
**Table. 4.8.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Rajidae based on 16S rRNA gene

	Di spAH1	Di spAH2	Di spBH1	Di joH1	Di joH2	Ok poH1
Di spAH1						
Di spAH2	0.010					
Di spBH1	0.019	0.016				
Di joH1	0.014	0.014	0.016			
Di joH2	0.016	0.016	0.017	0.002		
Ok poH1	0.067	0.069	0.070	0.070	0.072	

### Scyliorhinidae

The catsharks of four genera under the family scyliorhinidae were analysed. The amplified sequence length varied from 561 bp in *Cephaloscyllium silasi* to 568 bp in *Bythaelurus hispidus*. Multiple aligned sequences resulted in a total length of 561 bp sites including base pairs and gaps that included 481 Conserved, 80 Variable and 80 Parsimony Informative sites. A total of five haplotypes, one each from *Cephaloscyllium silasi*, *Bythaelurus hispidus* and *Halaelurus quagga* while two from *Apristurus* sp. A were observed. The analysis revealed the average nucleotide

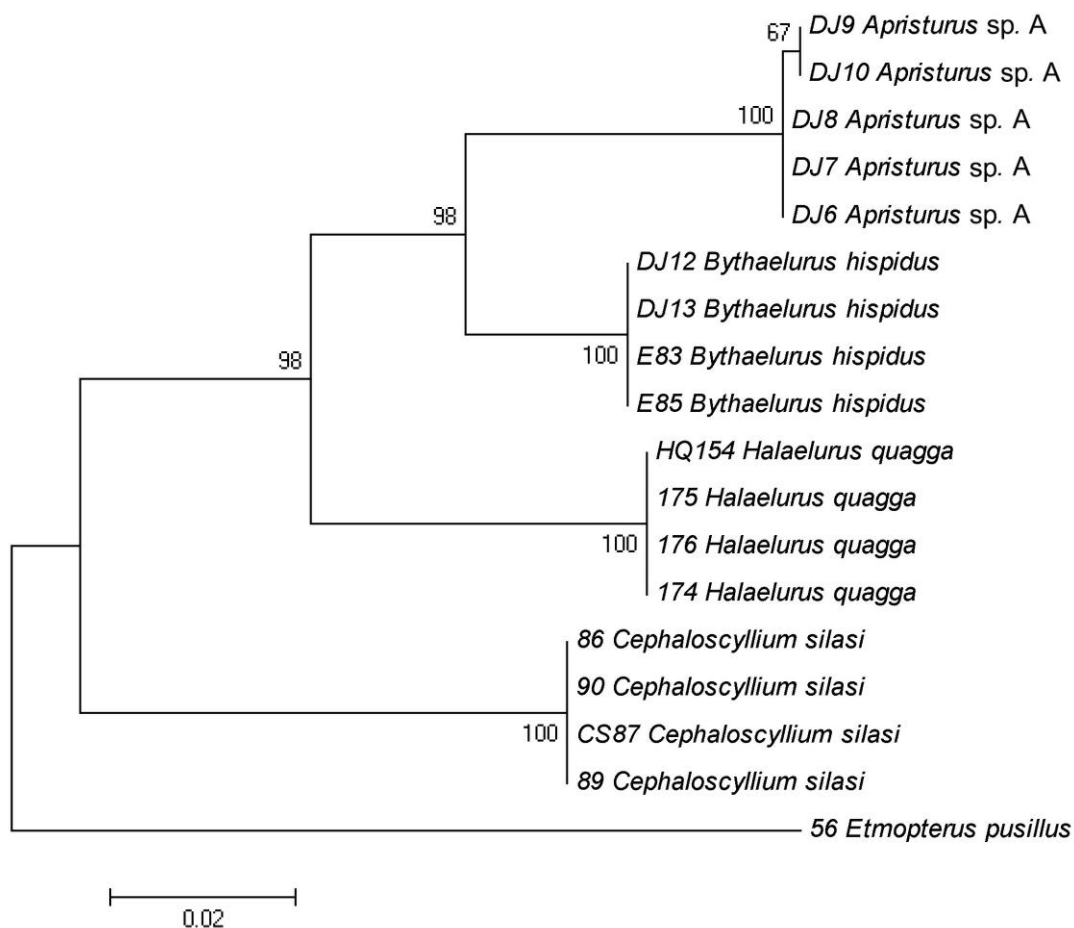
frequencies (%) as T = 28.4, C = 20.4, A = 31.9, and G = 19.3. The average transitional pairs (si = 22) were more frequent than transversional pairs (sv = 13) with an average ratio of R = 1.69.



**Fig. 4.13.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Rajidae inferred from DNA Sequences of mitochondrial gene 16S rRNA

The overall mean distance of individuals among the family was estimated as 0.074 (7.4%). The maximum interspecific K2P distance was 12.5% between *Cephaloscyllium silasi* and *Apristurus* sp. A, while minimum was 7.1% divergence between *Bythaelurus hispidus* and *Halaaelurus quagga*. The minimum intraspecies distance observed was zero in both *Bythaelurus hispidus* and *Halaaelurus quagga*, while maximum intraspecies distance observed was 0.2% in *Apristurus* sp. A. The pair-wise genetic divergence values of the haplotypes within the family Scyliorhinidae are given in Table 4.9. Three clades were recognised (Fig. 4.14) in

the NJ tree. The separations of *Bythaelurus hispidus* and *Apristurus* sp. A were observed in the first clade. The second clade was occupied by *Haelaelurus quagga* and *Cephaloscyllium silasi* placed in the third clade which distinctly separated from the first and second clades. All the nodes were supported by high bootstrap values of 100%.



**Fig. 4.14.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Scyliorhinidae inferred from mitochondrial 16S rRNA

**Table. 4.9.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Scyliorhinidae based on 16S rRNA gene

	Ce siH1	Ap spAH1	Ap spAH2	By hiH1	Ha quH1
Ce siH1					
Ap spAH1	0.129				
Ap spAH2	0.129	0.002			
By hiH1	0.116	0.052	0.054		
Ha quH1	0.119	0.087	0.090	0.073	



## 4.6. Discussions

### Identifying Chondrichthyan species

In this study, 38 species representing 9 orders and 20 families of Indian chondrichthyans species were characterized for DNA barcode variability. No insertions, deletions or stop codons were observed, indicating that all sequences were derived from functional mitochondrial COI sequences. Sequencing this c.650 bp region of mtDNA COI permits the discrimination of every one of these 38 species. The average degree of intraspecies divergence, 0.35%, is very similar to the values of 0.39% previously estimated for 143 species of teleosts and 64 species of chondrichthyans (Ward *et al.*, 2005) and 0.37% for 210 species of chondrichthyans (Ward *et al.*, 2008b).

During the present study, most of the reported deepwater sharks and rays species are barcoded. However, the average number of samples per species was only 5.03. This low value can be lead to an underestimate of intraspecific variation, especially of that portion attributable to spatial heterogeneity (Dasmahapatra and Mallet, 2006), and can reduce the precision of DNA barcoding (Moritz and Cicero, 2004). The six species collected here from both the east and west coasts of India showed no evidence for spatial differentiation (albeit sample sizes being very low), having similar within and between locality genetic distances. The extent of differentiation among populations in marine fish species is known to be generally small (Waples, 1998). Increasing sample size per species can also reveal any cryptic or unrecognized species, thereby increasing the precision of barcoding for identification purposes (Jaafar *et al.*, 2012).

DNA barcoding is a very efficient tool for differentiating Indian chondrichthyan species with a high degree of accuracy. Earlier molecular studies in fishes from India (Lakra *et al.*, 2009; Lakra *et al.*, 2010; Pavan-Kumar *et al.*, 2014) and adjacent countries (Ward *et al.*, 2005; Moura *et al.*, 2008; Ward *et al.*, 2008b) had already proven the robustness of COI as an excellent genetic marker for distinguishing fish species. The presence of unrecognized species in our study highlights the need for further detailed taxonomic examinations of several genera in Indian waters. Recent taxonomic studies of chondrichthyans in Indonesia and Australia have resulted in the description of many new species and better taxonomic

resolution of species complexes (Last *et al.*, 2008; Last *et al.*, 2010). This suggests that a systematic study of the chondrichthyan diversity in Indian waters could identify greater diversity of fauna and our DNA barcoding study support it by identifying new species using COI data. Although over 200 species of chondrichthyans have been reported in Indian waters, there are very few representative specimens in ichthyological collections and these are insufficient for detailed taxonomic studies. Many reported species are questionable and whether or not they truly occur needs to be resolved. However, it is clear that India does have a rich chondrichthyan biodiversity, and documentation of this fauna is essential. Without proper documentation, there is little scope for effective conservation and management of these vulnerable and exploited species in Indian waters.

During the present study, we found seven putative new species that await formal description, and a further 2 species that we were not confident to assign to any species because of possible confusion with closely related pre-existing or synonymised species. In addition to this, six elasmobranch species found in the landings, confirmed by morphological data and COI sequences, constitute first records in Indian waters, viz.: *Isurus paucus*, *Deania profundorum*, *Centrophorus zeehaani*, *Hexanchus griseus*, *Odontaspis noronhai* and *Zameus squamulosus*.

### **Scyliorhinidae**

Catsharks are ground sharks of the family Scyliorhinidae, with over 150 known species. These groups are represented by small size sharks and exclusively found in the deeper areas at depth ranges of 200-1000 m with no commercial values. Most of the species were discarded at sea and hence the knowledge on exact species diversity was very poor and most of the species described with very few numbers. There are 17 species under seven genera reported from Indian waters but eight species with questionable status (Akhilesh *et al.*, 2014). *Apristurus investigatoris* (Misra, 1962) was described from Andaman Sea based on single specimen. The present barcoded *Apristurus* is very different from *Apristurus investigatoris* based on morphological examination. The position of dorsal fins, interdorsal distance and teeth structures vary greatly between the above two species. The *Apristurus* sp. A sequence match with *Apristurus nakayai* (94%) and *Apristurus brunneus* (97%) on BLAST, based on COI and 16S rRNA gene sequences

respectively. Taxonomic clarification and description of these potentially new *Apristurus* is required.

The Quagga shark, *Halaelurus quagga* (Alcock, 1899), is one of the poorly known scyliorhinid (Carcharhiniformes) sharks of the world, described from a specimen collected from the Arabian Sea coast of India (off Malabar) and also reported from Somalia. Akhilesh *et al.*, (2011) redescribed *Halaelurus quagga* with two COI barcodes. In the present study, the four species collected from this family were found to be genetically distinct from each other and positioned into four groups that consistent with four genera without any haplotypes sharing or overlapping, based on partial sequence information of COI and 16S rRNA markers. *Bythaelurus hispidus* collected from wide geographic locations formed as one haplotypes with very low intraspecific variations.

## **Rajidae**

Skates (order Rajiformes, family Rajidae) are an extremely diverse group of fishes, characterized by a high morphological conservatism (McEachran and Dunn, 1998). *Dipturus* Rafinesque, 1810 is the second most speciose genus within the family Rajidae, with approximately 44 described species (Ebert and Compagno, 2007; Last *et al.*, 2008). The high level of species diversity coupled with morphological and ecological conservatism makes the species identification very difficult in the family Rajidae. There are 11 species reported from Indian waters with three questionable status (Akhilesh *et al.*, 2014). *Dipturus johannisdavisi* (Alcock, 1899) was the only one valid deepwater skate described based on single juvenile specimen from off Travancore coast. Taxonomic clarification is required due to the bad condition of the holotype. Another species *Dipturus* cf. *johannisdavisi* need more morphological comparison coupled with more genetic markers to confirm the species status. During this study, one species of *Dipturus* was confirmed to be new to science. Taxonomic clarification and description of these potentially new *Dipturus* species is given in detail in the chapter 4. *Dipturus* sp. A that was collected from off the Kerala coast, and off Chennai showing maximum of 1.3% and 1% intraspecies variation for COI and 16S rRNA genes respectively, warranting more taxonomic studies. The occurrence of *Raja miraletus*, *Raja texana* and *Rostroraja alba* may be questioned and these may be the possible misidentification of *Okamejei*

*powelli* and *Dipturus* sp. A respectively. *Dipturus* sp. B is not assigned to any species name. This species COI sequence match with *Dipturus* sp.1 (97.61%) on BLAST. Many specific efforts have been made to generate validated reference barcode sequences for skates globally (Ward *et al.*, 2008b; Serra-Pereira *et al.*, 2011; Coulson *et al.*, 2011). These reference sequences now facilitate various molecular studies of species identification, market mislabelling and forensic identification of species (Marko *et al.*, 2004; Wong and Hanner, 2008). However, for most of the Indian species reference sequences were not available in the public databases. In the present study, the four species collected from this family were found to be genetically distinct from each other and positioned into four groups without any haplotypes sharing based on partial sequence information of COI and 16S rRNA genes. The nucleotide variation among 16S rRNA and COI haplotypes showed the sequence divergences that clearly separated the four species of skates including *Dipturus* sp. A, *Dipturus* sp. B, *Dipturus* cf. *johannisdavisi* and *Okamejei powelli*. However, many earlier studies using 16S rRNA observed the low level of genetic divergence between some of the species, which is not within the range that frequently observed between species (Valsecchi *et al.*, 2005; Turan, 2008). In the present study, only 16S gene was consistently amplified and sequenced for all samples collected. Woodley *et al.*, (1994) found that 16S gene to be the best robust marker for catch verification of sharks. So for routine catch verification or identification of bycatch species from the commercial shrimp fishery, 16S gene will be sufficiently robust. Further analysis on a large data set from all described or recorded species of the family Rajidae coupled with additional nuclear genes will be needed to address to resolve the taxonomic incongruity.

### **Centrophoridae**

The family Centrophoridae consists of medium sized demersal sharks under two genera *Centrophorus* and *Deania*. The genus *Centrophorus* is the one of the most taxonomically complex and confusing group among the elasmobranchs. White *et al.*, (2013) in their revision of this group considered *Centrophorus acus* and *C. niaukang* as junior synonyms of *C. granulosus*. Eight species of Centrophoridae were reported in Indian waters and three of this species need confirmation (Akhilesh *et al.*, 2014). Targeted fisheries for these oil sharks from Indian waters have started very recently and it's a mixture of several species of different size class and sex

dominated by *C. atromarginatus* (Akhilesh *et al.*, 2011). In this study, only 16S gene was consistently amplified and sequenced in all samples, except *Centrophorus granulosus*. Many previous studies using the 16S gene also found the marker found to be best for catch verification and species discrimination (Woodley *et al.*, 1994; Daley *et al.*, 2012). In contrast, amplification and sequencing of the COI gene faced problems for samples collected from Tuticorin harbour. These samples are taken from trash that is being taken for poultry industry. Deepwater sharks that are caught as shrimp bycatch and used for oil extraction will never be stored under ideal condition for preservation. In these chances of DNA degradation is very high. In the present study, the 16S gene genetic distance was observed low as 0.002 between *Centrophorus atromarginatus* and *Centrophorus squamosus*. Similarly very low genetic distance of 0.001 was observed between *Centrophorus harrissoni* and *Centrophorus isodon* that was caught by Australian fisheries (Daley *et al.*, 2012). But distance between other main species caught by Australian fisheries showed 0.005 or greater and thus facilitates the 16S marker suitable for routine catch verification in Australia (Daley *et al.*, 2012). As in the case of Indian fisheries, the main species caught are *Centrophorus atromarginatus*, *Centrophorus zeehani* and *Centrophorus granulosus*. So the observed low genetic distance was consistent within the species and no haplotypes sharing between the species and thus makes the 16S marker suitable for future catch verification programme in India.

The comparison between the publicly available COI (Australia) sequences of *Centrophorus zeehani* with those obtained in the present study showed 100% similarity. Similarly, the same pattern of genetic matching also found by Naylor *et al.*, (2012) using NADH2 sequences that was generated from wide ranges (Australia and Atlantic Ocean). However, *Centrophorus zeehani* is considered to be a Southern Australian endemic species (White *et al.*, 2008). However, the existence of deep water marine superhighways that link regions together (Broecker, 1991) may be the possible reason for long distance movement and exchange of genetic material across their ranges. More studies on this group are needed to validate the species identity of *C. zeehani* in Indian waters.

### **Hexanchidae**

The family Hexanchidae comprises three widely recognised genera with four species. All the four species have been listed in the Indian fauna but the occurrence

of *Hexanchus nakamurai* and *Notorynchus cepedianus* need confirmation (Akhilesh *et al.*, 2014). The sixgill shark species, *Hexanchus griseus* and *H. nakamurai* are distinguishable by the presence of six distinctly comb-shaped lower teeth in the former and five comb-shaped lower teeth in the latter species (Ebert *et al.*, 2013). In the present study, the 16S sequence of *Hexanchus griseus* showed 7% divergence when compared with sequence of *H. nakamurai* available in the GenBank. Sample size of *Hexanchus griseus* collected from India was very limited (n=6) from off Kollam area. The range of distribution of *H. nakamurai* include Mauritius area, highlighting the possibility of occurrence of these species in Indian waters and more number of samples from wide geographic area needed to confirm the occurrence of this wide but patchy distribution of this species. The second species *Heptranchias perlo* matched 100% with a Portuguese specimen (EU869819) but 2% COI divergent from Western Australia specimens (EU869817-18). The NADH2 data of this species from around the oceans is variable and the Western Australia population is very distinct from other populations (Naylor pers. comm.). There is possibility of occurrence of the species *Heptranchias dakini* Whitley, 1931 described from Victoria, Australia, that is currently synonymised with *Heptranchias perlo*. This needs further morphological and genetic comparisons between widely-separated geographic samples.

## Conclusion

The present studies conclude that DNA barcoding can be used to distinguish chondrichthyan species with high degree of accuracy. Moreover, the reference library developed during this study, can be used to identify, the catches from shark fishery or bycatch, or even from the dried salted meat from the market. The 16S marker also distinguished Indian chondrichthyan species accurately using universal primers. 16S marker was consistently amplified in most of the samples collected when compared with COI gene. Hence, this marker is suitable for future catch verification programmes in Indian shark fishery for its conservation and management. Amplification of COI and 16S genes coupled with restriction digest fragment analysis will provide unambiguous identification of sharks and their relatives from Indian waters with high degree of accuracy.

**Chapter 5**

***Molecular identification of deep-sea  
teleost fishes***

## Chapter 5

# Molecular identification of deep-sea teleost fishes

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### 5.1. Introduction

Species discovery is being continued in fishes, with over 32,000 valid species, and more than 300 species described each year (Eschmeyer and Fricke, 2011). However, the deep-sea fishes remain the greatest challenge in the discovery of vertebrate species diversity due to many reasons such as lack of expertise, difficulty in sampling, sexual dimorphism, cryptic speciation etc. Some of the deep-sea groups like Myctophidae, Chlorophthalmidae, Lophidae, and Chiasmodontidae are very difficult to identify even by expert taxonomists and taxonomic ambiguity still remains. Many of the current confusions are mainly attributable to the meristic conservatism and individually variable nature of the morphology as in Chlorophthalmidae which group has to be re-examined to find out the natural species assemblages. There is a need to undertake a detailed study of many of the deep-sea fish families, including both morphological and molecular assessments, and also additional geographical, ecological, biological data should be collected (Galtier *et al.*, 2009; Padial *et al.*, 2010).

Mid-water species are often pelagic, occurring in many oceans and one of the least studied group of teleost fishes. Most of them spend their entire life at the deep bottom and show diurnal vertical migrations. Many studies on mid-water fishes such as lanternfishes, Macrouridae, Liparidae were published in the 1960s-1970s, with fewer studies published in the recent years (Eschmeyer *et al.*, 2010). However, the fauna of many deep-sea fish families of the Indo-Pacific is still poorly known (Prokofiev and Kukuev, 2007; Gomon *et al.*, 2014). More number of new species are expected from the deep-water habitat as deep-sea trawling continues for commercial catch and research explorations. Moreover, the presence of cryptic species, individually possessing same morphology, but being genetically divergent, in the marine environment is likely to be of importance for management purpose. Recently, Zahuranec *et al.*, (2012) found cryptic species in the mesopelagic fish genus *Benthosema* using molecular markers. In addition, many other marine fish families also show the presence of cryptic species that highlights the need of DNA



barcoding for critical re-examination of marine fishes to find the existence of putative new species (Hubert *et al.*, 2012; Puckridge *et al.*, 2013; Uiblein and Gouws, 2014).

The deep-sea fish diversity has been studied by Alcock during the *RIMS Investigator* expeditions in the Indian Ocean and adjacent seas during the period 1884-1914. After that, many other expeditions covered extensively both shallow and deepwater zones to explore the deep-sea ichthyofaunal diversity. Many deepwater species have been reported from Indian waters during the 1960s and 1970s (Jones and Kumaran, 1964, 1965; Tholasilingam *et al.*, 1964; Silas and Regunathan, 1974). The exploratory survey by FORV *Sagar Sampada* has increased the knowledge and museum collections of deep-sea fishes collected beyond 200 m depth. The major studies based on these collections include Sivakami *et al.*, (1998); Kurup *et al.*, (2005) and Jayaprakash *et al.*, (2006). Recently, Manjebrayakath *et al.*, (2012) reported 188 species of fishes, which belong to 136 genera, 80 families and 25 orders from the Indian EEZ based on the exploratory surveys conducted by FORV *Sagar Sampada*. In addition to that, many studies on the taxonomy of deep-sea fishes from the southern coast of India have been published, including the redescription of *Glyptophidium oceanium* (Kurup *et al.*, 2009), deep-sea eel *Bassozetus robustus* (Cubelio *et al.*, 2009a), *Dicrolene nigricaudis* (Cubelio *et al.*, 2009b) and rare batfish species *Halicmetus ruber* (Benjamin *et al.*, 2013). Based on the deep-sea shrimp trawl bycatch several interesting deep-sea fishes were reported from Tuticorin (Kannan *et al.*, 2013a; Kannan *et al.*, 2013b; Kannan *et al.*, 2014).

Deep-sea teleost fishes are a highly diverse group of fishes inhabiting deeper areas and their classification become very difficult, especially at morphological level. Taxonomy of deep-sea fishes becomes very difficult due to many factors such as sampling difficulty, good quality reference materials, bad conditions of holotypes or syntypes, lack of taxonomic expertise etc. *Holanthias perumali* Talwar, 1976, *Lestidium blanci* Kartha, 1971 are two deep-sea fishes described from off Kollam, India were synonymised with *Odontanthias rhodopeplus* (Günther, 1872) and *Arctozenus risso* (Bonaparte, 1840) respectively. Although many deep-sea fish species such as *Chlorophthalmus agassizi*, *C. punctatus*, *C. nigromarginatus*, *Priacanthus macracanthus*, *Notacanthus sexspinis*, *Bathypterois dubius*, *Chaunax pictus*, *Bufoceratias wedli*, *Oneirodes krefftii*, *Astronesthes lucifer*, *Bathyclupea*

*elongate*, *Parasudis truculent*, *Beryx decadactylus*, *Beryx splendens*, *Chelidoperca pleurospilus*, *Owstonia weberi*, *Sphenanthias simoterus*, *Rexea solandri* and *Saurida isarankurai* are reported from Indian waters, the validity of these species occurrences may be questioned as no detailed descriptions of species were given in publications discussing their occurrence. These studies show the need for taxonomic revisions of most of the deep-sea fish families from the Indian waters, supported by wide geographical comparisons and molecular approaches.

More recently, molecular techniques have entered the realm of systematic studies and application of molecular tools can provide valuable information for species identification and complement the traditional taxonomic data and validation of systematic position of any living organism. The mitochondrial 16S rRNA and COI genes are useful in resolving taxonomic ambiguities, resurrection of species, redescription and market mislabelling in fishes (Iwatsuki *et al.*, 2012; Ward *et al.*, 2005; Lee *et al.*, 2013; Keskin and Atar, 2012). Presently, DNA based species identification has become very popular technique due to its ease of use, application to all life stages including eggs and larvae and even cooked food items. In India, molecular markers have been used for identifying marine and fresh water fishes and that concluded with very promising result in the taxonomical applications. However, these collections do not include any deep-sea fish species. During the present study, the specimens were well preserved in formalin to maximise their usability for further morphological examinations and deposited in the collections of national museums. The aim of the study is to provide species specific molecular reference sequences of COI and 16S rRNA genes of deep-sea fishes collected from landing centres and exploratory surveys. This chapter also provides molecular confirmations on the occurrence of several deep-sea fishes that have not previously been reported from the Indian waters.

## 5.2. Material and methods

Details given in chapter 4.

## 5.3. Results

The sequence data sets generated in the present study included 605 individual sequences generated from 82 species of 43 families used for partial sequence analysis of 16S rRNA and COI genes. Sequences of a few additional

species (*Brachirus annularis*, *Scombrolabrax heterolepis*, *Neoscopelus microchir*, *Glyptophidium argenteum*, *Alepisaurus ferox*, *Bembrops platyrhynchus* and *Opisthognathus nigromarginatus*) were excluded in the analysis due to bad sequence quality and short sequence size. All sequences were compared with NCBI GenBank and BOLD for identification confirmation. The partial sequences of mt DNA generated in this study were deposited in the GenBank and BOLD public database. Species, family, voucher number and GenBank accession numbers are given in appendix VII and appendix VIII.

### 5.3.1 Taxon diversity

About 10 species of the deep-sea fishes collected during the present study were confirmed as new distributional record for Indian waters. These species records include *Chelidoperca occipitalis* Kotthaus, 1973, *Priacanthus blochii* Bleeker, 1853, *Aphanopus intermedius* Parin, 1983, *Psenes cyanophrys* Valenciennes, 1833, *Psenes arafurensis* Günther, 1889, *Chlorophthalmus acutifrons* Hiyama, 1940, *Bufoceratias thele* (Uwate, 1979), *Pontinus nigerimum* Eschmeyer, 1983, *Beryx mollis* Abe, 1959 and *Diaphus garmani* Gilbert, 1906. During the present study, eight species were confirmed as putative new species. Out of this eight, five species are formally described in detail (Chapter 6) that include *Chelidoperca maculicauda*, *Plectranthias alcocki*, *Liopropoma randalli*, *Symphysanodon xanthopterygion* and *Opisthognathus pardus*. Another three species were confirmed as new by comparing with closely related species using morphological and molecular markers and await formal species description. These species include *Glossanodon* sp. A, *Chaunax* sp. A and *Bathyclupea* sp. A.

### 5.3.2 Cytochrome oxidase sub unit I (COI) and Barcoding

A total of 426 fishes belonging to 82 species, 61 genera, 43 families and 14 orders were barcoded with a minimum of 651 bp. The collections included 18 fish species that were not previously reported from Indian waters. Eight of these taxa were confirmed as putative new species and ten of them identified as new record for Indian waters. All amplified sequences were >650 bp with no insertions, deletions, stop codons and NUMTs. Amplified sequence length varied among species and families but consistent within species. The shortest sequence observed was 588 bp in *Saurida* sp. A, longest with 675 bp in *Ebosia falcata*. Sequences were aligned and

multiple alignments resulted in consensus length of 651 bp per taxon was used for analysis. All sequences were compared with NCBI GenBank and BOLD ([www.barcodinglife.org](http://www.barcodinglife.org), see Ratnasingham and Hebert, 2007) for initial identification confirmation. Out of the total 651 sites obtained 324 (49.77%) were constant, 327 (50.23%) variable, 5 singleton and 322 (49.46%) parsimony informative sites. The polymorphic sites of selected families are illustrated in Fig. 5.8. A total of 183 haplotypes were observed across the taxa. Within each species (n=5), minimum number of haplotypes was one (*Priacanthus hamrur*, *Chelidoperca investigatoris*, *Symphysanodon xanthopterygion* and *Chascanopsetta lugubris*) and maximum was seven in *Diaphus watasei*. The overall mean distance of individuals among the deep-sea teleost fishes under this study was estimated as 0.233 (23.3%). The maximum interspecific K2P distance was 0.326 (32.6%) between *Samaris* sp and *Priacanthus blochii* and minimum was 0.049 (4.9%) divergence between *Notacanthus indicus* and *Notacanthus* sp. A. The minimum intraspecies distance observed was 0.2% in *Obliquogobius cometes* while maximum intraspecies distance observed was 0.011 (1.1%) in *Priacanthus prolixus*. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 5.1 to 5.7.

Overall nucleotide contents across all samples were estimated. The average percentage of the different nucleotides were T = 28.7, C = 29.5, A = 23.2, G = 18.5. As expected, the average transitional pairs (si=74) were more frequent than transversional pairs (sv=55) across all the taxa. The average transition to transversion rate (si/sv) estimate was 1.36.

### 5.3.3 Comments on some individual families

#### Priacanthidae

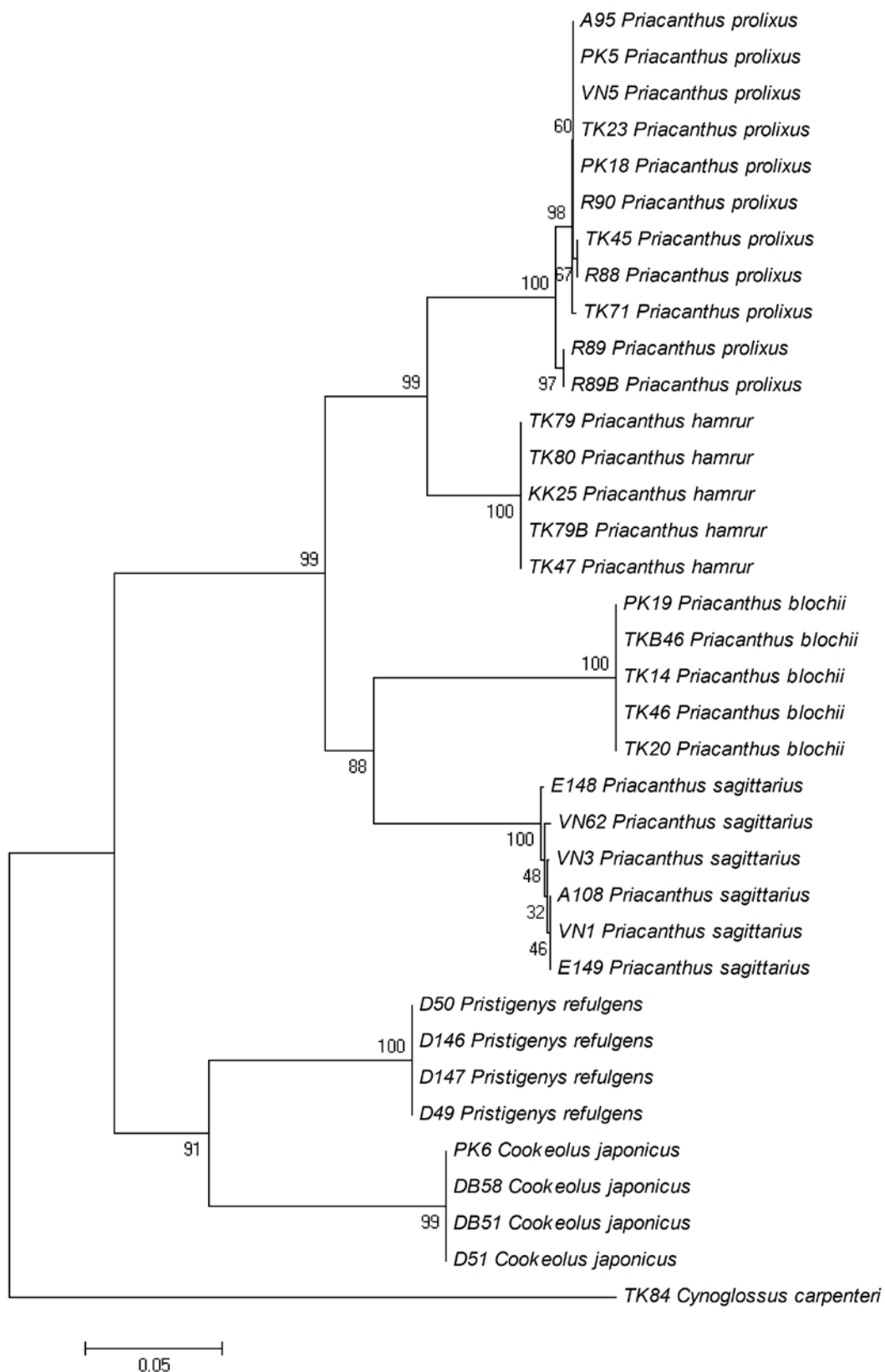
Six species under three genera belonging to the family Priacanthidae were investigated in the present study. The overall mean distance of individuals showed a high value of 17.1%. The maximum interspecific K2P distance was 31.3% between *Priacanthus prolixus* and *Cookeolus japonicus* and minimum was 8.7% divergence between *P. prolixus* and *P. hamrur*. The minimum intraspecies distance observed was 0.2% in *P. sagittarius* while maximum intraspecies distance observed was 0.011 (1.1%) in *P. prolixus*. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 5.1. The amplified sequence length varied

from 655 bp in *C. japonicus* to 672 bp in *P. prolixus*. Out of the total 655 sites obtained 460 were Constant, 195 Variable, 147 Parsimony sites and 48 Singleton sites. The polymorphic sites of selected family are illustrated in figure 5.8 (5.22.3). The analysis revealed the average nucleotide frequencies (%) as T = 27.5, C = 30.7, A = 23.0, and G = 18.7. The average transitional pairs (si = 55) were more frequent than transversional pairs (sv = 27) with an average ratio of R = 2.04.

A total of 12 haplotypes were observed across the taxa. Within each species (n=5), minimum number of haplotypes was one (*C. japonicus*, *P. hamrur*, *C. japonicus*) and maximum was 4 in *P. prolixus*. Two major clades were observed in Neighbour-Joining analysis for the family Priacanthidae (Fig. 5.1). All the species were separated from each other forming clusters, indicating sister groups in the family priacanthidae. All four *Priacanthus* species were seen as one clade and *C. japonicus* and *P. refulgens* separated into another clade. In the first major clade, *P. prolixus* and *P. hamrur* appear as sister clades. All these clades were supported by high bootstrap values.

**Table 5.1.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Priacanthidae based on COI gene

	Pr prH1	Pr prH2	Pr prH3	Pr prH4	Pr blH1	Pr saH1	Pr saH2	Pr saH3	Pr saH4	Pr haH1	Pr reH1	Co jaH1
Pr prH1												
Pr prH2	0.002											
Pr prH3	0.002	0.003										
Pr prH4	0.009	0.011	0.011									
Pr blH1	0.201	0.199	0.198	0.190								
Pr saH1	0.177	0.175	0.174	0.166	0.153							
Pr saH2	0.172	0.170	0.170	0.162	0.155	0.003						
Pr saH3	0.175	0.172	0.172	0.164	0.155	0.002	0.002					
Pr saH4	0.177	0.174	0.174	0.166	0.153	0.003	0.006	0.005				
Pr haH1	0.088	0.089	0.089	0.087	0.185	0.160	0.160	0.162	0.160			
Pr reH1	0.285	0.288	0.289	0.284	0.295	0.269	0.272	0.272	0.276	0.276		
Co jaH1	0.309	0.309	0.313	0.304	0.300	0.287	0.281	0.284	0.280	0.281	0.163	



**Figure 5.1.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Priacanthidae inferred from mitochondrial COI sequence analysis

## Myctophidae

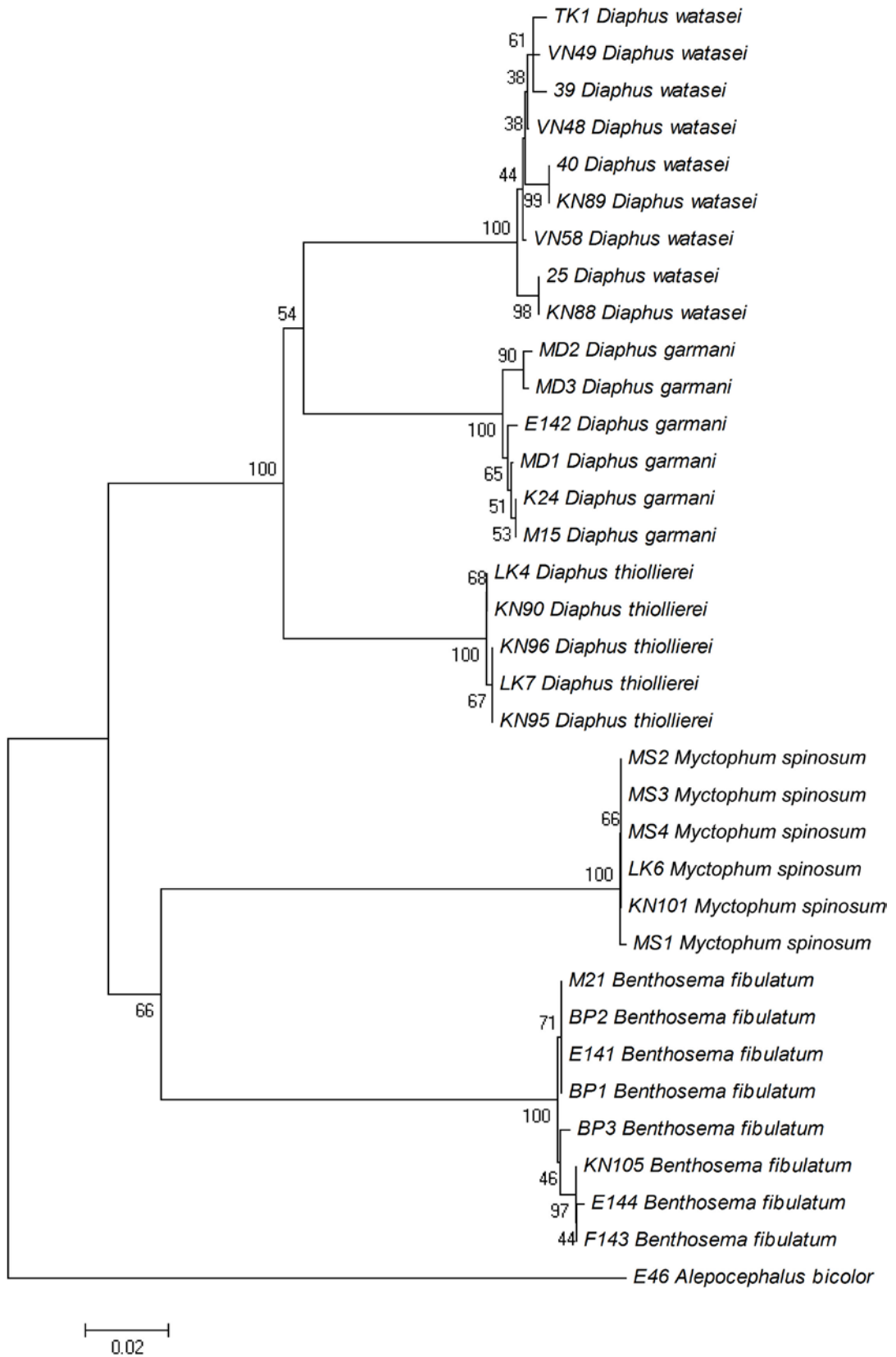
We used 34 individuals of five nominal species of lanternfishes such as *Benthoosema fibulatum*, *Myctophum spinosum*, *Diaphus watasei*, *Diaphus garmani* and *Diaphus thiollierei*. PCR amplification of this group of fishes was very challenging and many species did not produce good amplification even after repeat trials. The amplified sequence length was varied from 651 bp in *Diaphus thiollierei* to 668 bp in *Myctophum spinosum*. Out of the total 651 sites obtained 466 (71.58%) were constant, 185 (28.41%) variable, 182 (27.95%) parsimony sites and 3 (0.46%) singleton sites. The polymorphic sites of selected family are illustrated in figure 5.8 (5.22.6). The analysis revealed the average nucleotide frequencies (%) as T = 26.7, C = 32.7, A = 22.6, and G = 18.0. The average transitional pairs (si = 52) were more frequent than transversional pairs (sv = 30) with an average ratio of R = 1.74.

A total of 20 haplotypes were observed across the taxa. Within each species (n=5), minimum number of haplotypes was two in *Myctophum spinosum* and maximum was seven in *Diaphus watasei*. The overall mean distance of individuals among the family was estimated as 0.152 (15.2%). The maximum interspecific K2P distance was 0.186 (18.6%) between *Myctophum spinosum* and *Diaphus watasei* and minimum was 0.83 (8.3%) divergence between *Diaphus watasei* and *Diaphus garmani*. The minimum intraspecies distance observed was 0.1% in *Myctophum spinosum* while maximum intraspecies distance observed was 0.012 (1.2%) in *Diaphus watasei*. The pair-wise genetic divergence values of the haplotypes within the families are given in table 5.2. Family-wise phylogenetic tree was constructed using *Alepocephalus bicolor* as outgroup (Fig. 5.2). All three *Diaphus* species were seen to occupy one hand of the clade and *Myctophum spinosum* and *Benthoosema fibulatum* separated into another clade. All these clades were supported by high bootstrap values.

**Table 5.2.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Myctophidae based on COI gene

	Be fiH1	Be fiH2	Be fiH3	Be fiH4	Di thH1	Di thH2	Di gaH1	Di gaH2	Di gaH3	Di gaH4	Di gaH5	Di waH1	Di waH2	Di waH3	Di waH4	Di waH5	Di waH6	Di waH7	My spH1	My spH2
Be fiH1																				
Be fiH2	0.006																			
Be fiH3	0.005	0.001																		
Be fiH4	0.003	0.006	0.005																	
Di thH1	0.168	0.173	0.171	0.169																
Di thH2	0.166	0.171	0.170	0.168	0.001															
Di gaH1	0.157	0.162	0.160	0.159	0.086	0.088														
Di gaH2	0.155	0.160	0.158	0.157	0.086	0.088	0.003													
Di gaH3	0.157	0.161	0.160	0.158	0.088	0.089	0.004	0.001												
Di gaH4	0.157	0.162	0.160	0.159	0.088	0.086	0.009	0.009	0.010											
Di gaH5	0.155	0.160	0.158	0.157	0.088	0.086	0.006	0.006	0.008	0.003										
Di waH1	0.165	0.168	0.166	0.166	0.089	0.091	0.088	0.088	0.086	0.091	0.091									
Di waH2	0.165	0.168	0.166	0.166	0.089	0.091	0.089	0.089	0.088	0.092	0.092	0.010								
Di waH3	0.163	0.166	0.165	0.165	0.091	0.092	0.087	0.086	0.085	0.089	0.089	0.012	0.009							
Di waH4	0.164	0.168	0.166	0.166	0.090	0.092	0.090	0.089	0.088	0.092	0.092	0.010	0.005	0.009						
Di waH5	0.164	0.168	0.166	0.166	0.088	0.089	0.086	0.086	0.085	0.089	0.089	0.006	0.004	0.005	0.004					
Di waH6	0.166	0.170	0.168	0.168	0.091	0.092	0.088	0.088	0.087	0.091	0.091	0.008	0.004	0.008	0.004	0.003				
Di waH7	0.163	0.166	0.165	0.164	0.086	0.088	0.085	0.085	0.083	0.088	0.088	0.005	0.005	0.006	0.005	0.001	0.004			
My spH1	0.160	0.169	0.167	0.162	0.165	0.167	0.165	0.164	0.165	0.168	0.169	0.181	0.180	0.181	0.185	0.181	0.183	0.179		
My spH2	0.160	0.169	0.167	0.162	0.167	0.168	0.165	0.164	0.165	0.170	0.169	0.183	0.181	0.183	0.186	0.183	0.185	0.181	0.001	





**Figure 5.2.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Myctophidae inferred from DNA Sequences of mitochondrial gene COI

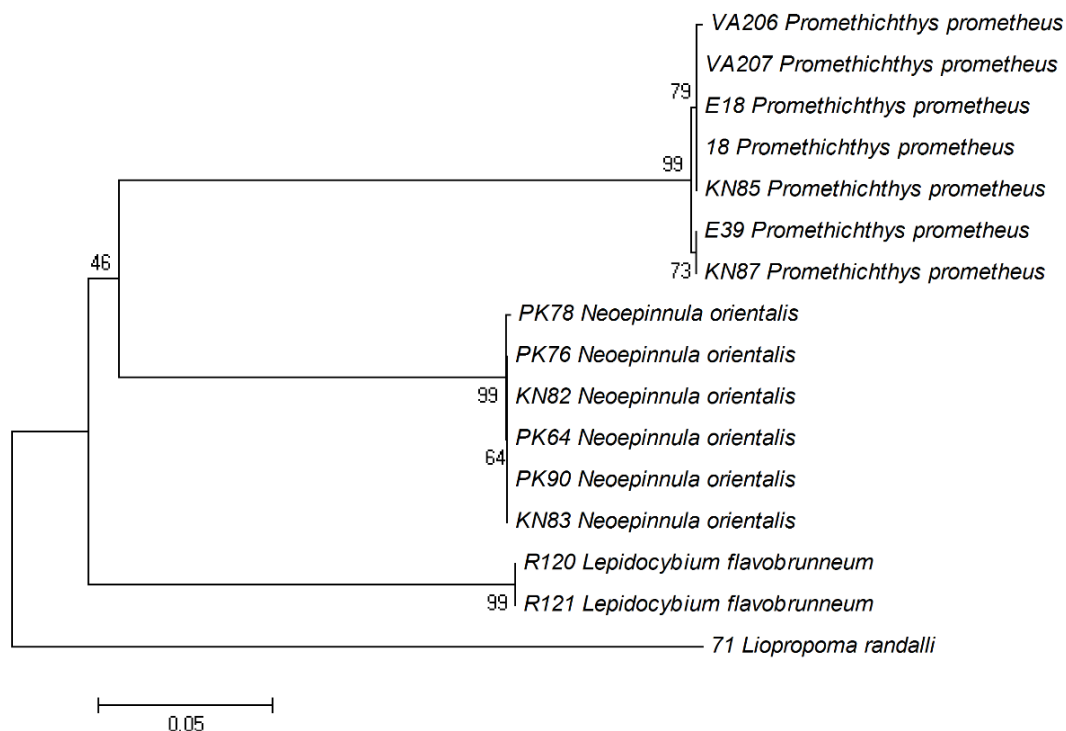
## Gempylidae

We used three nominal species of gempylids from Indian waters. PCR reactions did not produce amplifications for two species (*Rexea bengalensis* and *Ruvettus pretiosus*). The amplified sequence length from three species, *Promethichthys prometheus*, *Neoepinnula orientalis* and *Lepidocybium flavobrunneum* varied from 664 bp in *N. orientalis* to 669 bp in *L. flavobrunneum*. Out of the total 662 sites obtained 510 were Constant, 151 Variable and 151 Parsimony sites. The polymorphic sites of selected family are illustrated in figure 5.8 (5.22.2). The analysis revealed the average nucleotide frequencies (%) as T = 28.1, C = 29.32, A = 24.2, and G = 18.43. The average transitional pairs (si = 42) were more frequent than transversional pairs (sv = 30) with an average ratio of R = 1.39.

A total of 6 haplotypes were observed across the taxa. Within each species (n=5), minimum number of haplotypes was one (*L. flavobrunneum*) and maximum was 3 (*P. prometheus*). The overall mean distance of individuals among the family was estimated as 0.173 (17.3%). The maximum interspecific K2P distance was 0.292 (29.2%) between *L. flavobrunneum* and *P. prometheus* while minimum was 0.232 (23.2%) divergence between *Neoepinnula orientalis* and *L. flavobrunneum*. The minimum intraspecies distance observed was 0.0% in *P. prometheus* and *N. orientalis* while maximum intraspecies distance observed was 0.5% in *P. prometheus*. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 5.3. Comparison of present COI dataset with sequences mined from GenBank showed high intraspecific diversity in three species ie. *N. orientalis* (7.1%), *L. flavobrunneum* (2.9%) and *P. prometheus* (8.9%). Family-wise phylogenetic tree show that *P. prometheus* and *N. orientalis* occupy one hand of the clade and *L. flavobrunneum* forms the sister group to the other two gempylids (Fig. 5.3). Family-wise phylogenetic tree was constructed using *Liopropoma randalli* as out-group.

**Table 5.3.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Gempylidae based on COI gene

	Pr proH1	Pr proH2	Pr proH3	Ne orH1	Ne orH2	Le flH1
Pr proH1						
Pr proH2	0.002					
Pr proH3	0.003	0.005				
Ne orH1	0.27	0.274	0.27			
Ne orH2	0.27	0.274	0.27	0.002		
Le flH1	0.293	0.289	0.298	0.239	0.236	

**Figure 5.3.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Gempylidae inferred from DNA Sequences of mitochondrial gene COI

### Synodontidae

Six species of lizardfishes *Saurida* cf. *micropectoralis*, *Saurida tumbil*, *Saurida longimanus*, *Saurida undosquamis*, *Saurida* sp. A and *Saurida* sp. B were examined. The amplified sequence length varied from 588 bp in *Saurida* sp. A to 667 bp in *Saurida longimanus*. Multiple aligned sequences resulted in a total length of 588 bp sites with no gaps/indels. Out of the total 588 sites obtained 410 were Constant, 178 Variable, 164 Parsimony sites and 14 were Singleton sites. The polymorphic sites of selected family are illustrated in figure 5.8 (5.22.5). The analysis revealed the average nucleotide frequencies (%) as T = 28.3, C = 31.6, A = 21.0, and G = 19.0. The average transitional pairs (si = 61) were more frequent than transversional pairs (sv = 16) with an average ratio of R = 3.71.

A total of 19 haplotypes were observed across the taxa. Within each species (n=5), minimum number of haplotypes was one in *Saurida cf. micropectoralis* and maximum was five in *Saurida tumbil*. The overall mean distance of individuals among the family was estimated as 11.6%. The maximum interspecific K2P distance was 18.5% between *Saurida cf. micropectoralis* and *Saurida sp. B* and minimum was 9.3% divergence between *Saurida cf. micropectoralis* and *Saurida tumbil*. The minimum intraspecies distance observed was 0.1% in *Saurida sp. B* while maximum intraspecies distance observed was 0.8% in *Saurida longimanus*. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 5.4.

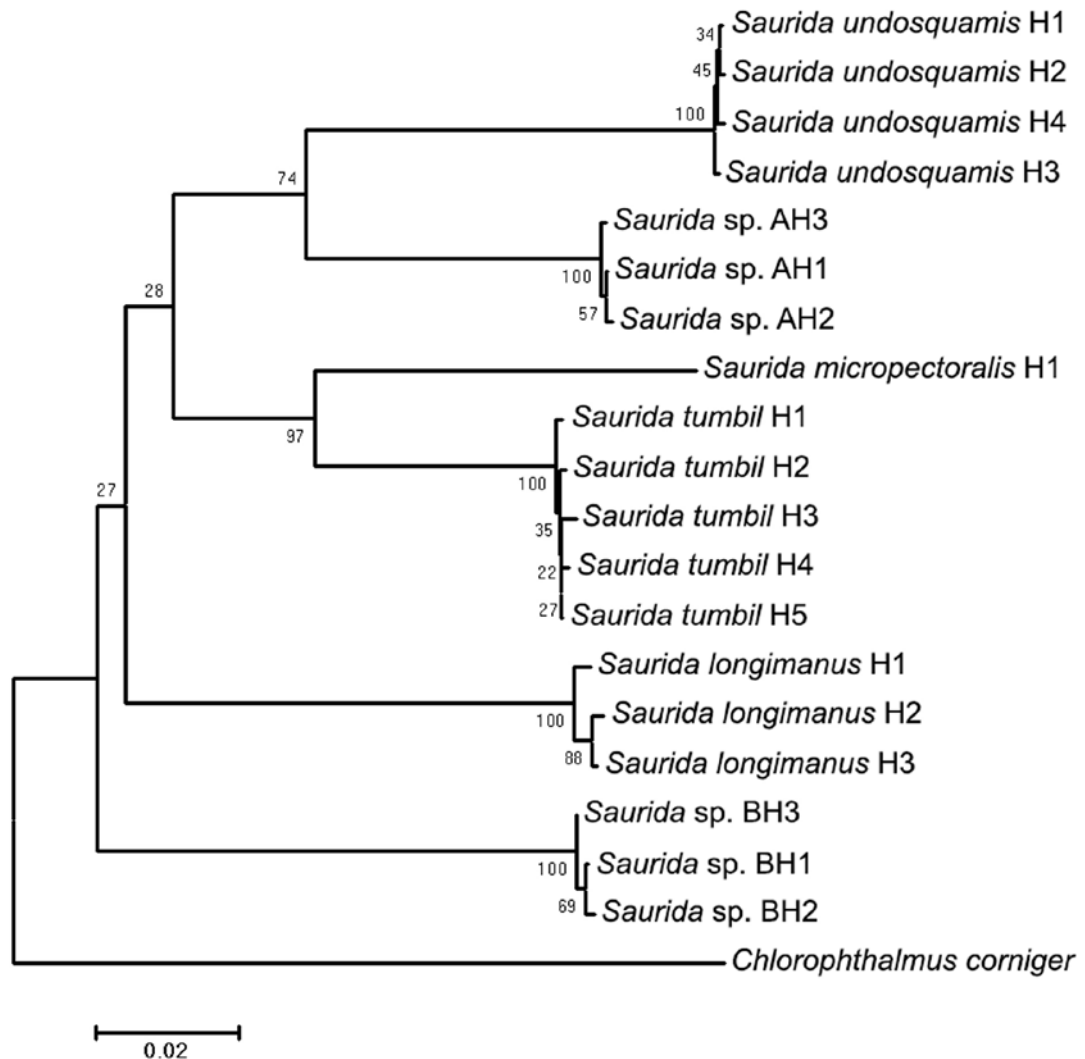
Family-wise phylogenetic tree was constructed using NJ (Fig. 5.4) method. The NJ tree of 16S rRNA gene and COI gene showed the same topology. *Saurida undosquamis* and *Saurida sp. A* were seen to occupy one hand of the clade and *Saurida micropectoralis* and *Saurida tumbil* separated into another clade. *Saurida sp. B* had separately occupied different clade. All these clades were supported by high bootstrap values ranges from 75 to 97%. *Chlorophthalmus corniger* was used as out-group.

### **Nomeidae**

Four species of driftfishes *Cubiceps whiteleggii*, *Cubiceps sp.*, *Psenes arafurensis* and *Psenes cyanophrys* were examined. The amplified sequence length varied from 656 bp in *Cubiceps whiteleggii* to 657 bp in *Psenes arafurensis*. Out of the total 639 sites obtained 484 were constant, 155 variable, 94 parsimony sites and 61 were Singleton sites. The polymorphic sites of selected family are illustrated in figure 5.8 (5.22.4). The analysis revealed the average nucleotide frequencies (%) as T = 29.4, C = 27.2, A = 25.2, and G = 18.1. The average transitional pairs (si = 36) were more frequent than transversional pairs (sv = 15) with an average ratio of R = 2.42.

A total of 10 haplotypes were observed across the taxa. Within each species, minimum number of haplotypes was one (*Psenes arafurensis*) and maximum was 3 (*Cubiceps sp.*). The overall mean distance of individuals among the family was estimated as 4.5%. The maximum interspecific K2P distance was 9.0% between *Cubiceps whiteleggii* and *Psenes arafurensis* while minimum was 2.7% divergence

between *Cubiceps* sp and *Cubiceps whiteleggii*. The maximum intraspecies distance observed was 0.5% in *Cubiceps* sp. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 5.5. Two major clades were observed in the NJ tree of family Nomeidae (Fig. 5.5). *Cubiceps whiteleggii* and *Cubiceps* sp were seen to occupy the first clade and *Psenes arafurensis* and *Psenes cyanophrys* occupied the second clade. All these clades were supported by high bootstrap values.



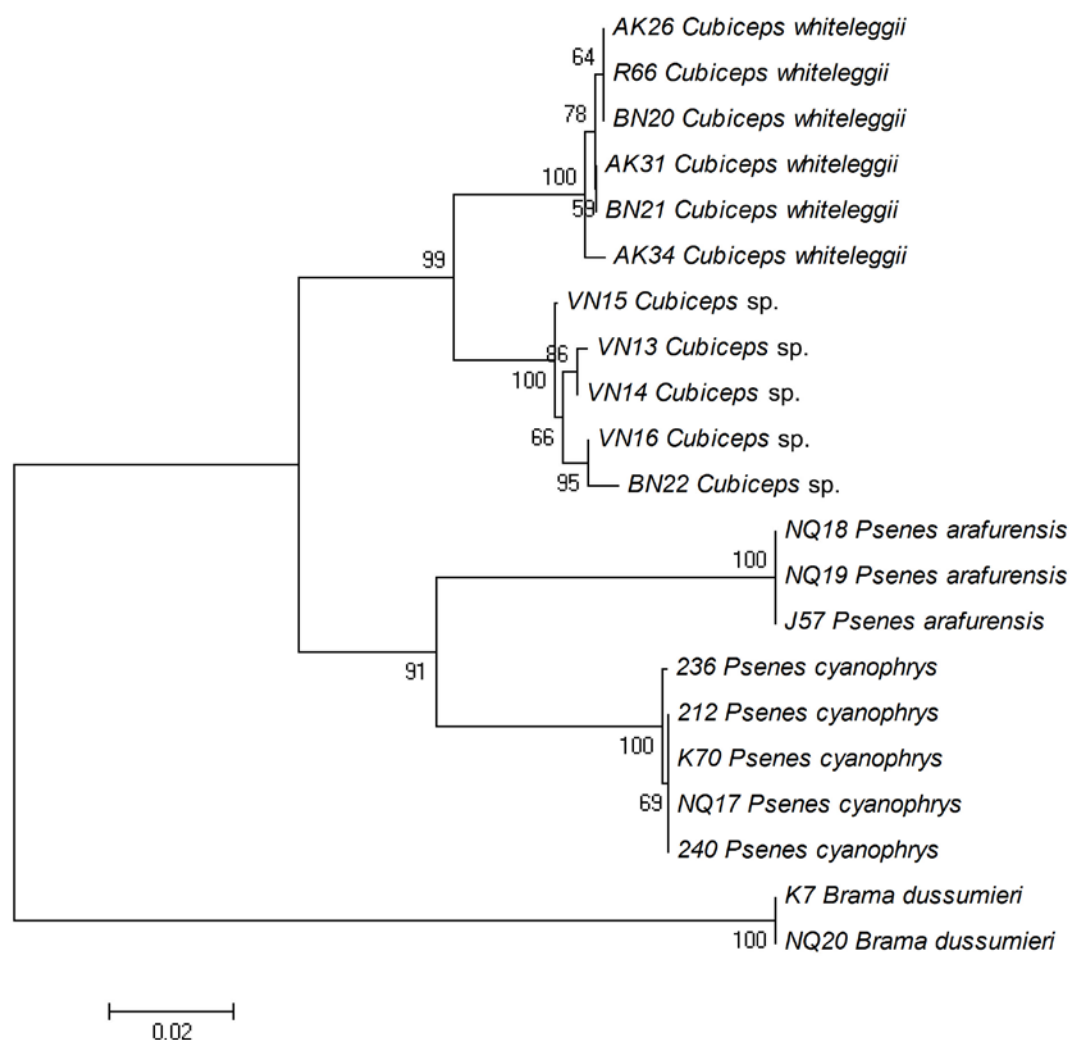
**Figure 5.4.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Synodontidae inferred from DNA Sequences of mitochondrial gene COI

**Table 5.4.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Synodontidae based on COI gene

	Sa loH1	Sa loH2	Sa loH3	Sa unH1	Sa unH2	Sa unH3	Sa unH4	Sa sp AH1	Sa sp AH2	Sa sp AH3	Sa miH1	Sa tuH2	Sa tuH3	Sa tuH4	Sa tuH1	Sa tuH5	Sa spBH1	Sa spBH2	Sa spBH3	
Sa loH1																				
Sa loH2	0.008																			
Sa loH3	0.005	0.003																		
Sa unH1	0.162	0.164	0.164																	
Sa unH2	0.162	0.164	0.164	0.001																
Sa unH3	0.160	0.162	0.162	0.001	0.003															
Sa unH4	0.162	0.164	0.164	0.001	0.003	0.003														
Sa sp AH1	0.148	0.147	0.147	0.103	0.103	0.105	0.104													
Sa sp AH2	0.148	0.147	0.147	0.103	0.103	0.105	0.104	0.001												
Sa sp AH3	0.147	0.145	0.145	0.102	0.102	0.103	0.102	0.003	0.001											
Sa miH1	0.149	0.158	0.156	0.158	0.158	0.156	0.158	0.129	0.130	0.129										
Sa tuH2	0.130	0.132	0.130	0.142	0.142	0.140	0.142	0.115	0.116	0.115	0.090									
Sa tuH3	0.130	0.132	0.130	0.143	0.143	0.142	0.144	0.118	0.119	0.118	0.091	0.004								
Sa tuH4	0.129	0.130	0.129	0.142	0.142	0.140	0.142	0.115	0.116	0.115	0.093	0.003	0.004							
Sa tuH1	0.130	0.132	0.130	0.142	0.142	0.140	0.142	0.115	0.116	0.115	0.090	0.003	0.004	0.003						
Sa tuH5	0.129	0.130	0.129	0.144	0.144	0.142	0.144	0.116	0.118	0.116	0.092	0.001	0.003	0.001	0.001					
Sa spBH1	0.145	0.149	0.147	0.135	0.135	0.135	0.133	0.136	0.134	0.136	0.185	0.135	0.135	0.135	0.138	0.137				
Sa spBH2	0.147	0.147	0.145	0.137	0.137	0.137	0.135	0.134	0.133	0.134	0.187	0.137	0.136	0.137	0.140	0.138	0.001			
Sa spBH3	0.144	0.147	0.145	0.134	0.134	0.134	0.132	0.134	0.133	0.134	0.183	0.133	0.133	0.133	0.137	0.135	0.001	0.003		

**Table 5.5.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Nomeidae based on COI gene

	Cu whH1	Cu whH2	Cu whH3	Cu spH1	Cu spH2	Cu spH3	Cu spH4	Cu spH5	Ps cyH1	Ps cyH2	Ps arH1
Cu whH1											
Cu whH2	0.001										
Cu whH3	0.004	0.003									
Cu spH1	0.032	0.031	0.032								
Cu spH2	0.031	0.029	0.031	0.001							
Cu spH3	0.028	0.027	0.028	0.003	0.002						
Cu spH4	0.032	0.031	0.032	0.005	0.004	0.004					
Cu spH5	0.035	0.034	0.035	0.009	0.008	0.008	0.003				
Ps cyH1	0.075	0.075	0.079	0.071	0.069	0.069	0.067	0.071			
Ps cyH2	0.074	0.074	0.078	0.072	0.071	0.071	0.068	0.072	0.001		
Ps arH1	0.09	0.09	0.09	0.083	0.082	0.081	0.079	0.084	0.063	0.064	

**Figure 5.5.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Nomeidae inferred from DNA Sequences of mitochondrial gene COI

## Nemipteridae

Three species of threadfin breams *Parascolopsis boesemani*, *P. eriomma* and *P. aspinosa* were examined. The amplified sequence length varied from 661 bp in *Parascolopsis boesemani* to 671 bp in *P. eriomma*. Multiple aligned sequences resulted in a total length of 633 bp sites with no gaps/indels. Out of the total 633 sites obtained 547 were Constant, 86 Variable, 33 Parsimony sites and 53 Singleton sites. The analysis revealed the average nucleotide frequencies (%) as T = 30.0, C = 28.0, A = 23.9, and G = 18.1. The average transitional pairs (si = 36) were more frequent than transversional pairs (sv = 13) with an average ratio of R = 2.72.

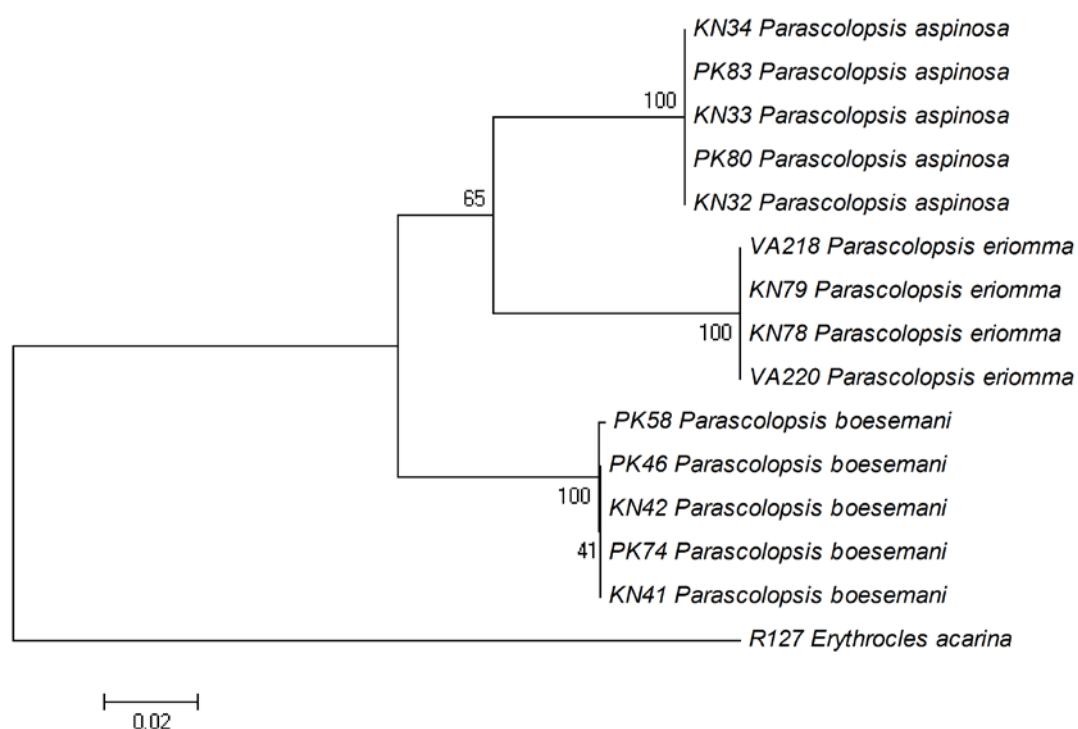
A total of 4 haplotypes were observed across the taxa. Within these species, minimum number of haplotypes was one (*Parascolopsis aspinosa* and *P. eriomma*) and maximum was two (*P. boesemani*). The overall mean distance of individuals among the family was estimated as 2%. The maximum interspecific K2P distance was 2.7% between *P. boesemani* and *P. eriomma* while minimum was 2.1% divergence between *Parascolopsis eriomma* and *P. aspinosa*. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 5.6.

Family-wise phylogenetic tree was constructed using NJ (Fig. 5.6) method including related species *Erythrocles acarina* as out-group, showed complete separation of outgroup from the Nemipterids by two major clades. One clade was seen with *Parascolopsis boesemani* and the other with *P. aspinosa* and *P. eriomma* forming sister clades. All these clades were supported by high bootstrap values.

**Table 5.6.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Nemipteridae based on COI gene

	Pa asH1	Pa boH1	Pa boH2	Pa erH1
Pa asH1				
Pa boH1	0.022			
Pa boH2	0.023	0.000		
Pa erH1	0.021	0.027	0.027	





**Figure 5.6.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Nemipteridae inferred from DNA Sequences of mitochondrial gene COI

### Serranidae

Seven species of serranids *Hyphorhodus octofasciatus*, *Liopropoma randalli*, *Sacura boulengeri*, *Odontanthias perumali*, *Chelidoperca occipitalis*, *Chelidoperca maculicauda* and *Chelidoperca investigatoris* were barcoded. The amplified sequence length varied from 642 bp in *Chelidoperca occipitalis* to 653 bp in *Chelidoperca investigatoris*. Multiple aligned sequences resulted in a total length of 642 bp sites with no gaps/indels. Out of the total 642 sites obtained 423 were Constant, 219 Variable, 197 Parsimony sites and 22 Singleton sites. The analysis revealed the average nucleotide frequencies (%) as T = 29.8, C = 28.5, A = 24.0 and G = 17.8. The average transitional pairs (si = 65) were more frequent than transversional pairs (sv = 41) with an average ratio of R = 1.59.

A total of 13 haplotypes were observed across the seven species of serranids. Within each species, minimum number of haplotypes was one (*Chelidoperca investigatoris*) and maximum was 3 (*Chelidoperca occipitalis*, *Liopropoma randalli*). The overall mean distance of individuals among the family was estimated as 25.5%. The maximum interspecific K2P distance was 34% between *Chelidoperca maculicauda* and *Liopropoma randalli* while minimum was 10.2% divergence

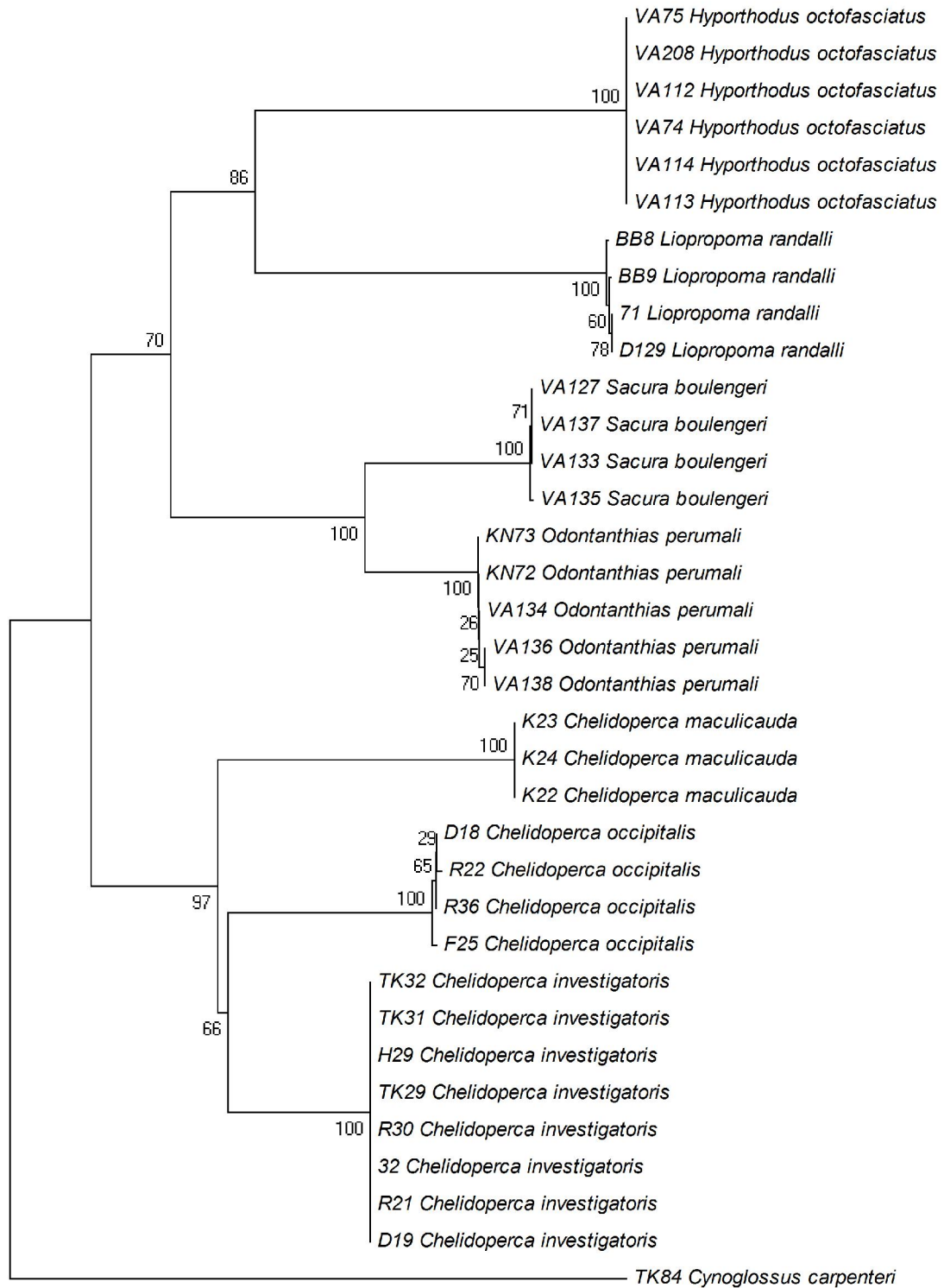
between *Sacura boulengeri*, *Odontanthias perumali*. The minimum intraspecies distance observed was 0.0% in *Chelidoperca maculicauda* while maximum intraspecies distance observed was 0.5% in *Chelidoperca occipitalis*. The pair-wise genetic divergence values of the haplotypes within the families are given in Table 5.7. The NJ tree shows the complete separation of outgroup, *Cynoglossus carpenteri*, from the serranids by two major clusters (Fig. 5.7). One clade was seen with *Chelidoperca maculicauda* and the other with *Chelidoperca occipitalis* and *Chelidoperca investigatoris* forming sister clades. All the clades were found to be separated with high bootstrap support of 86 to 100%.

### Other families

The family Chlorophthalmidae is represented by two species *Chlorophthalmus corniger* and *Chlorophthalmus acutifrons*. The amplified sequence length varied from 653 bp in *Chlorophthalmus corniger* to 655 bp in *C. acutifrons*. A total of nine haplotypes were observed across the two species of greeneyes. Within these species, the number of haplotypes was three in *C. corniger* and seven in *C. acutifrons*. The overall mean distance of individuals among the family was estimated as 3.7%. The interspecific pair-wise genetic distance was calculated as 7.0%. The maximum intraspecies distance observed was 0.5% in both species of greeneyes. Two species of deepsea spiny eels in the family Notacanthidae were barcoded. The amplified sequence length varied from 652 bp in *Notacanthus indicus* to 664 bp in *Notacanthus* sp. A. *Notacanthus* sp. A awaits formal species description. The overall mean distance of individuals among the family was estimated as 3%. The interspecific pair-wise genetic distance was calculated as 5%. *Setarches guentheri* and *S. longimanus* are the two species of deepsea bristly scorpionfishes under the family Setarchidae which were collected and barcoded. The overall mean distance of individuals among the family was estimated as 6.6%. The interspecific pair-wise genetic distance was calculated as 12.3%.

**Table 5.7.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Serranidae based on COI gene

	Ch inH1	Ch ocH1	Ch ocH2	Ch ocH3	Ch maH1	Hy ocH1	Li raH1	Li raH2	Li raH3	Od peH1	Od peH2	Sa boH1	Sa boH2
Ch inH1													
Ch ocH1	0.130												
Ch ocH2	0.132	0.002											
Ch ocH3	0.130	0.003	0.005										
Ch maH1	0.169	0.186	0.189	0.187									
Hy ocH1	0.326	0.333	0.336	0.340	0.316								
Li raH1	0.308	0.317	0.320	0.327	0.333	0.281							
Li raH2	0.308	0.317	0.320	0.327	0.340	0.274	0.003						
Li raH3	0.304	0.320	0.324	0.330	0.336	0.278	0.002	0.002					
Od peH1	0.245	0.305	0.308	0.314	0.298	0.291	0.290	0.290	0.293				
Od peH2	0.248	0.308	0.311	0.317	0.301	0.294	0.293	0.293	0.297	0.002			
Sa boH1	0.259	0.332	0.335	0.338	0.335	0.283	0.333	0.326	0.330	0.105	0.103		
Sa boH2	0.262	0.332	0.335	0.338	0.331	0.280	0.337	0.330	0.333	0.103	0.102	0.002	



**Figure 5.7.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Serranidae inferred from DNA Sequences of mitochondrial gene COI

5.22.1. SERRANIDAE

	1 1 1 1 2 2 2 2 3	3 3 3 4 4 4 4 4 5	5 5 6 6 6 7 7 7 8	8 8 9 9 9 0 0 0 1
Ch inH1	T C A T C G G A A C	T T A T C T C T C A	C A T C A C T T C A	A C C A T T T A C A
Ch oCH1	. . . . . A . . . . .	. . . . . C . C . . . . .	. . . . . . . . . . .	G . . . . . C G . G
Ch oCH2	. . . . . A . . . . .	. . . . . C . C . . . . .	. . . . . . . . . . .	G . . . . . C G . G
Ch oCH3	. . . . . A . . . . .	. . . . . C . C . . . . .	. . . . . . . . . . .	G . . . . . C G . G
Ch mAH1	. . . . . A . C . . .	C C . C . . . . . T G	. . . . . . . . . . .	. . . . . C . . . . .
Hy oCH1	. . . C T A . . . A	C C T C . G . A T .	T G A . G G A C A .	C . T G C . C . T T
Li RAH1	. . G C A A . . C A	C . . C . C T A T .	T . A . . A G C T G	. . . G C . C . T T
Li RAH2	. . G C A A . . C A	C . . C . C T A T .	T . A . . A G C T G	. . . G C . C . T T
Li RAH3	. . G C A A . . C A	C . . C . C T A T .	T . A . . A G C T G	. . . G C . C . T T
Od peH1	C . . C . A . . C A	C . . . . T A . C T .	A . A . . A G C . .	. . T . . . . C T T C
Od peH2	C . . C . A . . C A	C . . . . T A . C T .	A . A . . A G C . .	. . T . . . . C T T C
SA boH1	. T . C T A A . C A	C . . . . T A . . T .	A . A T . A G C . .	. . T . . . . T T T
SA boH2	. T . C T A A . C A	C . . . . T A . . T .	A . A T . A G C . .	. . T . . . . T T T

	1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1	1 1 1 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 2
Ch inH1	C C C T A T T T A T	A T A T A T G T A C	A A T C C C T A C A	T A C C A C A C A A
Ch oCH1	G . . . T . . . . .	. . . . . . . . . . T	G . . . T . . . T C	C . T . . . G . T .
Ch oCH2	G . . . T . . . . .	. . . . . . . . . . T	G . . . T . . . T C	C . T . . . G . T .
Ch oCH3	G . . . T . . . . .	. . . . . . . . . . T	G . . . T . . . T .	C . T . . . G . T .
Ch mAH1	. T . . . . . . . . .	. . G . . . . G C T	. . C . . G . T . C	. . . . G . . T . .
Hy oCH1	A . T C . . C A . .	G . T C . . A . . . .	. . . T . A C C . T	A G T T G . . T C
Li RAH1	. . . . . C A T . .	G . . C G A A . . . .	. G C A A A C . . C	A . T T . . . T T
Li RAH2	. . . . . C A T . .	G . . C G A A . . . .	. G C A A A C . . C	A . T T . . . T T
Li RAH3	. . . . . C A T . .	G . . C G A A . . . .	. G C A A A C . . C	A . T T . . . T T
Od peH1	A T T . . C . A . C	G . C A . . A . C . .	. . . T . T C . . C	A . T . . . . C .
Od peH2	A T T . . C . A . C	G . C A . . A . C . .	. . . T . T C . . C	A . T . . . . C .
SA boH1	G T T C . C . A . C	. C T G G C A C C .	. . . T . C . . T	A G . . . T . T T
SA boH2	G T T C . C . A . C	. C T G G C A C C .	. . . T . C . . T	A G . . . T . T T

	2 2 2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 3 3 3	3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3 3
Ch inH1	C C T C C C C T C	T A A G T A C G A A	A A G T T T T C C G	T A T C A C T A A A
Ch oCH1	A . C . . . T . . T	C . G A . . . A . C	. . A . C C C T T A	C . C . G . . . . .
Ch oCH2	A . C . . . T . . T	C . G A . . . A . C	. . A C C C C T T A	C . C . G . . . . .
Ch oCH3	A . C . . . T . . T	C . G A . . . A . C	. . A . C C C T T A	C . C . G . . . G . .
Ch mAH1	. . C . T . . T C .	. . G . . . T A T G	. . A . C . G . . A	. G C . G . . G . .
Hy oCH1	. A . . A . T T . T	. T T . A . G A T T	T C A . C C G . . A	. G C T . T . . T .
Li RAH1	T . C . T . T A . .	G G . . A . . T . G	C C A . C . . G . A	G C C . . . C . . .
Li RAH2	T . C . T . T A . .	G G . . A . . T . G	C C A . C . . G . A	G C C . . . C . . .
Li RAH3	T . C . T . T A . .	G G . . A . . T . G	C C A . C . . G . A	G C C . . . C . . .
Od peH1	T . A T T . A T . .	. C G . A G . . . T	C . . . . C G . . C	C C . . T . C . G C
Od peH2	T . A T T . A T . .	. C G . A G . . . T	C . . . . C G . . C	C C . . T . C . G C
SA boH1	T . A . . T A . C .	. C . . A . . . . T	C . A . A C A . . C	. C . . C T C . G C
SA boH2	T . A . . T A . C .	. C . . A . . . . T	C . A . A C A . . C	. C . . C T C . G C

	3 3 3 3 3 3 3 3 3 3	3 3 3 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4 4 4
	6 6 7 7 7 7 7 8 8 8	9 9 9 0 0 0 0 0 1 1	2 2 2 3 3 3 3 4 4 4	5 5 5 6 6 6 6 7 7 7
	4 7 0 1 3 6 9 2 5 8	1 2 4 0 1 3 6 9 2 8	1 4 7 0 3 6 9 2 5 8	1 4 7 0 3 6 9 2 5 8
Ch inH1	T C C C A A C T C C	C C A T A T C C T A	C T T C C A A T T T	G A C C C C T A C G
Ch oCH1	A A T . . . . . T T	. . . C . . . A . .	A . . T . . . . . .	. . A T . T A . . A
Ch oCH2	A A T . . . . . T T	. . . C . . . A . .	A . . T . . . . . .	. . A T . T A . . A
Ch oCH3	A A T . . . . . T T	. . . C . . . A . .	A . . T . . . . . .	. . A T . T A . . A
Ch mAH1	C A T T . . . C T G	. . G C G G . A C T	T . . . . . . C . .	A . . T T T C . . A
Hy oCH1	. T . . T T T C . G	. T . G G C G A . .	T . . T T T C . C C	. . . T T T . . T A
Li RAH1	. A T . C . . C . .	. . C A . C A G . .	. C C T T . T . C C	A . . . A T C . . .
Li RAH2	. A . . C . . C . .	. . C A . C A G . .	. C C T T . T . C C	A . . . A T C . . .
Li RAH3	. A . . C . . C . .	. . C A . C A G . .	. C C T T . T . C C	A . . . A T C . . .
Od peH1	C T T T . . T . . A	T . . A G C . T . G	T . C T T . T . . C	A G . . . . C G T A
Od peH2	C T T T . . T . . A	T . . A G C . T . G	T . C T T . T . . C	A G . . . . C G T A
SA boH1	C T . T . . T . . A	T T . A G . . T . G	. . C T T . T . . C	. G . T T . C . T A
SA boH2	C T . T . . T . . A	T T . A G . . T . G	T . C T T . T . . C	. G . T T . C . T A

	4 4 4 4 4 4 4 4 5 5	5 5 5 5 5 5 5 5 5 5	5 5 5 5 5 5 5 5 5 5	5 5 5 5 5 5 5 5 5 5
	8 8 8 9 9 9 9 9 0 0	1 1 1 2 2 2 2 2 2 3	3 3 4 4 4 5 5 5 6 6	6 6 6 7 7 7 8 8 8 8
	1 4 7 0 3 6 7 9 2 3	1 4 7 0 3 4 6 7 9 2	5 8 1 4 7 0 3 9 2 3	5 6 8 1 4 7 0 3 6 9
Ch inH1	C C G T T A T T A C	T C C T C C T T A C	G A T T C C C T G C	T T A C C A T T T A
Ch oCH1	. T A . C . . C . .	. . . . . . C C G .	. . . . . . . . . .	C . . . . . . . . .
Ch oCH2	. T A . C . . C . .	. . . . . . C C G .	C . . . . . . . . .	C . . . . . . . . .
Ch oCH3	. T A . C . . C . .	. . . . . . C C G .	C . . . . . . . . .	C . . . . . . . . .
Ch mAH1	A T C . G . . C C T	. A . . T . A . . .	C . . C T . T A A .	. . . T . . . G . .
Hy oCH1	A T . . A . G C . T	G A A . A . C C T .	C T . . . . T A . .	A . . T T T C C C C
Li RAH1	T T C . A G G C . .	. T . A T T A C T G	T G . C T T T A A T	G C . A T T . . C T
Li RAH2	T T C . A G G C . .	. T . A T T A C T G	T G . C T T T A A T	G C . A T T . . C T
Li RAH3	T T C . A G G C . .	. T . A T T A C T G	T G . C T T T A A T	G C . A T T . . C T
Od peH1	. A C . . . G C T .	. A T C T . . . . .	. . C C . T T . . .	. . G A . . C C C C
Od peH2	. A C C . . G C T .	. A T C T . . . . .	. . C C . T T . . .	. . G A . . C C C C
SA boH1	. G C C . . G C T T	. A T C T . . . . .	. . C . . T T C . .	. . . A . . C . . C
SA boH2	. G C C . . G C T T	. A T C T . . . . .	. . C . . T T C . .	. . . A . . C . . C

5.22.2. GEMPYLIDAE

			1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1
	1 1 1 1 2 3 3 4	4 4 4 5 5 5 6 7 7 7	8 9 9 9 0 0 0 0 1 2	2 3 3 3 6 6 6 7 7 7
	4 7 0 1 3 6 8 1 7 0	3 6 7 2 5 8 7 3 5 9	2 2 4 7 0 3 6 9 2 1	7 0 3 9 6 7 9 2 5 8
Pr prH1	C C A A T T C A A C	T G C T G A T G G G	C T A G T T G C C T	A C T C A A T A C C
Pr prH2	. . . . . . . . . .	. . . . . . . . . .	. . . . . . . . . .	. . . . . . . . . .
Pr prH3	. . . . . . . . . .	. . . . . . . . . .	. . . . . . . . . .	. . . . . . . . . .
Ne orH1	A T . G A . . C G .	C T T C A C G A C A	A C C C . . . . . .	G A . T T . C . T A
Ne orH2	A T . G A . . C G .	C C T C A C G A C A	A C C C . . . . . .	G A . T T . C . T A
Le flH1	G T C G A C T T G G	A C . C C C . A . A	A C C A C C A T T C	G A C . . G C G . A
	1 1 1 1 1 1 2 2 2 2	2 2 2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 2	3 3 3 3 3 3 3 3 3 3
	8 8 9 9 9 9 0 0 1 2	2 3 4 4 5 5 6 6 6 6	7 7 8 8 8 8 8 9 9 9	0 0 1 1 1 2 2 2 3 4
	1 7 0 3 6 7 2 8 4 3	6 5 1 4 0 3 2 3 5 8	1 7 0 3 6 7 9 2 5 8	1 8 0 3 6 2 5 8 4 0
Pr prH1	A G T A C G C A A C	A C A T G T T C C C	C C T C G C C T T T	G T A G C T A A T T
Pr prH2	. . . . . . . . . .	. . . . . . . . . .	. . . . . . . . . .	. . . . . . . . . .
Pr prH3	G . . . . . . . . .	. . . . . . . . . .	. . . . . . . . . .	. . . . . . . . . .
Ne orH1	T A C G A A . G G T	G . . C A C C T A .	. T C . . . G A C C	A G . C T . . . C C
Ne orH2	T A C G A A . G G T	G . . C A C C T A .	. T C . . . G A C C	A G . C T . . . C C
Le flH1	. . C . T A T . . .	. T G . A C . . G T	A . C G A T A C . A	A G C A T C G G A .

	3 3 3 3 3 3 3 3 3 3	3 3 3 3 4 4 4 4 4 4	4 4 4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 5 5 5
	4 4 5 5 5 5 6 6 6 7	7 7 8 9 0 0 1 1 1 2	2 3 3 5 5 5 6 6 7 7	7 7 8 8 9 9 9 0 0 0
	4 9 0 2 5 6 1 4 7 3	6 9 8 7 0 3 2 3 8 4	7 6 9 1 4 5 0 3 2 5	8 9 1 4 3 6 9 5 8 9
Pr prH1	C T A C T C C C G A	A A T T G T A G C T	T C C G C C C A T T	G A G G A T A G G G
Pr prH2	. . . . .	. . . . .	. . . . .	. . . . .
Pr prH3	. . . . .	. . . . .	. . . . .	. . . . .
Ne orH1	T C G A C . A . C T	C C C G C . . A T C	G . T A T A T . A .	T T C . C C . T A T
Ne orH2	T C G A C . A . C T	C C C G C . . A T C	G . T A T A T . A .	T T C . C C . T A T
Le flH1	. C G T . T . T T .	C T C C . C G A . C	C T T A T A . G A C	C T C A . C G A . .

	5 5 5 5 5 5 5 5 5 5	5 5 5 5 5 5 5 5 5 5	5 6 6 6 6 6 6 6 6 6	
	1 2 2 2 3 3 3 3 4 4	5 5 5 6 6 7 7 8 8 9	9 0 0 1 1 2 2 2 3 5	
	4 3 6 9 2 5 8 9 1 7	3 6 9 2 5 4 7 0 6 2	5 4 7 0 6 2 5 8 4 0	
Pr prH1	C A A A C G T C A C	T T C T G A G A T T	C C C T C A G C T C	
Pr prH2	. . . . .	. . . C . . . . .	. . . . .	
Pr prH3	. . . . .	. . . . .	. . . . . T . . .	
Ne orH1	A T C T G C . . . G	A A T . A G T C C .	T T . C . G C A A .	
Ne orH2	A T C T G C . . . G	A A T . A G T C C .	T T . C . G C A A .	
Le flH1	T . C C A T A T G .	C A T C A . A T C C	T . T . T T A A A T	

5.22.3. PRIACANTHIDAE

				1	1 1 1 1 1 1 1 1 1 1
	1 1 1 1 2 2 3	3 4 4 4 4 5 5 6 6 6	7 7 8 8 8 8 9 9 9 0	0	0 0 0 1 1 2 3 3 4 6
	2 4 8 1 3 6 9 2 5 1	7 3 6 7 9 5 8 1 4 7	0 3 2 5 6 8 1 4 7 0	0	3 6 7 2 8 7 0 3 8 3
Pr prH1	T A T A T T G C G C	C T T T A C C C T A	G C C C T T T C G C	T	T A A C C A C C C A
Pr prH2	. . . . .	. . . . .	. . . . .		. . . . .
Pr prH3	. . . . .	. . . . .	. . . . .		. . . . .
Pr prH4	. . . . . A T	. . . . . C .	. . . . .		. . . . .
Pr blH1	C . . . . A . A .	. . . C . T . C .	. . . A A . . C G . T	C . G . A . . . . .	
PrsAH1	C G . . . . . A .	. . . . . T T . C G	. A . . . . . C . A .	. . . . . T . . . . G	
PrsAH2	C G . . . . . A .	. . . . . T T . C G	. A . . . . . C . A .	. . . . . T . . . . G	
PrsAH3	C G . . . . . A .	. . . . . T T . C G	. A . . . . . C . A .	. . . . . T . . . . G	
PrsAH4	C G . . . . . A .	. . . . . T T . C G	. A . . . . . C . A .	. . . . . T . . . . G	
Pr hAH1	. . . . .	. . . . . T . . .	. A . . . . . C . A .	. . . T A G . . . .	
Pr reH1	C C C G A . T T . .	G A C . G T T . G .	A T . . G C . A . .	C . . T A . A T T G	
Co jAH1	C C C G A C T T A .	A A . . . G G T G .	A T A . G C . A . .	C G . . A . A T T G	

	1 1 1 1 1 1 1 1 1 1	1 1 1 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 2
	6 6 6 7 7 7 8 8 8 9	9 9 9 0 0 1 1 1 2 2	2 3 3 4 5 5 6 6 6 7	7 8 8 8 8 8 9 9 9 9
	6 7 9 2 5 8 1 4 7 0	3 6 9 2 3 1 4 7 0 3	6 2 5 1 0 3 2 5 8 1	4 0 3 6 7 9 0 2 5 8
Pr prH1	T G A A T G C C C T	G C C C C T A A C C	A C C G A C T C A C	T C G A C A A C T G
Pr prH2	. . . . .	. . . . .	. . . . .	. . . . .
Pr prH3	. . . . .	. . . . .	. . . A . . . . .	. . . . .
Pr prH4	. . . . .	. . . . .	. . . . .	. . . . .
Pr blH1	A . T G C A T T A C	. T T . . C G G T .	. T T A . . . A . T	. . C C T . . . C A
PrsAH1	A . . . . . T A C	A T T . . C G . T .	G . T A G T . A C .	. T T . . . . C A
PrsAH2	A . . . . . T A C	A T T . . C G . T .	G . T A G T . A C .	. T T . . . . C A
PrsAH3	A . . . . . T A C	A T T . . C G . T .	G . T A G T . A C .	. T T . . . . C A
PrsAH4	A . . . . . T A C	A T T . . C . . T .	G . T A G T . A C .	. T T . . . . C A
Pr hAH1	. . . . . T .	. . . . . T .	. T . . . . . T . .	. . T G . . . T . A
Pr reH1	A A T . . A G . A C	A . . . . T . G C . T	G T T . G T . T T .	A A T . . . G . . C
Co jAH1	A A T G C A A . A .	A T . A . C . C . .	G . T . G . C . T T	G T T G . T G . . C

	3 3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3 3	3 3 3 3 4 4 4 4 4 4
	0 0 0 0 1 1 1 1 2 2	2 3 3 4 4 4 4 4 5 5	5 6 6 7 7 7 7 8 8 8	8 9 9 9 0 0 0 0 1 1
	0 1 4 7 0 3 6 9 2 5	8 1 4 0 3 4 6 9 2 5	8 1 7 0 3 6 9 2 5 6	8 1 4 7 0 3 4 9 3 5
Pr prH1	C A A A T A A G T A	A G T C G C G C T T	A C A G C T A C A G	C C C A A T T A A C
Pr prH2	.	.	.	.
Pr prH3	.	.	.	.
Pr prH4	.	.	.	.
Pr bh1	. G . . . . .	.	. T . . . . .	.
PrsAH1	. C . G . G G . A G	. A C A . . . C C	G . G A . . T . . .	A . T T T C . C . .
PrsAH2	. C T G C G T A A .	G A A . . . A T . C	G . . A . G T . . .	T . T . T C . . . .
PrsAH3	. C T G C G T A A .	G A A . . . A T . C	G . . A . G T . . .	T . T . T C . . . .
PrsAH4	. C T G C G T A A .	G A A . . . A T . C	G . . A . G T . . .	T . T . T C . . . .
Pr hAH1	. . . . A G . . . .	. A . . A T A . C C	G . . A . . T . G .	. . T . . C . . . .
Pr reH1	G . . G G T . A C G	. A . . T . A . C C	T . T A A C T T T A	A T . C C . C . . T
Co jAH1	G . . . C T . A A .	G A . . A T A . G C	C T T . . C T . C A	. T T T T C C T G T
	4 4 4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4 4 5	5 5 5 5 5 5 5 5 5 5
	1 2 2 2 2 3 3 4 4 5	5 5 5 6 6 7 7 7 8 8	8 8 9 9 9 9 9 9 9 0	0 1 1 1 2 2 2 2 3 3
	8 1 4 5 7 3 6 5 8 1	4 6 7 3 9 2 5 9 1 3	4 7 0 1 2 3 6 7 9 2	5 1 4 7 0 3 6 9 2 5
Pr prH1	C G T C A C C C G A	C C C A C T C T C T	T C G G T T C T A C	C C G G T A T T T C
Pr prH2	.	.	.	.
Pr prH3	.	.	.	.
Pr prH4	.	.	.	.
Pr bh1	. A . . . . .	.	.	.
PrsAH1	. A C . . T . . A .	T . T G . . T A . .	G . . A C C . C T .	. . C A . G . A G .
PrsAH2	T A . T . T . T A .	T . T . . . . . . .	C . A A C A T . . .	. . T A . . C C A .
PrsAH3	T A . T . T . T A .	T . . . . . . . . .	C . A A C A T . . .	. . T A . . C C A .
PrsAH4	T A . T . T . T A .	T . T . . . . . . .	C . A A C A T . . .	. . T A . . C C A .
Pr hAH1	. A . . G . T T A .	T . T . . . A . . . .	. T . . . . . C C .	. . . A . . . G . .
Pr reH1	T A C . . . . . A T	T T T G T C . . . A A	A . A A C C . . . T	A T T A . T G . C T
Co jAH1	. C C . . . T T A .	T T . G T G . . . A A	A . A A C C A C . T	T . C A C T . C . A
	5 5 5 5 5 5 5 5 5 5	5 5 5 5 5 5 5 5 5 5	5 6 6 6 6 6 6 6 6 6	6 6 6 6 6
	3 4 4 4 4 5 5 5 5 6	6 6 7 7 7 8 8 8 9 9	9 0 0 1 2 2 2 3 3 3	4 4 4 4 5
	8 1 2 4 7 0 3 6 9 2	5 8 1 4 7 0 3 6 2 5	8 1 7 6 2 5 8 1 4 7	0 3 6 9 2
Pr prH1	T G G T T C C G T A	C C T A T C A T T A	C A C G A G A C A C	G C A C G
Pr prH2	.	. C . . . . .	.	.
Pr prH3	.	.	.	.
Pr prH4	.	.	.	.
Pr bh1	. C C . C C . . A . T	. . C G C A . C C .	. . T . G A G T G .	. . G . A
PrsAH1	. A . C . A . . . T	. . C . C A . C C .	. G . T G A G . . T	T . . . A
PrsAH2	. A . C . A . . . T	. . C . C A . C C .	. G . T G A G . . T	T . . . A
PrsAH3	. A . C . A . . . T	. . C . C A . C C .	. G . T G A G . . T	T . . . A
PrsAH4	. A . C . A . . . T	. . C . C A . C C .	. G . T G A G . . T	T . . . A
Pr hAH1	C A . C . T . . . .	T T . . . A . . C .	. . . T . . . . .	. . . . .
Pr reH1	C C T . . A T T C .	G T . G C . . . C C	. T . A . A G . C .	. . T . T A
Co jAH1	C A T C C A T C C .	G . A G C . C C C T	T T . T . . . . G T	. . . . .



5.22.4. NOMEIDAE

		1 1 1	1 1 1 1 1 1 1 2 2 2	2 2 2 2 2 2 2 2 2 2	2 2 2 3 3 3 3 3 3 3
	2 2 3 5 7 8 9 1 2 2	2 6 6 7 8 8 9 2 2 4	4 4 4 5 5 6 6 7 7 8	8 9 9 0 0 1 2 2 2 3	
	4 7 0 5 5 4 0 7 1 3	9 5 8 7 0 6 8 5 8 0	3 6 9 2 8 4 7 0 6 2	8 4 7 0 6 8 1 4 7 0	
Cu whH1	T A C C C T G C T C	A A T G C A A G C T	A A C C A C A T T T	T T T C T T T T C A	
Cu whH2	.	.	.	.	
Cu whH3	.	.	.	.	C . . . . .
Cu spH1	A . . . . . A . . . . .	G . . . . .	C . T C . G C C .	. . . T . A . . . . .	
Cu spH2	A . . . . . A . . . . .	G . . . . .	C . T C . G C C .	. . . T . A . . . . .	
Cu spH3	A . . . . . A . . . . .	G . . . . .	C . T C . G C C .	. . . T . A . . . . .	
Cu spH4	A . . . . . A . . . . .	G . . . . .	C T T C . G C C .	. . . T . A . . . . .	
Cu spH5	A . . . . . A . G . . . . .	G G . . . . . G	C T T C . G C C .	. . . T . A . . . . .	
Ps CyH1	A . . T T C . T . . . . .	G . A T C G . T . . . . .	T . T G . . . A	A C . . . A . G T .	
Ps CyH2	A . . T T C . T . . . . .	G . A T C G . T . . . . .	T . T G . . . A	A C . . . A . G T .	
Ps ArH1	A G T T T C A T . T . . . . .	G G . A T C G A . . . . .	G T T . C T . G . C	A . G . C A . A . G	

	3 3 3 3 3 3 3 3 3 3	3 3 3 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4 4 4	4 4 5 5 5 5 5 5 5 5
	3 3 3 4 5 6 6 7 7 8	8 9 9 0 2 2 2 4 4 5	5 6 6 7 7 7 8 8 8 9	9 9 1 1 1 2 2 2 3 3
	3 6 9 2 4 0 6 2 8 4	7 0 9 8 3 6 9 4 7 3	6 2 8 1 4 7 0 3 9 5	8 9 0 3 9 2 5 8 1 4
Cu whH1	C C C G A A C T C C	T A T C T C T T G T	C C G C G T T G C T	C C C C C T A C A A
Cu whH2	.	.	.	.
Cu whH3	G . . . . .	.	.	.
Cu spH1	. T . A . . . . T A . . . . .	. A . . . T . C . . . . .	. A . A C C A . . . . .	T T . . . . .
Cu spH2	. T . A . . . . T A . . . . .	. A . . . T . C . . . . .	. A . A C C A . . . . .	T T . . . . .
Cu spH3	. T . A . . . . T . . . . .	. A . . . T . C . . . . .	. A . A C C A . . . . .	T T . . . . .
Cu spH4	. T . A . . . . T A . . . . .	. G . . . T C C . . . . .	. A . A C C A . . . . .	T T . . . . .
Cu spH5	. T . A . . . . T A . . . . .	. G . . . T C C . . . . .	. A . A C C A . . . . .	T T . . . . .
Ps CyH1	. T T A . C T C . A C . A . . . . .	. T C C . . . . .	A T A T . A C C T C	T T A T A A T T G .
Ps CyH2	. T T A . C T C . A C . A . . . . .	. T C C . . . . .	A T A T . A C C T C	T T A T A A T T G .
Ps ArH1	T A . C G . . C . G . . . . .	. G G T C T . C A C	A T A T A A G A T C	T . G . T A . . G C

5.22.5. SYNODONTIDAE

	1 1 1 1 2 2 2 3	3 3 3 4 4 4 5 5 5 6	6 6 6 7 7 7 8 8 8 9	1 1 1 1 1 1 1 1
	3 6 0 2 8 9 1 4 7 0	3 6 9 2 5 8 1 4 7 0	3 6 9 2 5 8 1 4 7 0	3 6 2 5 8 6 2 5 8 1
SA loH1	C C C G C C G T T T	A C C G A G C T C G	C C G C C T G C T C	A T C G A G T A T C
SA loH2	.	.	.	.
SA loH3	.	.	.	.
SA unH1	. T . . . T A . . C . . . . .	. T T . G . . . . A	T T A T . . . A . C .	T C . T . . C G . T
SA unH2	. T . . . T A . . C . . . . .	. T T . G . . . . A	T T A T . . . A . C .	T C . T . . C G . T
SA unH3	. T . . . T A . . C . . . . .	. T T . G . . . . A	T T A T . . . A . C .	T C . T . . C G . T
SA unH4	. T . . . T A . . C . . . . .	. T T . G . . . . A	T T A T . . . A . C .	T C . T . . C G . T
SA sp AH1	. T . A . T A . . C . . . . .	. T T A G . . . . A	T T A . . . . . C .	C C . T . . C G C .
SA sp AH2	. T . A . T A . . C . . . . .	. T T A G . . . . A	T T A . . . . . C .	C C . T . . C G C .
SA sp AH3	. T . A . T A . . C . . . . .	. T T A G . . . . A	T T A . . . . . C .	C C . T . . C G C .
SA miH1	. G . A A T . . G C . . . . .	G G . A . A . . A . . . . .	. A T . C A T . G	. C T A . . . G C .
SA TuH2	. T . A . T A C . C . . . . .	. T . A . A . . A . . . . .	. A T T . A T . . . . .	. C . A . A . G C .
SA TuH3	. T . A . T A C . C . . . . .	. T . A . A . . A . . . . .	. A T T . A T . . . . .	. C . A . A . G C .
SA TuH4	. T . A . T A C . C . . . . .	. T . A . A . . A . . . . .	. A T T . A T . . . . .	. C . A . A . G C .
SA TuH1	. T . A . T A C . C . . . . .	. T . A . A . . A . . . . .	. A T T . A T . . . . .	. C . A . A . G C .
SA TuH5	. T . A . T A C . C . . . . .	. T . A . A . . A . . . . .	. A T T . A T . . . . .	. C . A . A . G C .
SA spBH1	A . T A . . A . A C . . . . .	. . . A C . G C . . . . .	. T A . . . . A . C .	G C . T G A . G . G
SA spBH2	A . T A . . A . A C . . . . .	. . . A C . G C . . . . .	. T A . . . . A . C .	G C . T G A . G . G
SA spBH3	A . T A . . A . A C . . . . .	. . . A C . G C . . . . .	. T A . . . . A . C .	G C . T G A . G . G

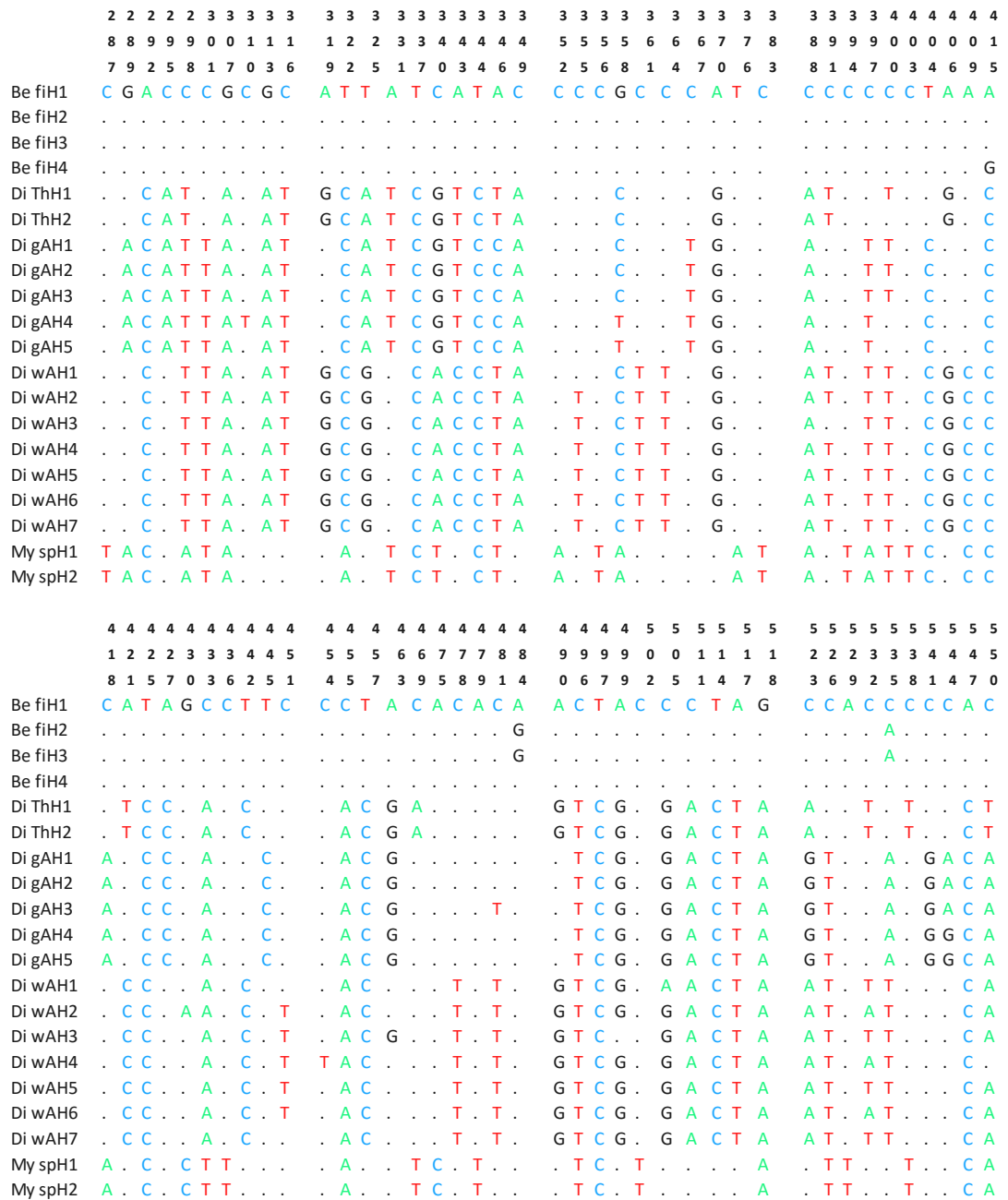
	1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 2 2	2 2 2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 2
	4 4 5 5 5 5 5 6 6 7	7 7 8 8 8 9 9 9 0 0	0 1 1 1 1 2 2 2 3 4	4 4 4 4 4 5 6 6 6 7
	4 7 0 3 6 7 9 2 8 1	4 7 3 6 9 2 5 8 1 4	7 0 3 6 9 2 5 8 7 0	3 4 6 7 9 2 1 4 7 0
SA loH1	C C A C A C T T T G	T A C C G A T C T G	C C A T C A T T C T	T C T C C A T C T A
SA loH2	. . . . .	. G . . . . .	T . . . . .	. . . . .
SA loH3	. . . . .	. G . . . . .	T . . . . .	. . . . .
SA unH1	A T . . G T A . A .	C T T . A . C T C .	. . G C . G . . T C	C . C T G C . A A .
SA unH2	A T . . G T G . A .	C T T . A . C T C .	. . G C . G . . T C	C . C T G C . A A .
SA unH3	A T . . G T A . A .	C T T . A . C T C .	. . G C . G . . T C	C . C T G C . A A .
SA unH4	A T . . G T A . A .	C T T . A . C T C .	. . G C . G . . T C	C . C T G C . A A .
SA sp AH1	G . . . . . A .	. C T . . . . . C A	T . . C . . C G T C	C . A . . C . A A G
SA sp AH2	G . . . . . A .	. C T . . . . . C A	T . . C . . C G T C	C . G . . C . A A G
SA sp AH3	G . . . . . A .	. C T . . . . . C A	T . . C . . C G T C	C . G . . C . A A G
SA miH1	A . G T . . . . A	C . T . . . . C T A A	. . G C T . C . . C	C T A . T T C G . .
SA TuH2	A . . . . . C . . .	. G . T . . . . . A	. . G C T . C G . C	C T A . T T . G . .
SA TuH3	A . . . . . C C . .	. G . T . . . . . A	. . G C T . C G . C	C T A . T T . G . .
SA TuH4	A . . . . . C . . .	. G . T . . . . . A	. . G C T . C G . C	C T A . T T . G . .
SA TuH1	A . . . . . C . . .	. G . T . . . . . A	. . G C T . C G . C	C T A . T T . G . .
SA TuH5	A . . . . . C . . .	. G . T . . . . . A	. . G C T . C G . C	C T A . T T . G . .
SA spBH1	. T . . . . C C . .	. T T T . G . . . A	. T G C . . . . . C	C . G . T . C A A G
SA spBH2	. T . . . . C C . .	. T T T . G . . . A	. T G C . . . . . C	C . G . T . C A A G
SA spBH3	. T . . . . C C . .	. T T T . G . . . A	. T G C . . . . . C	C . G . T . C A A G
	2 2 2 2 2 2 2 3 3	3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3
	7 7 7 8 8 8 9 9 0 0	0 0 0 1 1 1 2 2 2 3	3 3 4 4 4 4 5 5 5 6	6 6 6 6 7 7 7 8 8 8
	3 6 9 2 5 8 4 7 0 3	6 7 9 2 5 8 1 4 7 0	3 9 2 5 6 8 1 4 7 0	3 6 7 9 2 5 8 1 4 7
SA loH1	C A C A C A G G T C	T C A A C T C T C C	G C G C C A C C T C	C C C A G A T C C T
SA loH2	. . T . . . . .	. . . . .	. . . . T . . . .	. . . . .
SA loH3	. . T . . . . .	. . . . .	. . . . T . . . .	. . . . .
SA unH1	. G T . . G A C C G	C . G G . . . . C T T	T . C . . G . . T C T	G T . . . T . T T C
SA unH2	. G T . . G A C C G	C . G G . . . . C T T	T . C . . G . . T C T	G T . . . T . T T C
SA unH3	. G T . . G A C C G	C . G G . . . . C T T	T . C . . G . . T C T	G T . . . T . T T C
SA unH4	. G T . . G A C C G	C . G G . . . . C T T	T . C . . G . . T C T	G T . . . T . T T C
SA sp AH1	. G T . T G A C C G	C T . . . G C . C .	C . T . . . T . . T	T . . G A G C . . C
SA sp AH2	. G T . T G A C C G	C T . . . G C . C .	C . T . . . T . . T	T . . G A G C . . C
SA sp AH3	. G T . T G A C C G	C T . . . G C . C .	C . T . . . T . . T	T . . G A G C . . C
SA miH1	A G . . T C A T C G	C . . G . C . C . .	A . T . . . . T C .	G . . G A G C . . C
SA TuH2	. . . G . . A T C G	C . G C T C . C . .	. T C T T . T T . A	. . . . A T C . T C
SA TuH3	. . . G . . T C G	C . G G T C . C . .	. T C T T . T T . A	. . . . A T C . T C
SA TuH4	. . . G . . T C G	C . G C T C . C . .	. T C T T . T T . A	. . . . A T C . T C
SA TuH1	. . . G . . T C G	C . G C T C . C . .	. T C T T . T T . A	. . . . A T C . T C
SA TuH5	. . . G . . T C G	C . G C T C . C . .	. T C T T . T T . A	. . . . A T C . T C
SA spBH1	. . . G . . A T . .	. . G . A C T . . .	C . C T T G . T C .	A T T G A G . T T .
SA spBH2	. . . G . . A T . .	. . G . A C T . . .	C . C T T G . T C .	A T T G A G . T T .
SA spBH3	. . . G . . A T . .	. . G . A C T . . .	C . C T T G . T C .	A T T G A G . T T .
	3 3 3 3 4 4 4 4 4	4 4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4 4	4 4 4 4 5 5 5 5 5
	9 9 9 9 0 1 1 1 2 2	2 2 3 3 3 4 4 4 5 5	5 5 6 6 6 6 7 7 7 8	8 9 9 9 0 0 0 1 1 1
	0 3 6 9 2 1 4 7 0 3	6 9 2 5 8 1 4 7 0 3	6 9 0 2 5 8 1 4 7 6	9 2 5 8 1 4 7 0 3 6
SA loH1	T A C T T T A T T T	G G A T T T A A C A	C C C C T T G G C T	C T C T G C T T C C
SA loH2	. . . . .	. . . . .	. . . . .	. . . . .
SA loH3	. . . . .	. . . . .	. . . . .	. . . . .
SA unH1	A G T . . . . . C	A . C . C C C . . .	. . T A . A . A T C	. C T . . T C C . T
SA unH2	A G T . . . . . C	A . C . C C C . . .	. . T A . A . A T C	. C T . . T C C . T
SA unH3	A G T . . . . . C	A . C . C C C . . .	. . T A . A . A T C	. C T . . T C C . T
SA unH4	A G T . . . . . C	A . C . C C C . . .	. . T A . A . A T C	. C T . . T C C . T
SA sp AH1	G G T . . . . . C	. A C . C C . . T .	. G . A . G A A T .	. C T C C . C C . .
SA sp AH2	G G T . . . . . C	. A C . C C . . T .	. G . A . G A A T .	. C T C C . C C . .
SA sp AH3	G G T . . . . . C	. A C . C C . . T .	. G . A . G A A T .	. C T C C . C C . .
SA miH1	C G . . C C C C C C	. . T . . . . G T G	. A . A . C A A . C	T C . . C . C . T .
SA TuH2	G G . . C . . C . C	. . T C . . . . T .	. A . A . A A . .	. C . . C . C T T
SA TuH3	G G . . C . . C . C	. . T C . . . . T .	. A . A . A A . .	. C . . C . C T T
SA TuH4	G G . . C . . C . C	. . T C . . . . T .	. A . A . A A . .	. C . . C . C T T
SA TuH1	G G . . C . . C . C	. . T C . . . . T .	. A . A . A A . .	. C . . C . C T T
SA TuH5	G G . . C . . C . C	. . T C . . . . T .	. A . A . A A . .	. C . . C . C T T
SA spBH1	A G T C . . . . .	. A C C C C . . . .	G . . G C A . A . C	. . T C T . . C . T
SA spBH2	A G T C . . . . .	. A C C C C . . . .	G . . G C A . A . C	. . T C T . . C . T
SA spBH3	A G T C . . . . .	. A C C C C . . . .	G . . G C A . A . C	. . T C T . . C . T

5.22.6. MYCTOPHIDAE

	1	1	1	1	1	1	1	1	2	2	3	3	3	4	4	4	4	5	5	6	6	7	7	7	8	8	9	9	9	0	1	1	1	1	1	1	1	1	1	1
	3	4	5	6	7	0	1	3	6	5	8	1	4	7	0	6	9	3	8	1	7	3	4	6	2	5	1	4	7	0	3	6	9	2	5	4	7	0	3	4
Be fiH1	T	C	T	A	C	A	A	C	C	A	A	A	A	T	C	C	A	G	G	T	C	C	G	C	C	A	C	T	G	C	C	A	C	T	C	T	A	C	T	C
Be fiH2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
Be fiH3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
Be fiH4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Di ThH1	.	.	.	.	.	G	A	.	.	.	C	.	A	T	T	T	C	T	C	T	T	A	T	T	C	T	G	A	T	T	G	T	C	.	A	.	T	C	.	
Di ThH2	.	.	.	.	.	G	A	.	.	.	C	.	A	T	T	T	C	T	C	T	T	A	T	T	C	T	G	A	T	T	G	T	C	.	A	.	T	C	.	
Di gAH1	.	.	.	.	G	G	A	T	.	.	C	.	A	T	T	C	C	C	.	T	A	A	.	T	.	G	.	.	.	G	.	C	.	A	.	.	C	.	.	
Di gAH2	.	.	.	G	G	A	T	.	.	C	.	A	T	T	C	C	C	.	T	A	A	.	T	.	G	.	.	.	G	.	C	.	A	.	.	C	.	.		
Di gAH3	.	.	.	G	G	A	T	.	.	C	.	A	T	T	C	C	C	.	T	A	A	.	T	.	G	.	.	.	G	.	C	.	A	.	.	C	.	.		
Di gAH4	.	.	.	G	G	A	T	.	.	C	.	A	T	T	C	C	C	.	T	A	A	.	T	.	G	.	.	.	G	.	C	.	A	.	.	C	.	.		
Di gAH5	.	.	.	G	G	A	T	.	.	C	.	A	T	T	C	C	C	.	T	A	A	.	T	.	G	.	.	.	G	.	C	.	A	.	.	C	.	.		
Di wAH1	.	.	.	G	G	A	T	.	.	C	G	C	A	T	T	C	A	C	.	A	A	.	T	C	.	A	.	T	.	T	C	.	A	G	.	C	T	.		
Di wAH2	.	.	.	G	G	A	T	.	.	C	G	C	A	T	T	C	A	C	.	A	A	.	T	C	.	A	.	T	.	T	C	.	A	G	.	C	T	.		
Di wAH3	.	.	.	G	G	A	T	.	.	C	G	C	A	T	T	C	A	C	.	A	A	.	T	C	.	A	.	T	.	T	C	.	A	G	.	C	T	.		
Di wAH4	.	.	.	G	G	A	T	.	.	C	G	C	A	T	T	C	A	C	.	A	A	.	T	C	.	A	.	T	.	T	C	.	A	G	.	C	T	.		
Di wAH5	.	.	.	G	G	A	T	.	.	C	G	C	A	T	T	C	A	C	.	A	A	.	T	C	.	A	.	T	.	T	C	.	A	G	.	C	T	.		
Di wAH6	.	.	.	G	G	A	T	.	.	C	G	C	A	T	T	C	A	C	.	A	A	.	T	C	.	A	.	T	.	T	C	.	A	G	.	C	T	.		
Di wAH7	.	.	.	G	G	A	T	.	.	C	G	C	A	T	T	C	A	C	.	A	A	.	T	C	.	A	.	T	.	T	C	.	A	G	.	C	T	.		
My spH1	C	T	C	T	T	T	.	T	.	G	C	.	G	G	.	C	C	T	.	T	T	A	T	T	G	.	C	A	.	G	.	T	.	A	.	T	.	.	.	
My spH2	C	T	C	T	T	T	.	T	.	G	C	.	G	G	.	C	C	T	.	T	T	A	T	T	G	.	C	A	.	G	.	T	.	A	.	T	.	.	.	

	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
	5	6	6	6	6	7	7	7	7	8	8	9	9	9	9	0	0	0	1	1	1	2	2	2	2	3	4	4	4	5	5	6	6	6	7	7	7	8	8	8
	7	0	3	7	9	0	2	5	8	4	7	0	6	7	9	2	5	8	1	4	7	0	3	6	9	5	1	4	7	0	9	0	2	5	8	1	4	7	3	6
Be fiH1	A	C	G	C	T	C	A	T	A	C	A	C	C	G	C	C	A	A	C	C	C	C	C	G	C	T	A	C	C	A	A	C	T	A	A	A	G	C	C	T
Be fiH2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Be fiH3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Be fiH4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Di ThH1	.	A	.	A	C	A	G	C	G	T	.	A	A	T	.	T	G	.	T	.	T	T	A	.	.	.	T	G	G	.	C	T	C	T	C	.	.	C		
Di ThH2	.	A	.	A	C	A	G	C	G	T	.	A	A	T	.	T	G	.	T	.	T	T	A	.	.	.	T	G	G	.	C	T	C	T	C	.	.	C		
Di gAH1	G	T	A	A	C	A	G	C	.	T	G	.	T	A	.	G	C	G	.	T	.	A	.	.	T	C	.	.	G	.	.	C	T	C	.	.	T	C	.	
Di gAH2	G	T	A	A	C	A	G	C	.	T	G	.	T	A	.	C	G	.	T	.	A	.	.	T	C	.	.	G	.	.	C	C	C	.	.	T	C	.		
Di gAH3	G	T	A	A	C	A	G	C	.	T	G	.	T	A	.	C	G	.	T	.	A	.	.	T	C	.	.	G	.	.	C	C	C	.	.	T	C	.		
Di gAH4	G	T	A	A	C	A	G	C	.	T	G	.	A	A	.	C	G	.	T	.	A	.	.	T	C	.	.	G	.	.	C	T	C	.	.	T	C	.		
Di gAH5	G	T	A	A	C	A	G	C	.	T	G	.	T	A	.	C	G	.	T	.	A	.	.	T	C	.	.	G	.	.	C	T	C	.	.	T	C	.		
Di wAH1	.	.	A	C	A	G	.	G	T	G	T	A	A	.	C	G	.	T	.	.	.	.	T	C	.	.	.	.	T	A	T	C	T	C	.	.	C			
Di wAH2	.	.	A	C	A	G	.	G	T	G	T	A	A	.	C	G	.	T	.	.	.	.	T	C	.	.	.	.	T	A	T	C	T	C	.	.	C			
Di wAH3	.	.	A	C	A	G	.	G	T	G	T	A	A	.	C	G	.	T	.	.	.	.	T	C	.	.	.	.	T	A	T	C	T	C	.	.	C			
Di wAH4	.	.	A	C	A	G	.	G	T	G	T	A	A	.	C	G	.	T	.	.	.	.	T	C	.	.	.	.	T	A	T	C	T	C	.	.	C			
Di wAH5	.	.	A	C	A	G	.	G	T	G	T	A	A	.	C	G	.	T	.	.	.	.	T	C	.	.	.	.	T	A	T	C	T	C	.	.	C			
Di wAH6	.	.	A	C	A	G	.	G	T	G	T	A	A	.	C	G	.	T	.	.	.	.	T	C	.	.	.	.	T	A	T	C	T	C	.	.	C			
Di wAH7	.	.	A	C	A	G	.	G	T	G	T	A	A	.	C	G	.	T	.	.	.	.	T	C	.	.	.	.	T	A	T	C	T	C	.	.	C			
My spH1	.	A	.	A	C	A	.	C	.	T	.	A	A	.	T	T	.	A	G	A	T	A	A	C	.	T	.	.	.	C	T	T	A	T	T	.	.			
My spH2	.	A	.	A	C	A	.	C	.	T	.	G	A	.	T	T	.	A	G	A	T	A	A	C	.	T	.	.	.	C	T	T	A	T	T	.	.			



**Figure 5.8.** Alignment of partial DNA sequences of the mitochondrial COI gene of selected teleost fishes (only variable sites are reported)

### 5.3.4. 16S rRNA analysis

Mitochondrial partial 16S rRNA sequences that included both forward and reverse of about 232 sequences from 24 species of five families were used in the phylogenetic analysis. The selected families included Serranidae (n=3), Priacanthidae (n=6), Gempylidae (n=4), Synodontidae (n=6) and Myctophidae (n=7). The amplified sequence lengths varied from species to species and among families but were consistent within species. A total of 36 haplotypes were

observed across the taxa. The minimum number of haplotypes observed in within each species (n=5) was one (*Chelidoperca occipitalis*, *Neoepinnula orientalis*, *Benthoosema fibulatum*) and maximum was observed four (*Diaphus garmani*). The shortest sequence was observed in *Chelidoperca investigatoris* (608 bp) and the longest sequences in *Promethichthys prometheus* (624 bp). The sequences were aligned using BioEdit and the multiple alignments resulted in consensus length of 608 sites including base pairs and gaps. These includes 299 Conserved/Constant (C), 257 Variable/Polymorphic (V), 236 Parsimony Informative (Pi) and 21 singleton (S) sites. The polymorphic sites for 16S rRNA are illustrated in figure 5.14. A total of 36 haplotypes were observed. The analysis revealed the average nucleotide frequencies (%) as Thymine (T) = 21.4, Cytosine (C) = 26.1, Adenine (A) = 28.9, and Guanine (G) = 23.6. As expected the average transitional pairs (si = 46) were more frequent than transversional pairs (sv = 38) with an average ratio of R = 1.23.

The mean pair-wise genetic distance values (Kimura 2-parameter/K2P) estimated based on 16S rRNA using MEGA Version 5 (Tamura *et al.*, 2011) are given in Appendix X. The average genetic distance (K2P) of individuals among deep-sea fish species under this study was 0.167 (16.7%). The minimum K2P distance between species estimated was 0.020 (2.0%) (ie. between *Saurida undosquamis* and *Saurida* sp. A) observed in the family Synodontidae while maximum intergeneric divergence estimated was 0.25 (25%) observed between *Saurida undosquamis* and *Myctophum* sp. A. The minimum intraspecific genetic distance (K2P) of 0.002 (0.02%) in *Myctophum spinosum* and maximum was 0.011 (1.1%) in *Diaphus garmani* were reported within species among haplotypes in the family Myctophidae. The pair-wise genetic divergence values of the haplotypes (intergeneric and interspecies) within the various deep-sea fish families are given in Table 5.8 to 5.12

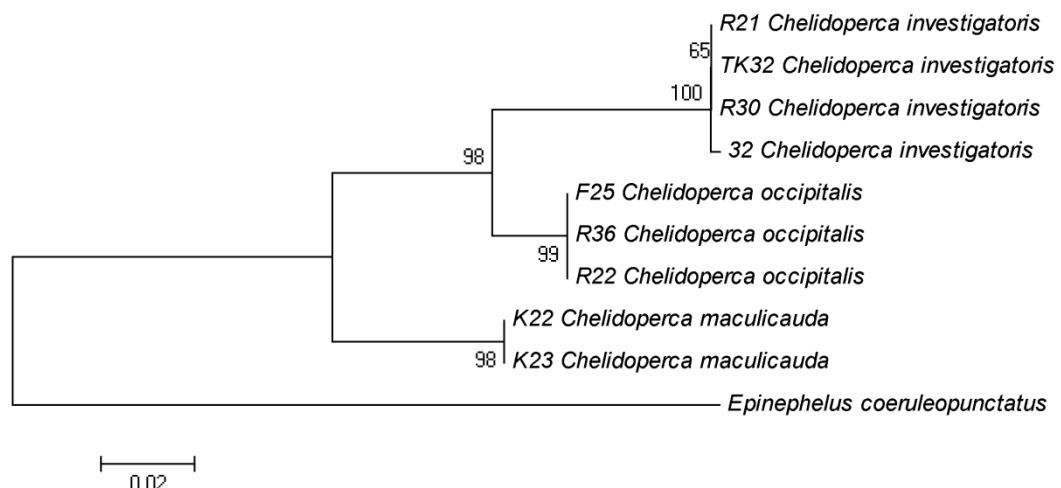
### **Serranidae**

Three species of serranids under the genus *Chelidoperca*: *Chelidoperca occipitalis*, *Chelidoperca maculicauda* and *Chelidoperca investigatoris* were examined. The amplified sequence length varied from 512 bp in *Chelidoperca occipitalis* to 516 bp in *Chelidoperca investigatoris*. Multiple aligned sequences resulted in a total length of 511 bp sites including base pairs and gaps that included 454 Conserved, 46 Variable, 57 Parsimony Informative and one Singleton sites. A

total of four haplotypes, one each from *Chelidoperca occipitalis* and *Chelidoperca maculicauda* and two from *Chelidoperca investigatoris* were observed. The analysis revealed the average nucleotide frequencies (%) as T = 24.8, C = 23.4, A = 29.5, and G = 22.4. The average transitional pairs (si = 19) were more frequent than transversional pairs (sv = 8) with an average ratio of R = 2.38.

The overall mean distance of individuals among the family was estimated as 0.062 (6.2%). The maximum interspecific K2P distance was 0.118 (11.8%) between *Chelidoperca investigatoris* and *Chelidoperca maculicauda* and minimum was 0.062 (6.2%) divergence between *Chelidoperca investigatoris* and *Chelidoperca occipitalis*. The minimum intraspecies distance observed was 0.0% in both *Chelidoperca maculicauda* and *Chelidoperca occipitalis* while maximum intraspecies distance observed was 0.002 (0.2%) in *Chelidoperca investigatoris*. The pair-wise genetic divergence values of the haplotypes within the genus *Chelidoperca* are given in table 5.8.

Family-wise phylogenetic tree was constructed using NJ (Fig. 5.9) method including related species *Epinephelus coeruleopunctatus* as outgroup. All the three species of genus *Chelidoperca* were clustered in one group. But, there was distinct separation of *C. maculicauda* from *C. investigatoris* and *C. occipitalis* and these three species formed two sister lineages. All the nodes were supported by high bootstrap values ranging from 98 to 100%.



**Figure 5.9.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Serranidae inferred from DNA Sequences of mitochondrial gene 16S rRNA

**Table 5.8.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Serranidae based on 16S rRNA gene

	Ch inH1	Ch inH2	Ch ocH1	Ch maH1
Ch inH1				
Ch inH2	0.002			
Ch ocH1	0.064	0.062		
Ch maH1	0.120	0.117	0.092	

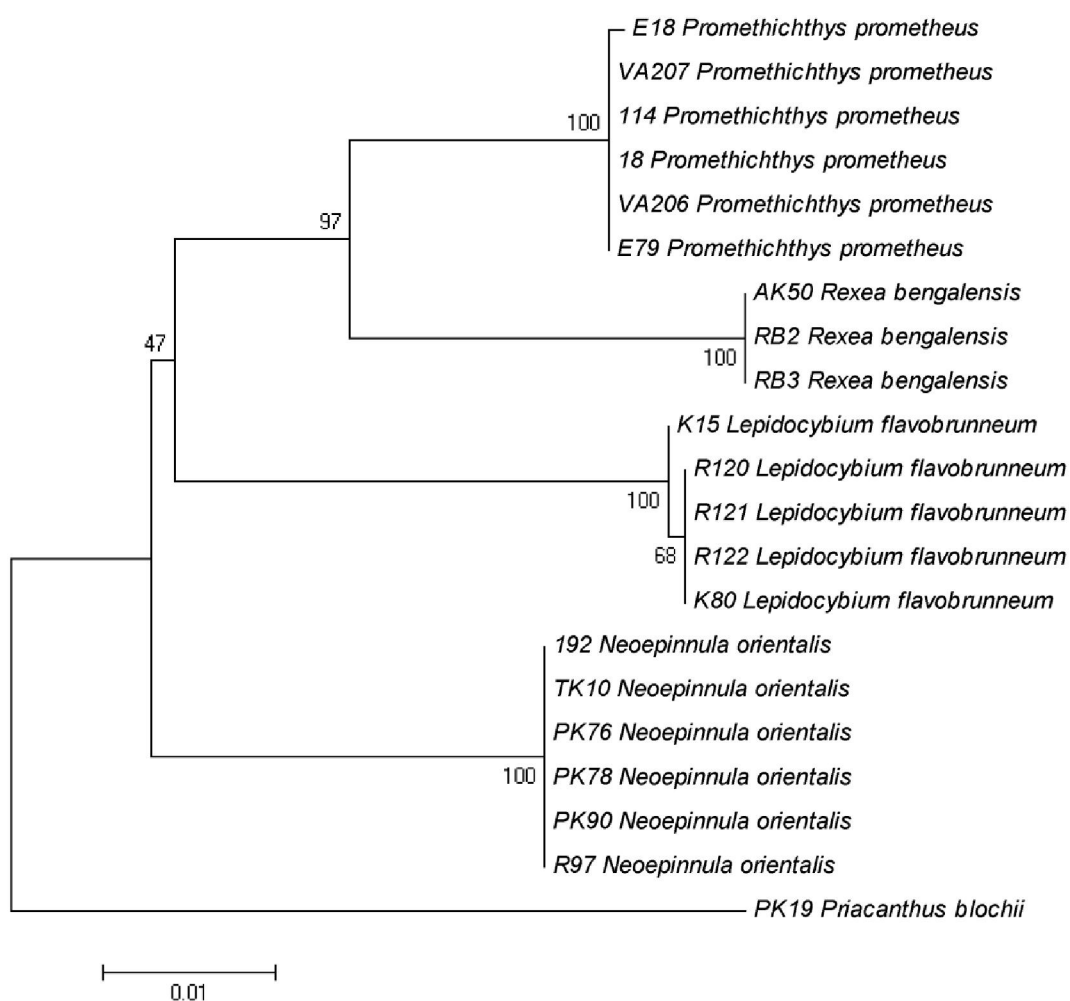
### Gempylidae

Four species of Gempylids, *Promethichthys prometheus*, *Neoepinnula orientalis*, *Rexea bengalensis* and *Lepidocybium flavobrunneum* were examined. The amplified sequence length varied from 583 bp in *Promethichthys prometheus* to 583 bp in *Lepidocybium flavobrunneum*. Multiple aligned sequences resulted in a total length of 583 bp sites including base pairs and gaps that included 483 Conserved, 105 Variable and 105 Parsimony Informative. A total of six haplotypes, one each from *Rexea bengalensis* and *Neoepinnula orientalis* and two each from *Promethichthys prometheus* and *Lepidocybium flavobrunneum* were observed. The analysis revealed the average nucleotide frequencies (%) as T = 23.6, C = 24.6, A = 28.6, and G = 23.2. The average transitional pairs (si = 28) were more frequent than transversional pairs (sv = 16) with an average ratio of R = 1.76.

The overall mean distance of individuals among the family was estimated as 0.037 (3.7%). The maximum interspecific K2P distance was 0.062 (6.2%) between *Lepidocybium flavobrunneum* and *Rexea bengalensis* and minimum was 0.037 (3.7%) divergence between *Promethichthys prometheus* and *Rexea bengalensis*. The minimum intraspecies distance observed was 0.0% in both *Rexea bengalensis* and *Neoepinnula orientalis* while maximum intraspecies distance observed was 0.002 (0.2%) in both *Promethichthys prometheus* and *Lepidocybium flavobrunneum*. The pair-wise genetic divergence values of the haplotypes within the family Gempylidae are given in table 5.9.

**Table 5.9.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Gempylidae based on 16S rRNA gene.

	Pr proH1	Pr proH2	Le flH1	Le flH2	Re beH1	Ne orH1
Pr proH1						
Pr proH2	0.002					
Le flH1	0.124	0.127				
Le flH2	0.122	0.124	0.002			
Re beH1	0.087	0.089	0.150	0.148		
Ne orH1	0.110	0.110	0.120	0.118	0.127	



**Figure 5.10.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Gempylidae inferred from DNA Sequences of mitochondrial gene 16S rRNA

### Myctophidae

Seven species of myctophids, *Benthoosema fibulatum*, *Benthoosema pterotum*, *Myctophum spinosum*, *Myctophum* sp A, *Diaphus garmani*, *Diaphus thiollierei* and *Diaphus* sp A were sequenced. The amplified sequence length varied from 530 bp in *Benthoosema pterotum* to 538 bp in *Myctophum spinosum*. Multiple aligned sequences resulted in a total length of 530 bp sites including base pairs and gaps that included 393 Conserved, 137 Variable, 128 Parsimony Informative and 9 Singleton sites. A total of 13 haplotypes, one each from *Benthoosema fibulatum*, *Diaphus* sp A, *Diaphus thiollierei* and *Myctophum* sp A. and maximum 4 from *Diaphus garmani* were observed. The analysis revealed the average nucleotide frequencies (%) as T = 20.8, C = 26.5, A = 29.1, and G = 23.6. The average transitional pairs (si = 32) were more frequent than transversional pairs (sv = 19) with an average ratio of R = 1.70.

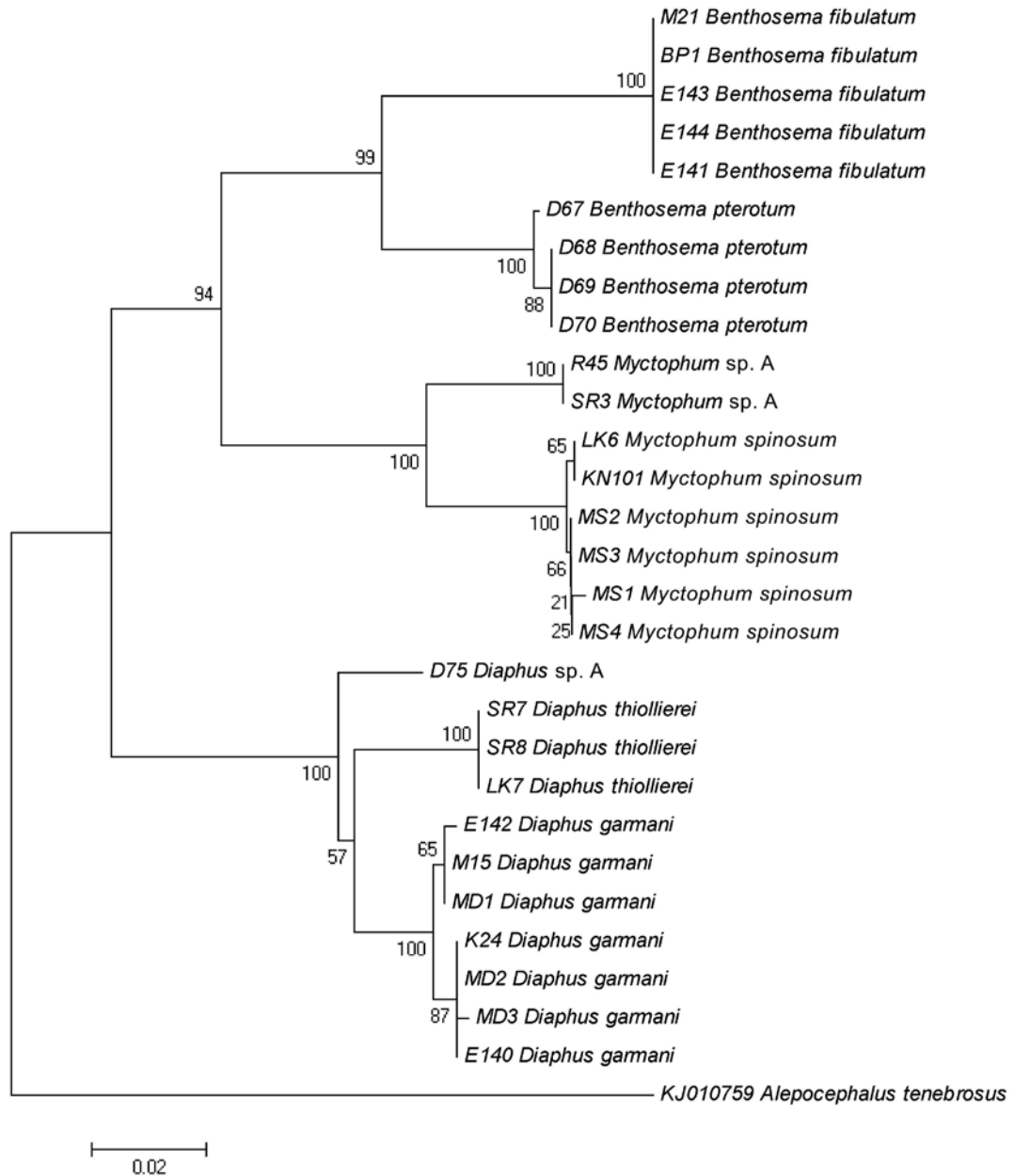


The overall mean distance of individuals among the family was estimated as 0.109 (10.9%). The maximum interspecific K2P distance was 0.168 (16.8%) between *Benthoosema fibulatum* and *Diaphus thiollierei* and minimum was 0.033 (3.3%) divergence between *Diaphus* sp A and *Diaphus garmani*. The minimum intraspecies distance observed was 0.0% in both *Benthoosema fibulatum* and *Diaphus thiollierei* while maximum intraspecies distance observed was 0.006 (0.6%) in both *Diaphus garmani*. The pair-wise genetic divergence values of the haplotypes within the family Myctophidae are given in table 5.10.

Family-wise phylogenetic tree was constructed using NJ (Fig. 5.11) method including *Alepocephalus tenebrosus* as outgroup. Two major groups were observed in the family Myctophidae. Group 1 was divided into two clades viz., one with *Benthoosema fibulatum* and *Benthoosema pterotum* forming two sub-clades and the other with two sub-clades containing *Myctophum spinosum* and *Myctophum* sp. A. Group 2 was separated into three sub-clades accommodating *Diaphus garmani*, *Diaphus thiollierei* and *Diaphus* sp. A. All the nodes were supported by high bootstrap values ranging from 94 to 100%.

**Table 5.10.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Myctophidae based on 16S rRNA gene

	Be ptH1	Be ptH2	Be fiH1	Di spH1	Di gaH1	Di gaH2	Di gaH3	Di gaH4	Dia spAH1	My spAH1	Yct neH1	Yct neH2	Yct neH3
Be ptH1													
Be ptH2	0.004												
Be fiH1	0.076	0.078											
Di spH1	0.142	0.147	0.167										
Di gaH1	0.144	0.144	0.161	0.041									
Di gaH2	0.144	0.144	0.162	0.043	0.002								
Di gaH3	0.144	0.144	0.161	0.041	0.008	0.010							
Di gaH4	0.141	0.141	0.159	0.039	0.006	0.008	0.002						
Dia spAH1	0.132	0.137	0.152	0.041	0.035	0.037	0.035	0.033					
My spAH1	0.120	0.125	0.137	0.138	0.144	0.147	0.144	0.142	0.140				
Yct neH1	0.132	0.134	0.138	0.141	0.150	0.150	0.150	0.147	0.143	0.050			
Yct neH2	0.129	0.132	0.136	0.138	0.147	0.147	0.147	0.145	0.140	0.048	0.002		
Yct neH3	0.132	0.134	0.138	0.138	0.147	0.148	0.147	0.145	0.140	0.050	0.004	0.002	



**Figure 5.11.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Myctophidae inferred from DNA Sequences of mitochondrial gene 16S rRNA

### Priacanthidae

Six species of priacanthids, *Priacanthus sagittarius*, *Priacanthus blochii*, *Priacanthus hamrur*, *Priacanthus prolixus*, *Pristigenys refulgens* and *Cookeolus japonicus* were sequenced. The amplified sequence length varied from 536 bp in *Priacanthus prolixus* to 542 bp in *Priacanthus blochii*. Multiple aligned sequences resulted in a total length of 536 bp sites including base pairs and gaps that included 433 conserved, 103 variable, 101 parsimony informative and 2 singleton sites. A total of 9 haplotypes, one each from *Priacanthus sagittarius*, *Priacanthus blochii*

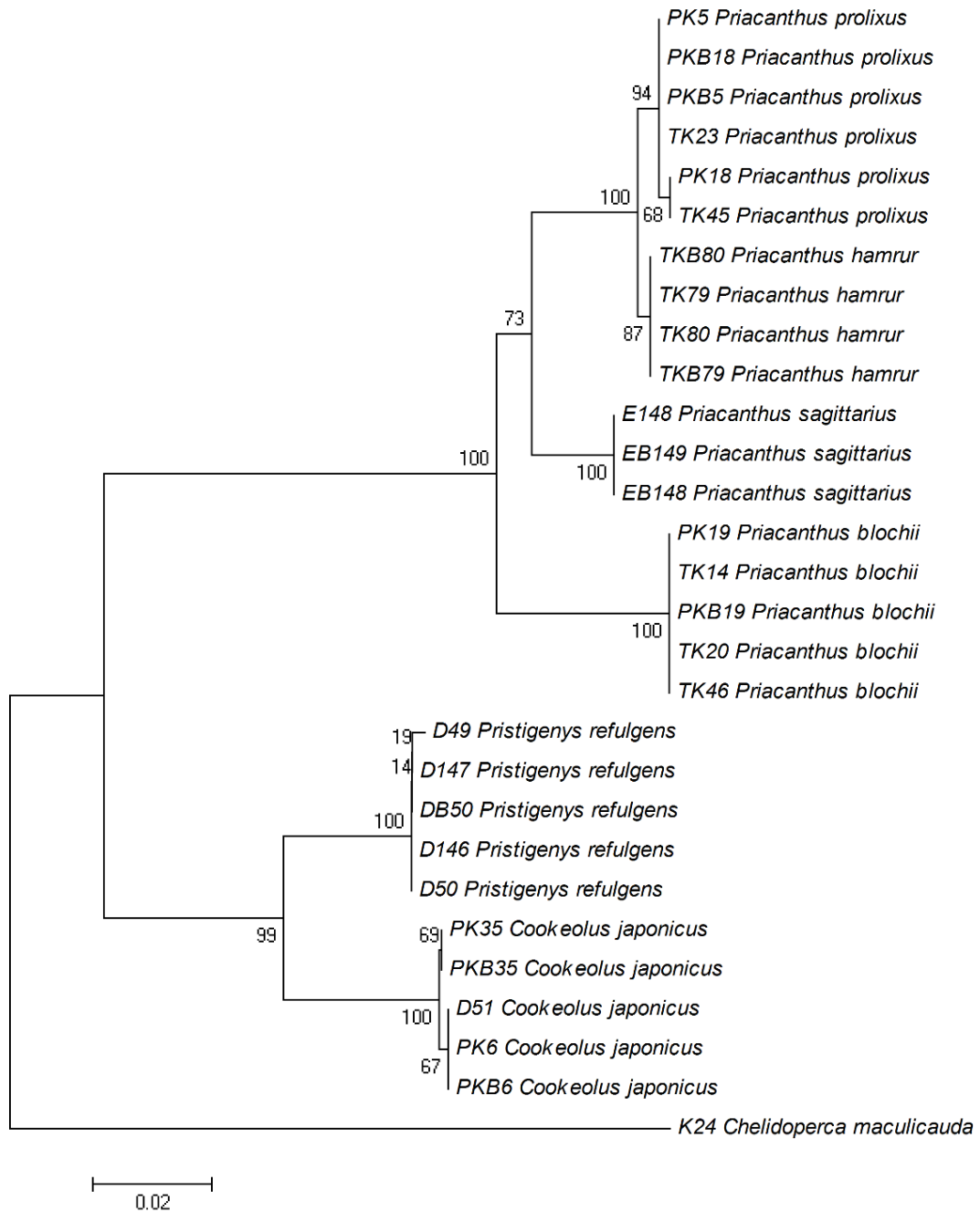
and *Priacanthus hamrur* and two each from *Priacanthus prolixus*, *Pristigenys refulgens* and *Cookeolus japonicus* were observed. The analysis revealed the average nucleotide frequencies (%) as T = 21.9, C = 25.7, A = 28.4, and G = 24.1. The average transitional pairs (si = 29) were more frequent than transversional pairs (sv = 14) with an average ratio of R = 2.08.

The overall mean distance of individuals among the family was estimated as 0.087 (8.7%). The maximum interspecific K2P distance was 0.154 (15.4%) between *Cookeolus japonicus* and *Priacanthus prolixus* and minimum was 0.006 (0.6%) divergence between *Priacanthus prolixus* and *Priacanthus hamrur*. The minimum intraspecies distance observed was 0.0% in *Priacanthus sagittarius*, *Priacanthus blochii* and *Priacanthus hamrur* while maximum intraspecies distance observed was 0.002 (0.2%) in *Priacanthus prolixus*. The pair-wise genetic divergence values of the haplotypes within the family Priacanthidae are given in table 5.11.

Family-wise phylogenetic tree was constructed using NJ (Fig. 5.12) method including *Chelidoperca maculicauda* as outgroup. Two major groups were observed in the family Priacanthidae. Group 1 was divided into three clades viz., *Priacanthus prolixus* and *Priacanthus hamrur* resolved as sister species in first sub-clade and the other two species *Priacanthus sagittarius* and *Priacanthus blochii* formed second sub-clade. Group 2 was separated with two sub-clades accommodating *Pristigenys refulgens* and *Cookeolus japonicus*. All the nodes were supported by high bootstrap values ranging from 99 to 100%.

**Table 5.11.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the family Priacanthidae based on 16S rRNA gene

	Pr reH1	Pr reH2	Co jaH1	Co jaH2	Pr saH1	Pr prH1	Pr prH2	Pr blH1	Pr haH1
Pr reH1									
Pr reH2	0.002								
Co jaH1	0.049	0.047							
Co jaH2	0.051	0.049	0.002						
Pr saH1	0.146	0.144	0.153	0.151					
Pr prH1	0.150	0.147	0.154	0.152	0.039				
Pr prH2	0.147	0.145	0.152	0.154	0.041	0.002			
Pr blH1	0.152	0.149	0.154	0.151	0.047	0.058	0.060		
Pr haH1	0.142	0.140	0.147	0.144	0.037	0.006	0.008	0.058	



**Figure 5.12.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to family Priacanthidae inferred from DNA Sequences of mitochondrial gene 16S rRNA

### Synodontidae

Six species of lizardfishes, *Saurida cf. micropectoralis*, *Saurida tumbil*, *Saurida longimanus*, *Saurida undosquamis*, *Saurida* sp. B and *Saurida* sp. A were examined. The amplified sequence length varied from 572 bp in *Saurida longimanus* to 578 bp in *Saurida undosquamis*. Multiple aligned sequences resulted in a total length of 572 bp sites including base pairs and gaps that included 494 Conserved, 78 Variable, and 78 Parsimony Informative sites. A total of 10 haplotypes, one each

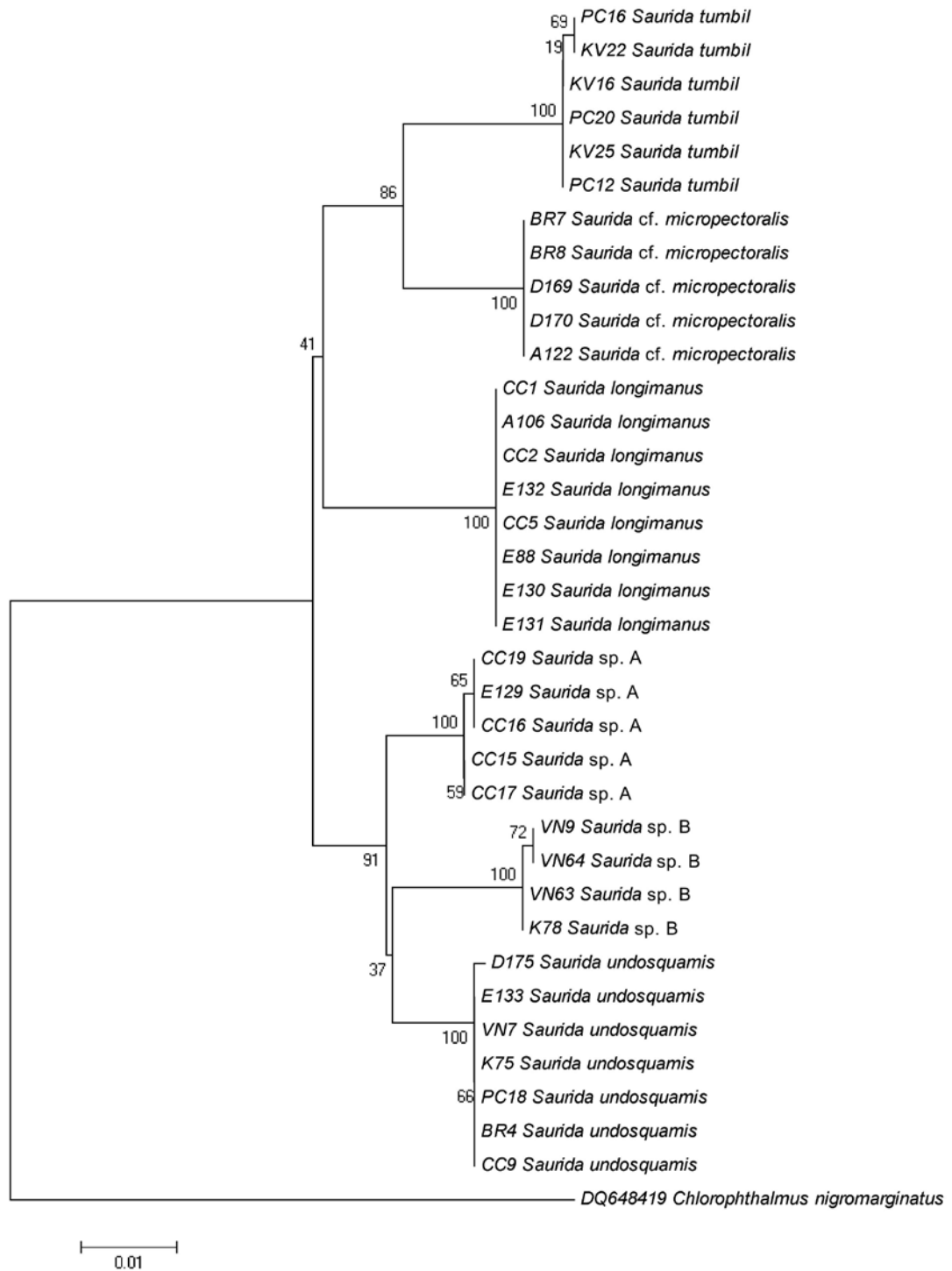
from *Saurida* cf. *micropectoralis*, *Saurida longimanus* and two each from *Saurida tumbil*, *Saurida undosquamis*, *Saurida* sp. B and *Saurida* sp. A were observed. The analysis revealed the average nucleotide frequencies (%) as T = 20.4, C = 28.3, A = 27.7, and G = 23.6. The average transitional pairs (si = 20) were more frequent than transversional pairs (sv = 8.0) with an average ratio of R = 2.37.

The overall mean distance of individuals among the family was estimated as 0.034 (3.4%). The maximum interspecific K2P distance was 0.051 (5.1%) between *Saurida tumbil* and *Saurida* sp. B and minimum was 0.017 (1.7%) divergence between *Saurida* sp. A and *Saurida undosquamis*. The minimum intraspecies distance observed was 0.0% in *Saurida longimanus* while maximum intraspecies distance observed was 0.001 (0.1%) in *Saurida* sp. B, *Saurida* sp. A, *Saurida undosquamis*. The pair-wise genetic divergence values of the haplotypes within the genus *Saurida* are given in Table 5.12.

Family-wise phylogenetic tree was constructed using NJ (Fig. 5.13) method including *Chlorophthalmus nigromarginatus* as outgroup. Two major groups were observed in the genus *Saurida*. Group 1 was divided into three clades viz., *Saurida tumbil* and *Saurida* cf. *micropectoralis* occupied as sister species in first sub-clade and *Saurida longimanus* occupied distantly in second sub-clade. Group 2 was separated with two sub-clades accommodating *Saurida* sp. A in first sub-clade and *Saurida* sp. B and *Saurida undosquamis* in second sub-clade. All the nodes were supported by high bootstrap values ranging from 86 to 91%.

**Table 5.12.** Pair-wise genetic distance (Kimura 2-parameter) among haplotypes of fishes belonging to the genus *Saurida* of the family Synodontidae based on 16S rRNA gene

	Sa unH1	Sa unH2	Sa cfmiH1	Sa loH1	Sa spAH1	Sa spAH2	Sa tuH1	Sa tuH2	Sa spBH1	Sa spBH2
Sa unH1										
Sa unH2	0.001									
Sa cfmiH1	0.033	0.032								
Sa loH1	0.034	0.036	0.039							
Sa spAH1	0.017	0.018	0.038	0.036						
Sa spAH2	0.018	0.019	0.039	0.037	0.001					
Sa tuH1	0.043	0.041	0.028	0.041	0.043	0.044				
Sa tuH2	0.044	0.043	0.030	0.042	0.044	0.045	0.001			
Sa spBH1	0.021	0.022	0.041	0.038	0.022	0.023	0.050	0.051		
Sa spBH2	0.022	0.023	0.042	0.039	0.023	0.024	0.049	0.050	0.001	



**Figure 5.13.** Neighbour joining (NJ) phylogenetic tree of fishes belonging to the genus *Saurida* of the family Synodontidae inferred from DNA Sequences of mitochondrial 16S rRNA gene.

5.22.1. CHELIDOPERCA

		1	1 1 1 1 1 1 1 1 2 2	2 2 2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 2
	2 3 4 5 6 8 8 9 0	1 4 5 5 5 8 9 9 0 0	0 0 1 1 1 1 1 1 1 2	2 2 2 2 2 3 3 3 3 4	
	4 0 0 6 9 3 0 1 3 9	0 9 0 1 2 1 2 8 1 4	5 7 0 3 4 6 7 8 9 0	2 3 5 7 8 2 6 7 9 0	
Ch inH1	C T A T - A C A T C	T A T G T - A C T C	A T C C A T T T T T	A T G C C C A T G T	
Ch inH2	. . . . .	. . . . .	. . . . .	. . . T . . . . .	
Ch oCH1	T . G C - . T C A .	. . C A C - . . . T	T . . T G . . A . .	G C . T G . . . . .	
Ch mAH1	T C G C A G T C G T	C G . A G C G T C T	. A A . - C A C C A	. G A T G A G C A C	
	2 2 2 2 2 2 2 2 3 3	3 3 3 3 3 3 3 3 4			
	4 4 5 5 5 5 9 9 0 0	1 1 1 4 4 7 8 9 5			
	1 9 0 1 3 4 6 8 2 9	1 2 8 1 5 0 3 5 2			
Ch inH1	T T T C A T T T A A	T C T A T T A A A			
Ch inH2	. . . . .	. . . . .			
Ch oCH1	. C C T G . . . . G	C T C G G A . . G			
Ch mAH1	A . C T G C C A C .	C . C G C A C G G			

5.22.2. GEMPYLIDAE

		1 1 1 1	1 1 1 1 1 1 1 1 2 2	2 2 2 2 2 2 2 2 2 2
	1 1 2 4 4 4 5	7 7 8 8 8 9 0 0 1 3	3 4 6 7 7 7 7 9 1 1	1 2 2 2 3 3 3 3 4 4
	6 7 8 6 7 7 6 7 9 7	3 9 0 8 9 4 2 7 8 0	5 1 3 5 6 7 8 8 0 4	8 3 4 9 3 4 5 9 0 1
Pr prH1	A C C C G T C C A G	C T C G G T T T T T	T A G C T A C A A C	G T C T A C C T T A
Pr prH2	. . . . .	. . . . .	. . . T . . . . .	. . . . .
Le flH1	C T G . T . T T G .	T . T A . G A C C C	. . A . C C . G G -	. . . . . A C . G
Le flH2	C T G . T . T T G .	T . T A . G A C C C	. . A . C C . G G -	. . . . . A C . G
Re beH1	C . T T . . . . .	. . . . . A . .	A G . . . . A . . A	. . . C T T A C . .
Ne orH1	C . A . T A T T . A	. C T A A . . . . .	A . . A A T A . . .	A C T . G . A C C .
	2 2 2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 3	3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3 3 3
	4 4 4 4 5 5 6 6 6 6	7 7 7 7 7 7 8 8 8 0	0 0 1 1 1 1 1 1 2 2	3 3 3 3 3 4 4 4 4 4
	2 3 4 8 2 5 0 1 7 9	1 3 5 6 8 9 0 4 8 2	3 5 0 1 2 3 4 5 3 4	0 1 6 7 9 0 1 2 3 4
Pr prH1	A A T A A A C T G G	C A G C C C T T A C	C G A T A A A A G A	A T A T C T - - - -
Pr prH2	. . . . .	. . . . .	. . . . .	. . . . .
Le flH1	. . C G G G T A . A	. C . . T C C . A	T . G C . . G C A C	. . . C . T C T T
Le flH2	. . C G G G T A . A	. C . . T C C . A	T . . C . . G C A C	. . . C . T C T T
Re beH1	. . . G G G T A . .	A . A . T . . . . .	. A . . G T . . . .	C . G A T A - - - -
Ne orH1	T T . G . . T A A A	. . . T . . - . T G	. . . . . A C	G C . C . . T A - -
	3 3 3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 4 4 4	4 4 4 4 4 4 4 4 4 5	5 5 5 5 5 5
	4 4 4 4 4 5 5 5 5 5	5 6 6 7 8 8 8 0 0 0	0 1 2 2 3 3 4 7 7 2	2 3 3 4 4 6
	5 6 7 8 9 0 1 2 4 8	9 3 4 2 1 2 3 3 4 5	8 9 0 7 2 6 5 5 8 1	4 2 4 2 5 8
Pr prH1	- - - - - A C T T	T C T G A T T - - T	T A C T A A T T C T	T C T T G T
Pr prH2	. . . . .	. . . . .	. . . . .	. . . . .
Le flH1	T T T T T C T . C .	A G C . G C A A C .	A . . C G . C C A .	. T A A . A
Le flH2	T T T T T C T . C .	A G C . G C A A C .	A . . C G . C C A .	. T A A . A
Re beH1	- - - - - . T . .	A . . A . G . - - A	- C . C G . . C A C	C . G . A A
Ne orH1	- - - - - . C C	A . C A G . G - - .	A . A C G G . C A .	. . G . . A



5.22.3. MYCTOPHIDAE

	1 1 1 1 1 1										1 1 2 2 2 2 2 4 5 6										7 7 7 8 8 8 8 9 9 0										1 1 1 1 1 1 1 1 1 1											
	4 5 7 8 9 1 2 3 4 7										8 9 1 3 4 5 8 4 5 9										1 7 8 3 5 6 7 1 2 0										5 2 8 8 5 9 8 2 5 6											
Be pTH1	G	C	T	G	C	-	A	G	T	-	C	A	T	T	-	G	A	T	A	C	C	T	T	A	G	A	A	T	A	C	T	G	G	T	T	A	T	G	T	T		
Be pTH2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
Be fiH1	.	.	C	.	.	C	.	.	.	-	A	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	A	C	.	.	.	A	A	C	.	.		
Di spH1	C	G	.	.	C	C	C	.	C	.	T	.	.	-	T	C	C	G	.	.	C	.	.	G	.	C	T	A	.	.	C	.	G	.	A	C	.	.	A	C		
Di gAH1	.	.	.	.	C	.	.	C	.	.	T	.	.	-	.	C	C	G	.	.	C	.	.	G	.	C	T	A	.	.	C	C	G	.	.	A	C	.	.	A	C	
Di gAH2	.	.	.	.	C	.	.	C	.	.	T	.	.	-	.	C	C	C	.	.	C	.	.	G	.	C	T	A	.	.	C	C	G	.	.	A	C	.	.	A	C	
Di gAH3	.	.	.	.	C	.	.	C	.	.	T	.	.	-	.	C	C	G	.	.	C	.	.	G	.	C	T	A	.	.	C	C	G	C	.	A	C	.	.	A	C	
Di gAH4	.	.	.	.	C	.	.	C	.	.	T	.	.	-	.	C	C	G	.	.	C	.	.	G	.	C	T	A	.	.	C	C	G	.	.	A	C	.	.	A	C	
Di spAH1	.	G	.	T	G	C	.	A	C	.	T	.	.	-	.	C	.	G	.	.	C	.	.	G	.	C	T	A	.	C	.	C	C	G	.	.	A	C	.	.	A	C
My spAH1	.	.	.	.	C	.	.	-	.	.	T	.	A	.	T	A	.	G	A	A	.	C	T	.	G	G	.	T	.	A	A	C	.	.	.	A	A	.	.	A	A	
YCT neH1	.	.	.	.	C	.	.	-	.	.	A	-	A	A	-	A	.	.	A	A	C	C	T	A	G	G	.	T	.	A	.	C	.	.	.	A	.	.	.	A	.	
YCT neH2	.	.	.	.	C	.	.	-	.	.	A	-	A	A	-	A	.	.	A	A	C	C	T	A	G	G	.	T	.	A	.	C	.	.	.	A	.	.	.	A	.	
YCT neH3	.	.	.	.	C	.	.	-	.	.	A	-	A	A	-	A	.	.	A	A	C	C	T	A	G	G	.	T	.	A	.	C	.	.	.	A	.	.	.	A	.	

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	8 8 8 2 2 2 3 3 3 3										3 3 3 3 3 4 4 4 4 5										5 6 6 6 6 7 7 7 7 8										8 8 9 9 9 9 9 9 9 0									
	7 8 9 0 4 9 0 1 2 4										5 6 7 8 9 0 5 7 9 0										5 0 1 7 8 5 6 7 8 3										4 5 0 1 3 5 6 8 9 0									
Be pTH1	A	C	T	A	C	A	G	T	T	A	A	C	C	C	A	T	T	A	T	A	-	-	-	C	T	A	G	A	A	A	A	T	A	A	C	T	A	T	G	A
Be pTH2	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Be fiH1	G	.	T	.	.	C	.	.	.	.	C	T	.	A	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Di spH1	.	.	.	G	A	C	-	G	.	.	.	.	.	C	.	A	-	.	.	C	T	C	T	A	.	A	T	G	G	.	A	.	T	C	G	C	.	.	.	.
Di gAH1	G	.	C	G	.	G	A	C	-	G	.	.	.	C	C	C	A	-	.	A	T	C	T	A	.	A	T	G	.	.	A	G	.	T	C	G	C	.	.	.
Di gAH2	G	.	C	G	.	G	A	C	-	G	.	.	.	C	C	C	A	-	.	A	T	C	T	A	.	A	T	G	.	.	A	G	.	T	C	G	C	.	.	.
Di gAH3	G	.	C	G	.	G	A	C	-	G	.	.	.	C	C	C	A	-	.	A	T	C	T	A	.	A	T	G	.	.	A	.	T	C	G	C	.	.	.	.
Di gAH4	G	.	C	G	.	G	A	C	-	G	.	.	.	C	C	C	A	-	.	A	T	C	T	A	.	A	T	G	.	.	A	.	T	C	G	C	.	.	.	.
Di spAH1	G	.	.	G	.	C	-	G	.	.	.	.	.	C	C	.	A	C	.	-	T	C	T	A	.	A	T	G	.	.	A	.	T	C	G	C	.	.	.	.
My spAH1	.	A	A	.	T	.	C	-	.	.	.	T	.	.	C	.	.	.	.	-	-	-	-	T	C	C	G	.	G	A	.	G	.	G	C	A	G			
YCT neH1	.	A	A	T	T	.	C	-	.	.	.	T	.	.	C	.	C	.	.	-	-	-	-	A	T	G	G	.	A	.	G	.	G	C	A	G				
YCT neH2	.	A	A	T	T	.	C	-	.	.	.	T	.	.	C	.	C	.	.	-	-	-	-	A	T	G	G	.	A	.	G	.	G	C	A	G				
YCT neH3	.	A	A	T	T	.	C	-	.	.	.	T	.	.	C	.	C	.	.	-	-	-	-	A	T	G	G	.	A	.	G	G	.	G	C	A	G			

	3 3 3 3 3 3 3 3 3 3										3 3 3 3 3 3 3 3 3 3										3 3 3 3 3 3 3 3 3 3										3 3 3 3 3 4 4 4 4 4									
	0 1 1 2 2 3 3 4 4 4										5 5 5 5 6 6 6 6 6 6										7 7 7 7 8 8 8 8 9 9										9 9 9 9 9 0 0 0 0 0									
	1 8 9 4 8 3 9 0 1 9										0 1 6 9 1 5 6 7 8 9										0 3 4 9 0 1 3 8 0 1										4 5 7 8 9 0 1 5 7 8									
Be pTH1	T	G	C	G	G	A	T	A	A	A	T	A	C	A	G	C	T	A	-	C	C	T	C	C	-	T	G	A	A	G	C	A	G	C	T	A	G	T	C	T
Be pTH2	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Be fiH1	C	A	T	.	A	.	C	.	G	G	C	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	A	T	.	T	A	A	.	C	.	
Di spH1	A	.	.	.	G	C	C	.	.	.	C	.	T	G	.	A	.	T	A	G	.	T	.	C	C	.	C	G	A	.	.	A	T	C	.	A	.	T	.	.
Di gAH1	A	.	.	.	G	C	C	.	.	.	C	G	.	.	A	.	T	A	G	.	T	.	C	C	.	C	T	.	.	G	A	T	C	.	A	.	T	.		
Di gAH2	A	.	.	.	G	C	C	.	.	.	C	G	.	.	A	.	T	A	G	.	T	.	C	C	.	C	T	.	.	G	A	T	C	.	A	.	T	.		
Di gAH3	A	.	.	.	G	C	C	.	.	.	C	G	.	.	A	.	T	A	G	.	T	.	C	C	.	C	T	.	.	G	A	T	C	.	A	.	T	.		
Di gAH4	A	.	.	.	G	C	C	.	.	.	C	G	.	.	A	.	T	A	G	.	T	.	C	C	.	C	T	.	.	G	A	T	C	.	A	.	T	.		
Di spAH1	A	.	.	.	C	C	.	.	.	.	C	.	.	.	T	A	.	C	A	G	.	T	.	C	C	.	C	.	.	.	A	T	C	.	A	.	T	.	.	
My spAH1	C	.	.	A	.	G	.	.	.	.	C	.	G	.	.	C	-	A	G	C	T	.	A	.	A	.	.	.	T	.	A	T	.	A	.	.	.	.		
YCT neH1	.	.	.	A	.	C	.	.	.	.	C	.	G	.	.	C	-	A	G	C	T	T	A	.	.	.	.	.	T	.	A	T	C	.	A	A	.	.		
YCT neH2	.	.	.	A	.	C	.	.	.	.	C	.	G	.	.	C	-	A	G	C	T	T	A	.	.	.	.	.	T	.	A	T	C	.	A	A	.	.		
YCT neH3	.	.	.	A	.	C	.	.	.	.	C	.	G	.	.	C	-	A	G	C	T	T	A	.	.	.	.	.	T	.	A	T	C	.	A	A	.	.		



### 5.3.5 Intraspecific divergence in COI and possible cryptic taxa

***Acropoma japonicum***. Three sequences of the species from GenBank were analysed with Indian samples of *Acropoma japonicum*. Genetic divergence (K2P) among individuals of *Acropoma japonicum* from three wide geographic area such as Japan, South Africa and India, was very high value between 3.3 to 5.8%, indicating the possible presence of sibling species or synonymised species and geographically subdivided populations. The conspecific variations range within the lineages was 0.10% to 0.30% observed (Appendix IX).

***Cyttopsis rosea***. There are two valid species of *Cyttopsis* under the family Parazenidae. The analysis included four sequences of *Cyttopsis rosea* generated in the present study and one *C. cypho* sequence mined from GenBank. Genetic divergence (K2P) among individuals of *Cyttopsis rosea* from India (present study) and Portugal exhibit extensive divergence (4.4%) forming subclusters in the NJ tree. However, relatively little conspecific variation (0.2%) within lineages was observed.

***Promethichthys prometheus***. Similarly the *Promethichthys prometheus* show the highest overall mean distance (3.33%, Appendix IX) and that form three highly divergent lineages. One lineage (n=7) found off Kollam (Arabian Sea) was similar to one sequence from Japan (AP012504) and second lineage (n=10) collected from South Indian Sea. A third lineage (n=1) from Mid Atlantic bight is separated from other two lineage with high divergence of 5.5% and 4.6% for first and second lineage respectively.

***Neopinnula orientalis***. This species analysis represented by 11 samples from two localities, one from Arabian Sea (off Kollam) and other from Tugela Banks, South Africa. The specimens from both localities forms two distinct lineages with high intraspecific divergence value of 3.6%. There was a little (0.1%, Kollam) or no (Tugela deep) variation observed within lineages.

***Lepidocybium flavobrunneum***. *Lepidocybium flavobrunneum* was represented by 18 specimens that formed two distinct lineages with an overall mean distance of 1.5%. The first lineage formed by Indian and South African specimens and the second lineage was formed by samples from Brazil. These two lineages are separated by 2.7% divergence value.

***Gephyroberyx darwinii***. *Gephyroberyx darwinii* was represented by 14 specimens forming three lineages with an overall mean distance 1.5%. One lineage (n=6) was found off Kollam (Arabian Sea) and second lineage (n=3) off Tugela deep (South Africa). There was a little (0.1%, Kollam) or no (Tugela deep) variation within lineages, yet 1.1% divergence between them. A third lineage (n=5) from Tasman Sea (Australia) with 0.6% variation within lineage is separated by about 2.2% from other two lineages.

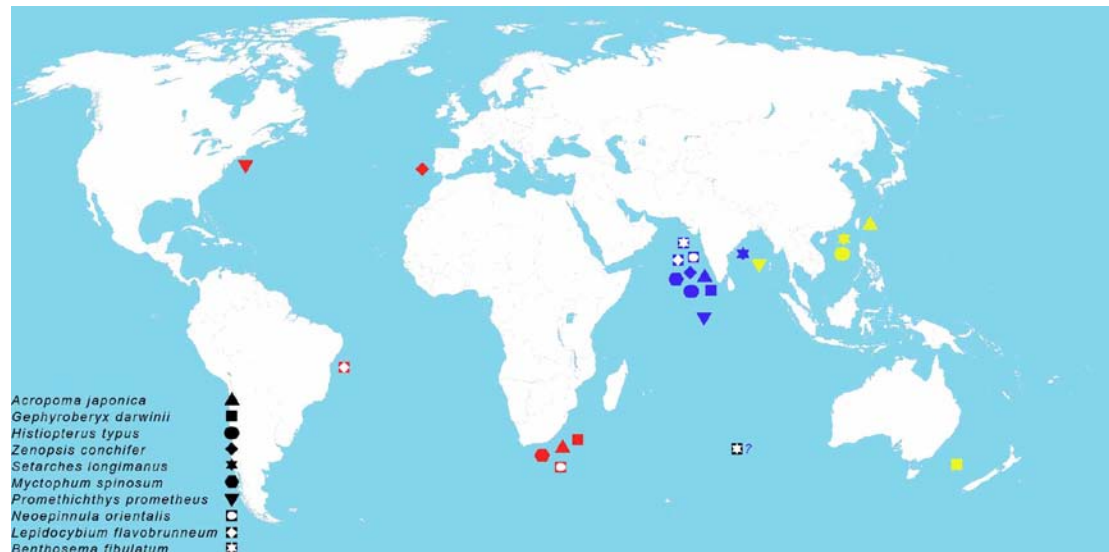
***Histioporus typus***. *Histioporus typus* was represented by 11 specimens forming two lineages. One lineage (n=9) was found off Kollam and Durban, South Africa. The second lineage was found off South China Sea. There was again little (0.1%, South Africa and 0.4%, South China Sea) variation within lineages, but 4.8% divergence between Indian and South China Sea lineages.

***Zenopsis conchifer***. Similarly *Histioporus typus* was also represented by 11 specimens forming two lineages. One lineage (n=7) formed by Indian samples and another one by samples from Portugal. There was an again very low intraspecific variation within the lineages (0.17%, India; 0.34%, Portugal) but high divergence of 4.15% between them.

***Setarches longimanus***. Two species of *Setarches* were identified, *Setarches longimanus* and *S. guentheri*. *Setarches longimanus* represented by 6 specimens, four from southwest India and two from South China Sea, with little (0.17%, India) or no (South China Sea) variation within geographical localities. However, slightly high divergence value (3.21%) was observed between the lineages.

***Myctophum spinosum***. *Myctophum spinosum* was represented by six samples from India and 3 samples from South Africa formed two distinct lineages. The overall mean distance calculated was 2.0%. The observed within lineage divergence was very low (0.12%, India and 0.24, South Africa), but 3.9% variation between them.

***Benthosema fibulatum***. *Benthosema fibulatum* was represented by 8 specimens collected off Kollam and three samples from GenBank formed three distinct lineages with high overall mean distance value of 3.39%. The sequence AP012253 and KJ555324 forms the second lineage was vary greatly (4.6%) with the sequence KJ555325. Again this lineage and Indian lineage show very high (8.1%) divergence value between them.



**Figure 5.15.** Map showing the distribution of cryptic species in the world oceans

#### 5.4. Discussion

Regardless of the type of biological study, reliable knowledge of the taxonomy and identification of the organisms under scrutiny forms a fundamental basis for all other kinds of knowledge produced (Bickford *et al.*, 2007; Bortolus, 2008). However the accurate identifications of deep-sea fishes can take a long time to determine depending on the taxonomic group and most of the deep-sea fish families still contain many undescribed or ill-defined species (Prokofiev and Kukuev, 2007; Gomon *et al.*, 2014). Deep-sea fishes are considered as one of the least explored groups of fishes with many taxonomic ambiguities in most of the families. Morphology based taxonomy is seen to be problematic in many of the deep-sea fish families and genetic tools have great potential to resolve the taxonomic status and find out the accurate fish diversity (Gomon *et al.*, 2014; Zahuranec *et al.*, 2012). Identification and taxonomy based on molecular data have been around for almost as long as the multiple molecular methods that supports them (Teletchea, 2009). Recently, many large-scale projects like the Barcode of life international project (Hebert *et al.*, 2003a) and the the Barcode of life Database BOLD (Ratnasingham and Hebert, 2007) started with stringent quality control and is one of the largest with a focus on taxonomy. Morphological identification of species is the traditional approach in fishes and the use of modern tools such as DNA markers for species identification make it more concrete (Hebert *et al.*, 2003a; 2004b; Ilves and Taylor, 2009). Species identification by using DNA barcoding is based upon the

principle that interspecific divergence sufficiently outcores intraspecific divergence and the biological species can be clearly demarcated by a threshold value (Hebert *et al.*, 2003a). However, despite extensive generation and application of DNA barcoding in species identification throughout the last decade, no universal standard threshold has been defined for interspecies differentiation (Chakraborty and Ghosh, 2014). The present study represents the first molecular survey of deep-sea fish diversity using COI and 16S rRNA data from the southern coast of India. This includes generation of COI barcodes for 82 species and confirmed eight new species based on morphology and molecular data.

#### 5.4.1. Cryptic taxa

Recent advances in molecular technique have discovered cryptic species, morphologically indistinguishable but genetically distinct species, in many habitats. Cryptic species have been found in various genera of fishes living in the different habitat and more recently found in the lanternfish genus *Benthoosema*, that are found on the mesopelagic area (Zahuranec *et al.*, 2012). Addition to that, DNA barcoding studies in the family Carangidae have successfully identified the cryptic species diversity within single known species (Jaafar *et al.*, 2012). The deep divergences within the valid species observed in many instances during barcoding can be due to species misidentification, synonymised species or cryptic or unrecognised species (Ward *et al.*, 2008a; Ward *et al.*, 2009). Hebert *et al.*, (2004a) proposed the '10x rule' for flagging possible cryptic species if they diverge by 10 times or more the average intraspecific variability in the group. In addition to this threshold value, Ward *et al.*, (2009) showed that 2% level distance or more can be used as an indicator for presence of unrecognised or cryptic species. However, both these threshold value is not applicable for some instance of barcoding due to very low or no COI divergence (Victor and Randall, 2014). We identified 11 possible cryptic species during the present study. On examination of genetic distance data for all these species, *Lepidocybium flavobrunneum* show the least average divergence of 1.5% (Appendix IX). This average value is much more if we use either 10x or 2% rule suggests that all these 9 species contains many provisional or unrecognised species. *Acropoma japonicum* has three genetic lineages across Arabian Sea, Taiwan and South Africa that all separated at divergence of >3%. The Indian and Taiwan lineage separated by about 3.5% and lineage between South Africa and Taiwan

diverge by 5.5%. This high threshold support presence of provisional species status for all three lineages of *A. japonicum*. *A. splendens*, Lloyd, 1909 described from Gulf of Oman is currently a synonym of *Acropoma japonicum*. Also some additional new species of *Acropoma* species is described very recently. To confirm the species status of these three provisional species, examination of voucher specimens is required and compared with closely related recently described species. However, the present study and retrieved sequence number per species was relatively limited, and it is likely that more cryptic species in the deep-sea fishes is yet to be discovered.

### **Serranidae**

This study provides a comprehensive molecular evidence for the species identification of *Chelidoperca* from Indian waters. The phylogenetic tree derived from two mitochondrial genes (COI and 16S) are identical and strongly suggest that these *Chelidoperca* fishes are divided into three species (*Chelidoperca investigatoris*, *C. occipitalis* and *C. maculicauda*). These three groups are not sharing any haplotypes based on both 16S rRNA and COI genes. In all the three species the intraspecies variation was low and this may be due to the low number of haplotypes. The observed transition versus transversion ratios in *Chelidoperca* is also comparable to those of many serranid fishes (Craig and Hastings, 2007). The GC content of 511 bp 16S rRNA (45.7%) and 675 bp COI (46.2%) was comparatively high in all of these fishes. The barcode sequences based on partial sequence information of COI gene has been widely used in species identification and validation of species identity (Ward *et al.*, 2005; Lakra *et al.*, 2009). Our result revealed that COI barcoding is an effective marker for molecular identification of *Chelidoperca* fishes from Indian waters.

### **Priacanthidae**

Our study provides the molecular evidence based on two mitochondrial genes (COI and 16S rRNA) for species identification of the family Priacanthidae. The six species of priacanthids from the Indian coast were found to be genetically distinct from each other and portioned into three groups without any haplotypes sharing. The high degree of K2P nucleotide divergence with 16S rRNA gene (interspecies 0.049-0.158; intergeneric 0.008-0.157), indicating its ability to adequately describe interrelationship of priacanthids species. The sequence analysis

of the 16S rRNA gene lacked the ability to resolve relationships in some marine taxa such as sparids and percoid fishes (Orrell and Carpenter, 2004). However, Lakra *et al.*, (2009) reported high nucleotide divergence among the sciaenids species in the Indian waters using 16S rRNA gene sequences and Iwatsuki (2013) showed similar results in *Acanthopagrus latus* complex, indicating the effectiveness of 16S rRNA gene sequence for accurate identification of species. The barcode sequences based on partial sequence information of COI gene has been widely used in species identification and validation of species identity (Ward *et al.*, 2005; Spies *et al.*, 2006; Lakra *et al.*, 2009, Lakra *et al.*, 2011; Ebert *et al.*, 2010). Our result revealed that COI barcoding is an effective marker for molecular identification of priacanthids from Indian waters.

*Priacanthus prolixus* is closely related to *P. arenatus* Cuvier, 1892, *P. hamrur* (Forsskal, 1775) and *P. meeki* Jenkins, 1904, the group sharing crescentic caudal fin, higher counts of dorsal-fin and anal fins rays (Starnes, 1988). The fresh body colour was similar in the above three species except that *P. prolixus* has a reddish-yellow pectoral fin (Motomura *et al.*, 2001). *P. prolixus* is very similar to *P. hamrur*, hence misidentification is evident from GenBank and BOLD database. The fish called *P. hamrur* collected from India (Eight sequences: EF609574-EF609577, KJ000235, KF830276, FJ265856 and FJ265857) should be *P. prolixus* for the value of the genetic distance within intraspecies ( $D=0.3\%$ ). The sequence FJ265856 shows 4.6% average divergence with other *P. prolixus* sequences which may be due to sequence errors or other species of *Priacanthus*. For the lack of voucher specimen, we could not decide on any species name. During the collection period, we observed that *P. prolixus* is moderately abundant in catches along with *P. hamrur* and *blochii* along the southwest coast of India. *Priacanthus prolixus* Starnes, 1988 originally described on the basis of 12 samples from the Arabian Sea (Off Somalia) is endemic to that area (Starnes, 1988). Later, colour description was given based on the colour photographs of freshly collected materials from Karnataka and Kerala (Motomura *et al.*, 2001). However, our collections of these species from Tuticorin, Chennai and Kolkata shows that *P. prolixus* is not endemic species for Arabian Sea but are widely distributed in the Indian Ocean including the Bay of Bengal. However, the *P. arenatus* sequences (JX124872, GU702522, JX124871 and GU702524) from Brazil cluster with *P. prolixus* sequences from India with mean interspecies distance of



0.9%. Therefore, *P. arenatus* may be senior synonym for *P. prolixus*. *P. arenatus* is very similar to *P. hamrur* and *P. prolixus* of the Indo-Pacific and *P. meeki* of the Hawaiian Pacific region, with difference only in meristic counts or morphometry (Starnes, 1988). However, Caldwell (1962) stated that *P. arenatus* might be synonymous with Indo-Pacific forms. Our sequence comparisons of COI genes propose that *P. arenatus* should be a senior synonym for *P. prolixus*. Therefore, there is a need to conduct further taxonomic inquiry for these two species whose statuses are found to be doubtful.

*Pristigenys refulgens* (Valenciennes 1862) described from Seychelles Islands was considered as junior synonym of *P. nipponia* (Cuvier 1829). However, more recently acquired materials have facilitated a reinvestigation, with a redescription and designation of a neotype for *Pristigenys refulgens* (Iwatsuki *et al.*, 2012). Nair and Geetha (2006) had recorded *Pristigenys nipponia* from Indian waters. This Indian report may be the possible misidentification of *P. refulgens* which is widely distributed in the Indian Ocean and Western Pacific (Iwatsuki *et al.*, 2012). The sequence of *P. nipponia* (JQ681466) from South China Sea shows 3.3% divergence with our sequence of *P. refulgens* from India. Our results based on the partial sequences of COI genes firstly supply the molecular evidence that *P. refulgens* should be a valid distinct species from *P. nipponia*. This is consistent with Iwatsuki *et al.*, (2012) on the morphological characteristics.

The family Priacanthidae separated into two major clades. The first clade included the genus *Priacanthus* and the second clade included the remaining three genera *Heteropriacanthus*, *Pristigenys* and *Cookeolus*. Yet, for *Heteropriacanthus cruentatus*, sequence grouped in two very different clades, one including all the samples from the Society Islands (French Polynesia), and the samples from the Belize. Bootstrap support was high >90% for all the relationship discussed above, except for the *Priacanthus* clades, where support was low. The distance between *P. prolixus* and *P. hamrur* was 9.5% while the distance between the two *Heteropriacanthus cruentatus* clades was 11.8%, a difference that was not statically significant. These may comprise of either some previously synonymised species or latent species. *Priacanthus argenteus* and *Priacanthus bonariensis* are the two questionable synonyms of *H. cruentatus* described from Molucca Islands, Indonesia and Buenos Aires, Argentina respectively. The fish called *Heteropriacanthus*

*cruentatus* (Three sequences: EU871696, EU871697 and JF952706) should be *Cookeolus japonicas* for the value of the genetic distance within intraspecies. Our study revealed that *P. prolixus* has often been misidentified as *P. hamrur*. Our results warn fisheries managers, ecologists, observers while dealing with the family priacanthidae about the possible cryptic species.

### **Myctophidae**

Lanternfishes, family Myctophidae comprise approximately 250 species that live in the mesopelagic habitat, usually between 200-1000 m depth. Myctophids are very abundant fishes and play an important role as a major link in the food web (Eastman, 1993). Most of the lanternfishes are fragile in nature and often in such poor condition of shape and loss of other identification marks which make their identification very difficult. Thus molecular approach is an important tool for their identification. The fishes of the family myctophidae are very difficult to identify morphologically and cryptic species are evident recently (Zahuranec *et al.*, 2012). PCR amplification was difficult compared to other deepsea fish families and though repeated for samples failed to get good amplification. This amplification failure was due to the presence of large quantities of lipids in the tissues, making DNA extractions difficult (Catul *et al.*, 2011) and are evident in recent studies of myctophids (Zahuranec *et al.*, 2012).

Our sampling contains relatively few numbers of species and low samples size. However, the generated datasets shows high divergence for both COI and 16S rRNA. For 16S rRNA, the sequence divergence among species was 10.9% and for COI was 15.2%. We observed that many species sequenced here shows very high genetic divergence with sequence in the GenBank and BOLD databases. This may indicate the presence of cryptic species in the family myctophidae as observed by Zahuranec *et al.*, (2012). Two species, *Myctophum* sp A and *Diaphus* sp A confirmed as new species based on the morphological analysis requires more samples to describe the species. Both COI and 16S shows as very good marker for species delineation in the family myctophidae based on the materials collected. To get the better understanding of genetic diversity and extent of cryptic diversity in the family myctophidae, wider geographical sampling is required, and additional nuclear markers should be sequenced along with mtDNA.

## Synodontidae

The genus *Saurida* represented the deep-sea fishes that significantly contribute the demersal fish catch compositions. There are eight species of *Saurida* listed from Indian waters (Kapoor *et al.*, 2002). However, the validity of *Saurida gracilis*, *Saurida isarankurai* and *Saurida wanieso* may be questioned. There has been considerable confusion about the genus *Saurida* in the family Synodontidae. The genus *Saurida* is in urgent need of revision and taxonomic validity of many species is still uncertain (Barry Russell pers. comm. 2013). Recent studies in the family Chlorophthalmidae using COI barcode region independently group specimens to natural species assemblages for verifying and correcting the species (Gomon *et al.*, 2014), however, till date DNA based studies were lacking in Indian waters. In the present study, six species of lizardfishes, *Saurida cf. micropectoralis*, *Saurida tumbil*, *Saurida longimanus*, *Saurida undosquamis*, *Saurida* sp. A and *Saurida* sp. B were examined. The two species of *Saurida*, *Saurida* sp. A and *Saurida* sp. B were confirmed as new species based on morphological analysis and their formal descriptions are awaiting. The species *Saurida isarankurai* Shindo and Yamada, 1972 was described from Prachanbririkan Province, Gulf of Thailand, South China Sea. We initially identified some samples from off Mangalore coast as *S. isarankurai*. However, later we collected samples from the Gulf of Thailand and compared both Mangalore (*Saurida* sp. B) and Thailand samples (*S. isarankurai*). Our results based on two markers, COI and 16S, shows that these two are two distinct species. This study provides a comprehensive molecular evidence for the species identification of the genus *Saurida* from Indian waters. The phylogenetic tree derived from two mitochondrial genes (COI and 16S) are almost identical and strongly suggest that these *Saurida* fishes are divided into six species. In all the six species except *S. longimanus* (0.8%) the intraspecies variation was low and this may be due to the low number of haplotypes. Further detailed research, including molecular markers, to know the taxonomic validity of many species in the genus *Saurida* is therefore urgently needed before an accurate assessments, biological study or GIS based resource mapping can be made.

## Chlorophthalmidae

The family Chlorophthalmidae is one of the taxonomically confusing fishes due to the meristic conservatism of the genus and the individually variable nature of

the morphology (Gomon *et al.*, 2014) and are represented by two species in the present study. In the initial stage of identification, identified as *C. bicornis*, and then re-identified as *C. corniger* after a detailed examination of the morphology of type series of *C. corniger* and *C. bicornis* revealed that the two species are identical, and *C. corniger* should be considered the senior synonym of *C. bicornis*. *Chlorophthalmus acutifrons* was first described by Hiyama (1940) from the Mediterranean Sea. In this study COI sequence information was generated for seven specimens of *C. acutifrons* from the southern coast of India. COI sequences of some specimens named as *C. punctatus* collected from India in GenBank (EU 392197-EU 392201) by Lakra *et al.*, (2011) are identified as *C. acutifrons* according to the value of the COI genetic distances (0.00%), when compared with sequences created during this study for *C. acutifrons*. Specimens reported as *C. agassizi* from Indian waters also could be *C. acutifrons*. The present study confirms the occurrence of *C. acutifrons* along the Indian coast.

## 5.5. Conclusion

It is concluded that partial sequence information of both mitochondrial 16S rRNA and COI genes can be used as a molecular marker for identification and resolution of taxonomic ambiguity in deep-sea fishes. The present study stresses the necessity to do the morphological taxonomy and revisions of most of the deep-sea families distributed along the Indian waters before using COI or 16S as a routine identification tool. The deep-sea fishery resources of India consist of deep-sea shrimps, sharks and fishes. The deep-sea shrimp fishery operating along the southern coast of India and with landings occurring in nearly eight locations and this fishery is poorly monitored and managed (Rajool *et al.*, 2012). The deep-sea fishery resources started harvesting is very recently and the baseline information such as taxonomy, catch composition and biology about these resources is very limited. The conservation of deep-sea resources is gaining momentum and there is an urgent need for baseline information. The increasing use of COI and 16S rRNA genes can help the morphological identification and clear the taxonomic resolution of most difficult groups by documenting the diversity of both genes sequences. The use of molecular sequencing approach helped to get better understanding of species composition and diversity of deep-sea fishes in the targeted deep-sea shrimp fishery along the southern coast of India.

DNA based approach can greatly improve the current understanding of species diversity and should be incorporated in species inventories and description (Lowenstein *et al.*, 2011) and is very useful for identification of new species (DeSalle *et al.*, 2005; Akhilesh *et al.*, 2012; Bineesh *et al.*, 2013). Given the criticisms and many limitations of single marker sets and DNA barcoding in species identification, it is very important that results should be cross verified with additional sampling and use of nuclear markers (Ilves *et al.*, 2010). More taxonomic sampling and generation of data from independent markers in the nuclear genome may provide a better understanding of diversity in the Indian deep-sea fishes.

The present study underscores the need of keeping voucher specimens for re-examination in case of doubts occurred due to misidentifications or sequence contaminations. Therefore all sequences must be associated with complete good voucher specimens. The voucher specimens should be preserved in formalin after the collection of tissue materials for molecular analysis. This helps to provide better information for taxonomic revisions and resolving the taxonomic ambiguity. Identifications using GenBank and BOLD data systems show the incompleteness of deep-sea fish database. BOLD and GenBank will give accurate result only when the database is complete for each taxonomic group.

**Chapter 6**

***Description of new deep-sea species***

## Chapter 6

# Descriptions of new deep-sea fish species

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### 6.1 Introduction

The basic diversity of fishes through species discovery and description is mostly complete for some areas of the world and many families are deeply studied. However, many species remains to be discovered. Deep-sea habitat is one of the areas identified as the habitat where most new marine taxa will likely be found. Mora *et al.*, (2008) used records from the Ocean Biogeographical Information System (OBIS) database to estimate the completeness of the global fish inventory and estimated that the global inventory is about 79% complete, or that 21% of marine fishes remain to be discovered. Descriptions of new fish species over time varies widely for different reasons. The advent of SCUBA resulted in the discovery of many new marine species since 1950. New explorations coupled with new techniques significantly contribute to the study of deep-sea and mid water species (Eschmeyer *et al.*, 2010). Recently, different molecular markers have been used to support the morphology based descriptions of many fish species. The diversity of deep-sea fish species along the Indian coast are poorly known, and are known from very few scattered studies. However the diversity is considered to be higher than thought earlier (Silas *et al.*, 1969; Kurup *et al.*, 2009; Akhilesh *et al.*, 2010; Bineesh *et al.*, 2013). This chapter deals with the descriptions of new deep-sea fish species confirmed during the present study.

### 6.2 *Chelidoperca maculicauda*

The family Serranidae (Perciformes) is one of the largest perciform family with 5 subfamilies, 64 genera and 529 valid species (Eschmeyer and Fong, 2012). The serranid fish genus *Chelidoperca* Boulenger (1895) includes 6 species: *Chelidoperca hirundinacea* (Valenciennes, 1831), *C. investigatoris* (Alcock, 1890), *C. lecromi* Fourmanoir, 1982, *C. margaritifera* Weber, 1913, *C. occipitalis* Kotthaus, 1973 and *C. pleurospilus* (Günther, 1880) (Eschmeyer and Fong, 2012). *Chelidoperca* are usually found on the continental shelf and slope with muddy bottoms in the Indo-Pacific (Nelson, 2006). Two species of *Chelidoperca*, namely *C.*

*investigatoris* and *C. occipitalis* are known from the Arabian Sea (Kotthaus 1973; Manilo and Bogorodsky, 2003; Jayaprakash *et al.*, 2006). *Chelidoperca maculicauda* represents the third known species in the genus *Chelidoperca* from the Arabian Sea.

Specimens of *Chelidoperca* were collected from commercial deep-sea shrimp trawler operated in the continental shelf of Arabian Sea, off Kollam at depths 180–320 m, and landed at Sakthikulangara Fisheries Harbour, Kerala, during April 2012. Measurements of formalin (5%) preserved specimens were taken following Hubbs and Lagler (1967). The morphometric characters of the specimens were measured with a digital vernier caliper with an accuracy of 0.1 mm and expressed as percentage of standard length (SL) or head length (HL). Gill raker counts were taken from the first gill arch of the right side. Vertebral and caudal-fin ray counts were taken from radiographs of the type specimens. In the description, values in parentheses refer to data for the paratypes when different from the holotype. Specimens examined are deposited in the collections at Central Marine Fisheries Research Institute, Zoological Survey of India and National Bureau of Fish Genetic Resources (NBFGR), Kerala, India.

***Chelidoperca maculicauda* Bineesh & Akhilesh, 2013**

(Figure 6.1–6.3; Table 6.1)

**Diagnosis.** A species of *Chelidoperca* with the following combination of characters: Dorsal fin rays X, 10; anal fin rays III, 6; pectoral-fin rays 15; lateral-line scales 42; gill rakers 3 + 9, caudal rays 17. Fourth dorsal spine longest, 2.8 (3) in HL, body depth 23.3 (22.8–24.5)% SL, 4.3 (4.1–4.4) in SL; head length 40.3 (42.3–42.6)% SL; orbital length 9.3 (8.9–9.1) in SL; 2.5–3 scales above lateral-line to dorsal origin; serrae on margin of preopercle 40–46.; dorsal fin continuous, with ninth dorsal spine shorter than tenth spine; longest dorsal soft ray (seventh) 2.4 (2.3–2.4) in HL; first anal-fin spine 8.7 (8.3–9.7) in HL, second anal-fin spine 3.49 (3.16 – 3.54) in HL; pelvic fin relatively short, 4 (4.6–4.3) in SL; head and body pinkish in colour, ventral side pale. Six white blotches on body. Numerous small bluish white circular spots on lower caudal fin, upper caudal fin with a small circular grey spot distally.



**Description.** (When counts vary, those from the holotype are given in parentheses, Table.6.1). Dorsal rays X, 10 ; anal rays III, 6; pectoral rays 15; pelvic rays I, 5; anal rays III, 6; caudal fin rays 17; lateral-line scales 42; 3 (4–5 as rudiments) + 9 (2–3 as rudiments) gill rakers on the first arch (total 7–8 +11–12); vertebrae 23. Body moderately elongate, cylindrical; body depth at dorsal origin 4.3 (4.1–4.4) in SL, 1.7 (1.8–1.9) in HL; body moderately compressed, width 1.3 (1.2–1.3) in body depth. Head moderately compressed, short, its length 40.3 (42.3–42.6)% SL, 2.5 (2.4) in SL; snout short, pointed, snout length 4.7 (4.8–4.9) in HL; orbit moderately large, greater than interorbital width and snout length, orbital diameter 9.3 (8.9–9.1) in SL, 3.7 (3.7–3.9) in HL; interorbital space flat, scaly, scales reaching to anterior edge of the orbit; Interorbital width 8.9 (8.8–9.) in HL; two opercular spines and three occipital spines covered with scales, above origin of lateral-line (Fig. 6.3); preopercle finely serrated (40–46). Predorsal length 37.5 (38.6–38.8) % SL. Caudal peduncle depth 10.7 (10.3–10.8) % SL; caudal peduncle length 24.5 (24.7–25.5) % SL.

Mouth terminal, large and oblique, jaws strong; lower jaw projecting in front of upper lip, upper jaw nearly reaching vertical to posterior margin of orbit, widest at end and maxilla truncate posteriorly, with rounded corners; supramaxilla relatively large and terminally positioned; upper jaw length 2.3 (2.3–2.4) in HL. Teeth in villiform bands in the premaxilla and palatines and in a small patch on the vomer, premaxilla with an outer row of moderate conical teeth; outer teeth anteriorly with irregular inner series of smaller conical teeth and a wide inner band of much smaller teeth; small canine teeth in mandible and at premaxillary symphysis. Tongue long, slender and spatulate anteriorly. Head fully scaled except lips, snout and maxilla; about 5–6 rows of scales from edge of orbit to corner of preopercle; scales ctenoid; lateral-line not in a straight line (wavy), 2.5–3 rows of scales below tenth dorsal-fin spine and the lateral line.

Dorsal fin continuous and deeply notched; dorsal fin originating in front of a vertical through opercular margin, and opposite pectoral anterior insertion and pelvic posterior insertion. Dorsal-fin spines relatively slender and straight without flexible tips; dorsal-fin origin above fifth lateral-line scale; first dorsal spine short, slender, 9.2 (10.7–11.1) in HL, 1.9 (2) in second spine; 4<sup>th</sup> dorsal spine longest, 2.8 (3) in HL; ninth dorsal spine slightly shorter than tenth spine, 1.3 (1.1–1.4) in tenth dorsal

spine; dorsal-fin rays branched, additional branches at tips, longest dorsal-fin ray (seventh or eighth) 2.4 (2.3–2.4) in HL. Pectoral fins long, reaching vertical through anal-fin origin, 24.5 (24.7–25.5)% SL, 1.6 (1.3–1.5) in HL; pelvic fins inserted in front of and beneath pectoral fins, not reaching to anal-fin origin, pelvic-fin length 1.6 (1.8–2) in HL, 24.9 (21.8–23)% SL. Anal-fin origin in a vertical below dorsal ray origin. Third anal-fin spine longer than second, 4.4 (3.4–4.8) in head length; longest anal-fin soft ray (fifth) 2.2 (1.9–2.1) in head length; Pre anal-fin length 61.7 (64.2–63.6) % SL. Anal-fin rays branched like dorsal-fin rays. Upper lobe of caudal fin longer and rounded.

**Colouration.** When fresh: Head and body pinkish in colour, belly and throat white. Bright yellow markings on the cheek and opercles. Bright red line on distal border of anal fin. Basal region of anal-fin rays with yellow spots, membrane of last anal ray yellow spotted. Six white blotches on body. Upper part of maxilla yellowish, lower grey. Upper rim of orbit with red spots. a narrow violet stripe below eye across distal part of maxilla. dorsal fin mostly yellowish, base of dorsal spines with pink spots; caudal fin pinkish red proximally, upper and lower margins yellow, upper margins yellow, scattered small blue white spots centrally on lower lobe; anal fin pale; upper caudal lobe with a small grey spot. Lower lobe yellowish with bluish-white spots. In preservative: Similar to above with the most markings and grey spot on caudal fin less apparent.

**Comparisons.** *Chelidoperca maculicauda* can be distinguished from all other species of the genus by its unique colour pattern, consisting of a completely pink body with six white blotches, a grey spot on the upper half of caudal fin, numerous small pale blue dots on the lower caudal fin, shape of the caudal fin, and preopercular serration 40–46. Other characters to distinguish amongst the Indian Ocean species of *Chelidoperca* are as follows. *C. maculicauda* is readily distinguished from *Chelidoperca pleurospilus* (Günther, 1880) in having a high preopercular serration 40–46 (vs 15–29 in *C. pleurospilus*), gill rakers 3+9 (vs 5–6+11–12), body depth 22.8–24.3 (vs 22.7–23.1% SL), and post orbital length 23.7–24.7 (vs 19.3–19.5% SL) (Akazaki, 1972; Park *et al.*, 2007). *Chelidoperca maculicauda* is distinguished from *C. occipitalis* in having an eye diameter 10.8–11.6 (vs 9.1–10.6% SL) and absence of a black band on the body (vs present). *Chelidoperca maculicauda* differs from *C. investigatoris* in the absence of

blackish bands (vs. dark bands on sides), body depth 22.8–24 (vs. 26.2–28% SL), head length 40.3–42.6 (vs 42–49% SL), interorbital 4.6–4.8 (vs 3.4–4.1% SL), and preanal length 61.7–64.2 (64.4–70% SL). *Chelidoperca investigatoris* Alcock, 1890 described from the collections of RIMS *Investigator* from the Chennai coast (Tamil Nadu), Bay of Bengal, was the only *Chelidoperca* species known from Indian waters until *C. occipitalis* was reported from the southwest coast of India (Bineesh *et al.*, 2014). All three species vary considerably in their COI sequence, indicating three distinct species in Indian waters.

**Distribution.** Presently known only from off the southwest coast of India in the Arabian Sea, at depths of 180–320 m.

**Etymology.** The new species is named *maculicauda* with *cauda* from the Latin, meaning tail, and *macula* from the Latin, meaning spot with reference to the distinctive grey spot on the tail.

**Proposed common name.** Indian perchlet

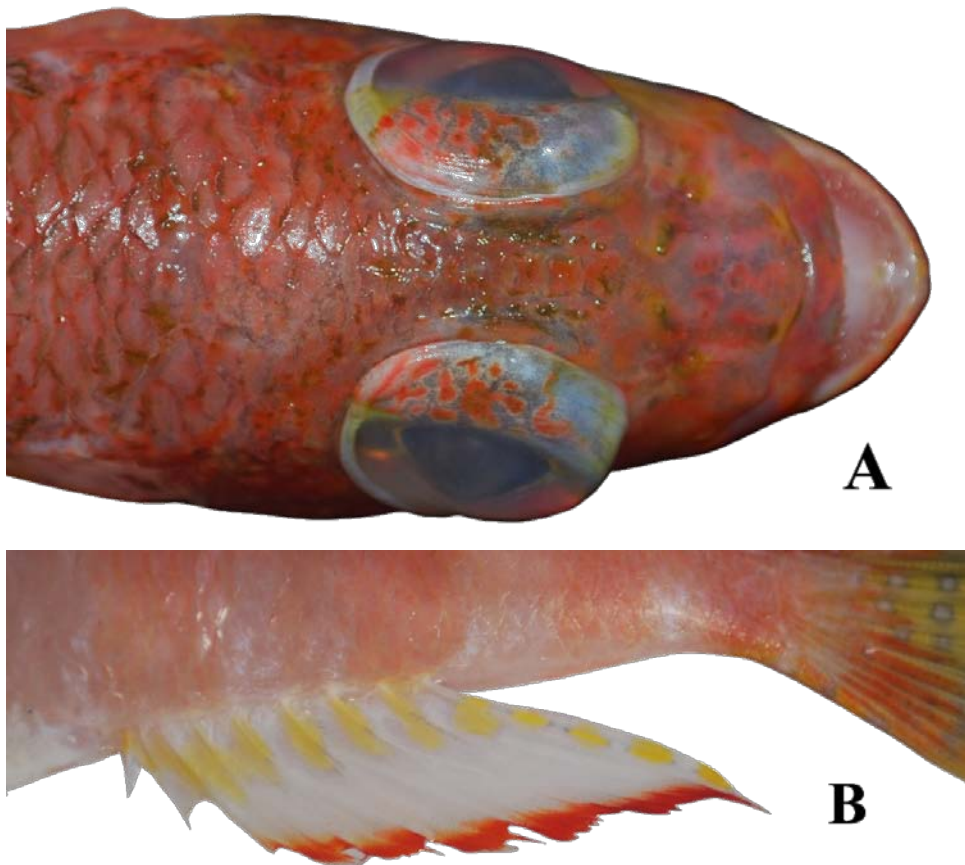
Holotype. CMFRIGB 31. 139.16. 2, 127 mm SL, off Kollam, Kerala coast, India, south eastern Arabian Sea, 180–320 m depth, collected by K.K. Bineesh and K.V. Akhilesh, 25 April 2012. NCBI GenBank Accession No: JX185308. Paratypes. CMFRI GB 31. 139. 16. 2.1, 128.9 mm SL, off Kollam, Kerala Coast, India, south eastern Arabian Sea, 180–320 m depth, collected by K.K. Bineesh and K.V. Akhilesh, 25 April 2012. NCBI GenBank Accession No: JX185309. CMFRI GB 31. 139. 16. 2.2, 122.9 mm SL, off Kollam, Kerala Coast, India, south eastern Arabian Sea, 180–320 m depth, collected by K.K. Bineesh and K.V. Akhilesh, 25 April 2012. NCBI GenBank Accession No: JX262929.

Other material examined during this study: *Chelidoperca investigatoris*– Syntype ZSI 12820, 107.5 mm SL, Syntype ZSI 12821, 103.4 mm SL, Off the Ganjam coast, Odisha, India, 180–187 m depth, R. I.M.S. *Investigator*. NBFGR CHN 3012– 3024, 13 specimens, 127.51–177.9 mm TL, off Kollam, Kerala coast, India, south eastern Arabian Sea (09°05' N, 75°52' E), 180–280 m depth, collected by K.K. Bineesh and K.V. Akhilesh, 08 February 2009. NCBI GenBank Accession No: JX185305, JX185307, JX185312 and JX185310. *Chelidoperca occipitalis*: Holotype. ZMH 5136, 114 mm SL, Off Socotra Islands, Arabian Sea, 190–290 m depth. CMFRI GB 31.139.16.1.1–3, 3 specimens, 135–153 mm TL, NBFGR CHN

3001– 3011, 11 specimens, 135–163 mm TL, off Kollam, India, Kerala coast, south eastern Arabian Sea (09°20' N, 75°51' E), 180–320 m depth, collected by K.K. Bineesh and K.V. Akhilesh, 22 April 2009. NCBI GenBank Accession No: JX185311, JX185313, JX185306 and JX185304.



**Figure 6.1.** *Chelidoperca maculicauda* holotype, CMFRIGB 31. 139.16. 2, 127 mm SL, off Kollam, Kerala, India.



**Figure 6.2.** A) Dorsal view of head *Chelidoperca maculicauda*, holotype, CMFRIGB 31. 139.16. 2, (B) Anal fin image, holotype, CMFRIGB 31. 139.16. 2.

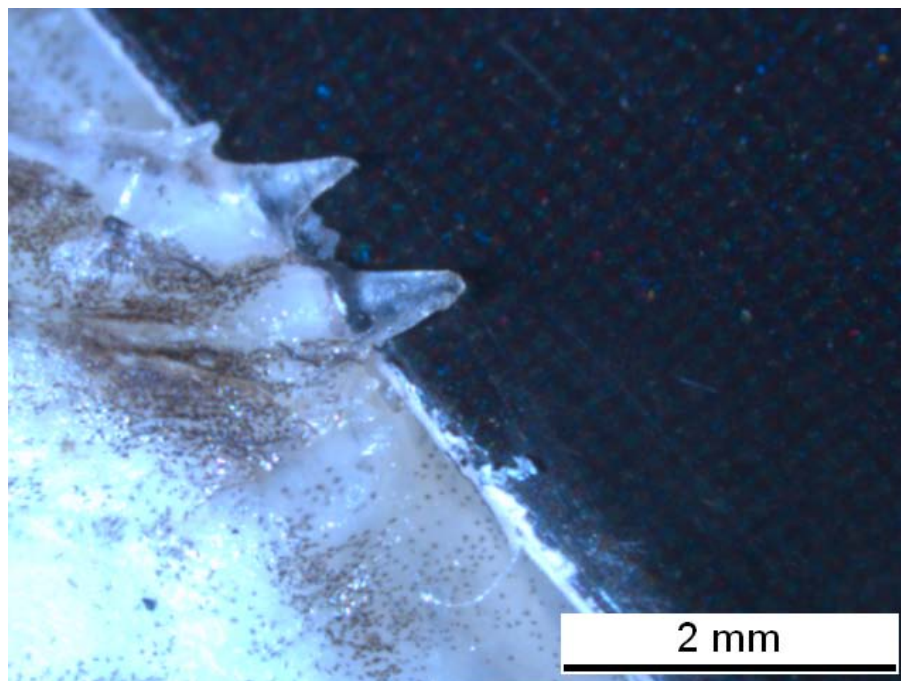
**Table 6.1.** Proportional measurements of the holotype (CMFRIGB 31. 139. 16. 2) and two paratypes of *Chelidoperca maculicauda* as percentage of standard length.

Measurements	Holotype	Paratype	Paratype
	GB 31. 139.16. 2	GB 31. 139.16. 2. 1	GB 31. 139.16. 2.2
Total length (mm)	156.6	160.7	153.4
Standard length (mm)	127.2	128.9	122.9
Body depth	23.3	24.3	22.8
Body width	18.5	18.8	18.8
Head length	40.3	42.6	42.3
Post orbital length	23.7	24.7	24
Snout length	8.6	8.7	8.8
Eye diameter	10.8	11	11.3
Upper jaw length	17.8	18	18.3
Interorbital width	4.6	4.8	4.7
Predorsal length	37.5	38.8	38.6
Prepectoral length	37.9	39.9	38.6
Prepelvic length	35.2	35.2	35.9
Preanal length	61.6	64.2	63.6
Pectoral fin length	24.5	24.7	25.5
Pelvic fin length	24.9	21.7	23
Caudal fin length	23.9	22.5	24.8
Caudal peduncle depth	10.7	10.3	10.8
Anal fin length	35.3	33.9	36.9
Anal fin base length	17.3	16.2	17.8
Dorsal fin length	60.1	60.9	62.3
Dorsal fin base length	46.5	47.6	47.9
Caudal peduncle length	17.9	20.9	20.3
First dorsal spine length	4.4	4	3.8
Second dorsal spine length	8.4	7.9	7.9
Third dorsal spine length	13.1	12.7	12.9
Fourth dorsal fin spine length	14.6	14.3	14.2
Ninth dorsal fin spine	7.9	8.2	7.4
Tenth dorsal fin spine length	10.3	9.1	10.4
Eighth dorsal fin spine length	8.1	7.5	8.4
First anal spine length	4.6	5.2	4.3
Second anal spine length	6.7	7.7	6.4
Third anal spine length	9.3	12.6	8.8
First anal ray length	13.3	12	12.1
Longest anal ray length	18.8	20	22

## 6.2 *Plectranthias alcocki*

The serranid fish genus *Plectranthias* (Serranidae: Anthiinae) was established by Bleeker (1873) for *Plectropoma anthioides* Günther, 1872, and contains small benthic species found in tropical and subtropical areas on coral or rocky reefs at depths of 20–300 m, hence not often caught in trawls. They are poorly represented in museum collections and nearly half of the valid species are known from only one or two specimens (Randall, 1980; Heemstra and Randall, 2009).

Recently, Wu *et al.*, (2011) described two new species based on single specimens from Taiwan. Randall (1980) recognized 30 species in an early revision of the genus. Forty-eight valid species of *Plectranthias* are currently known, of which only 13 occur in the Indian Ocean for at least part of their range (Heemstra and Randall, 2009). The most recently described species is *P. flammeus* Williams *et al.*, (2013) from the Marquesas Islands, French Polynesia. Only one species, *Plectranthias intermedius* (Kotthaus, 1973), is known from the Arabian Sea (Manilo and Bogorodsky, 2003). The purpose of this paper is to describe the second Arabian Sea species, *Plectranthias alcocki*, currently known only from off Kollam, southwest coast of India.



**Figure 6.3.** Occipital spines of *Chelidoperca maculicauda*, holotype, CMFRIGB 31.139.16.2.

Specimens of *Plectranthias* were collected from commercial deep-sea shrimp trawler operating over the continental shelf of the Arabian Sea, off Kollam at depths of 180–320 m, and landed at Sakthikulangara Fisheries Harbour, Kerala on 22 August 2012. Measurements of formalin (5%) preserved specimens were taken following Randall (1980). Gill-raker counts were made from the first gill arch, including all rudiments, with the raker at the angle of the arch included in the count for the lower limb. In the description, values in parentheses refer to data for the paratype when different from the holotype. The holotype and paratype are deposited in the collections at Central Marine Fisheries Research Institute, India.

***Plectranthias alcocki* Bineesh, Gopalakrishnan & Jena, 2014**

(Figure 6.4; Table 6.2)

**Diagnosis.** A species of *Plectranthias* with the following combination of characters: dorsal-fin rays X,15; anal-fin rays III,7; pectoral-fin rays 14; pelvic-fin rays I,5; lateral-line complete, the pored scales 28; scales above lateral line to origin of dorsal fin 1; scales dorsally on head extending to posterior nostrils; no scales on maxilla or chin; gill-rakers 5 + 11 (2 + 7 developed); circumpeduncular scales 10; fourth dorsal spine longest, 2.8 (2.6) in HL, body depth 34.4 (35)% SL; head length 46 (49.8)% SL; orbit diameter 8.6 in SL; margin of preopercle finely serrate, the serrae 33 (23), ventral edge without antrorse spines; dorsal fin slightly notched and continuous, with longest dorsal-fin soft ray (second) 2.4 (2.7) in HL; first anal-fin spine 4.9 (5.6) in HL, second anal-fin spine 2.2 (2.6) in HL; the dorsal fin with a black blotch at base of fourth to eighth spines, one at base of the last three spines, and two at base of soft portion of fin, the dark pigment extending onto adjacent body.

**Description.** Dorsal-fin rays X,15 (all rays branched, the last to base); anal-fin rays III,7 (all rays branched); pectoral-fin rays 14 (both sides counted; all unbranched); pelvic-fin rays I,5; 4<sup>th</sup> dorsal spine longest 16.5 (18.8)% SL, 2.8 (2.6) in HL; short cirrus behind tips of 3<sup>rd</sup> spine; caudal-fin rays broken, but possibly slightly emarginate, principal rays 19, branched rays 17; lateral line complete, not interrupted with 28 pored scales; one row of scales above lateral-line to origin of dorsal fin; circumpeduncular scales 10; gill rakers 5 + 11 (2 + 7 developed), first lower-limb gill raker on first gill arch adjacent to raker at angle longest, its length one-half orbit diameter; branchiostegal rays 7.

Body moderately elongate, depth 1.3 (1.4) in HL, 34.4 (35)% SL; and compressed, the width 2.1 (2.2) in body depth; head pointed, the lower jaw slightly projecting; dorsal profile of head smoothly convex; head length 46 (49.8)% SL, 2.2 (2) in SL; snout length 4.9 (5.2) in head; orbit diameter 4 (4.3) in HL; interorbital space flat, the least bony width 11.5 (10.8) in HL; least depth of caudal peduncle 3.6 (4.3) in HL; caudal peduncle length 2.3 (2.4) in HL. Opercle with three prominent flat spines posteriorly, the middle spine largest and terminating most posteriorly, curving slightly upward, its sharp tip at level of middle of pupil, closer to lower than upper spine; upper spine terminating most anteriorly and most pointed; lower and

middle spine very acute and sharp; margin of preopercle finely serrate, serrae 33 (28), ventral edge without antrorse spines; subopercle and interopercle smooth; opercular flap well-developed and at an angle upward in alignment with middle opercular spine. No scales on maxilla, snout, sub orbital or interorbital.

Mouth terminal, oblique and large; the maxilla extending posteriorly to a vertical through posterior third of orbit, the upper-jaw length 2.2 (2.4) in head length; snout, maxilla, suborbitals and lower jaw naked. Mouth with an incurved canine tooth on each side at front of upper jaw separated by a symphyseal gap without teeth; a band of villiform teeth in upper jaw that broadens anteriorly; posterior half of lower jaw with a narrow band of medially depressible conical teeth in three rows, those of outer row very small, those of inner row largest; a fixed recurved canine tooth in outer row at mid-side of lower jaw; tooth bands on lower jaws almost contiguous; vomer with villiform teeth in two to three irregular rows; palatines with a narrow band of villiform teeth in two to three irregular rows. Tongue narrowly triangular with a slightly rounded tip.

Dorsal fin continuous and slightly notched; origin of dorsal fin over second lateral-line scale; first dorsal spine 7.9 in HL; second dorsal spine nearly twice as long as first, 4.3 in HL; fourth dorsal spine longest, 2.8 (2.6) in HL, 16.5 (18.8)% SL, last dorsal spine 4.9 (5.8) in HL; second dorsal-fin soft ray longest, 2.4 (2.7) in HL; origin of anal fin below base of second dorsal-fin soft ray: first anal spine 4.9 (5.6) in HL; second anal spine 2.2 (2.6) in HL, longer than third spine; third anal spine 3 (3.5) in HL; fourth anal soft ray longest, 2 (2.4) in HL; caudal fin broken in both specimens but seems to have been emarginate; pectoral fin 1.3 in HL (longest rays ninth and tenth), reaching to second anal soft ray in vertical; origin of pelvic fins slightly anterior to vertical line from upper end of gill opening and pectorals; pelvic fins reaching 1–4 mm before anus, pelvic fin length 2 in HL (second ray longest).

**Colouration.** When collected, mainly red posterior to an oblique demarcation from mid nape to origin of anal fin, then brown anteriorly, the head suffused with orange-yellow anteriorly; body with blackish pigment on the scales increasingly darker dorsally and posteriorly, more blackish than red on posterior half of caudal peduncle; a black spot posteriorly on opercle, and a dusky spot ventrally on abdomen; fins translucent yellow, the dorsal with a black blotch at base of fourth to



eighth spines, one at base of the last three spines, and two at base of soft portion of fin, the dark pigment extending onto adjacent body (Fig. 6.4). Colour in formalin pale with traces of black blotches on the body.

**Distribution.** Presently known only from off the southwest coast of India in the Arabian Sea, at depths of 180–320 m.

**Etymology.** The species is named in honour of W. Alcock, in recognition of his contribution to the taxonomy of deep-sea fauna of Indian seas.

Proposed common name: Alcock's deep-reef basslet.



**Figure 6.4.** Holotype of *Plectranthias alcocki*, CMFRI GB.31.139.30.10, 72.2 mm SL, off Kollam, Kerala coast, India

**Comparisons.** *Plectranthias alcocki* can be distinguished from all other species of the genus *Plectranthias*, except *P. maugei* Randall, 1980 and *P. foresti* Fourmanoir, 1977, by its unique colour pattern, consisting of dorsal fin with a black blotch at base of fourth to eighth spines, one at base of the last three spines, and two at base of soft portion of fin, the dark pigment extending onto adjacent body and in several other morphometric characters (Randall, 1980; Heemstra and Randall, 2009). Amongst the Indian Ocean *Plectranthias*, *P. alcocki* is readily distinguished from *P. maugei* in having 14 pectoral-fin rays (13 rays in *P. maugei*), head length 46.0–49.8% SL (vs 42.1–43.1), eye diameter 4–4.3 in head length (vs 3.5–3.6), caudal peduncle length 2.3–2.4 in head length (vs 3.3–3.6), circumpeduncular scales 10 (vs 14 in *P. maugei*) (Randall, 1980; Heemstra and Randall, 2009).

**Table 6.2.** Proportional measurements of holotype and paratype of *Plectranthias alcocki* expressed as a percentage of standard length.

Measurements	Holotype	Paratype
	GB.31.139.30.10.	GB.31.139.30.10.1
Standard length (mm)	72.2	63.7
Head length	46	49.8
Snout length	9.5	9.6
Eye diameter	11.6	11.6
Post orbital length	26.3	29.5
Upper jaw length	20.7	20.7
Maxillary width	6.4	6.8
Interorbital width	4	4.6
Body depth	34.4	35
Body width	16.2	15.8
Pre-dorsal length	39.2	41.8
Pre-anal length	70.5	68.1
Pre-pelvic length	37.3	37.3
Dorsal-fin base length	52.3	53.9
Anal-fin base length	15.6	16.3
Anal fin length	30	broken
Pelvic fin length	23.1	25.3
Pectoral fin length	34.9	38.4
Pre-pectoral length	40.3	42.6
Caudal peduncle depth	12.8	11.5
Caudal peduncle length	19.7	20.8
First anal spine length	9.5	8.8
Second anal spine length	20.8	19.4
Third anal spine length	15.4	14.3
First dorsal spine length	5.8	6.3
Second dorsal spine length	10.8	11.5
Third dorsal spine length	16.1	16
Fourth dorsal spine length	16.5	18.8
Fifth dorsal spine length	14.8	18
Tenth dorsal spine length	9.4	8.7
Longest dorsal spine length	16.4	18.8
Longest dorsal soft ray length	18.8	18.3
Longest anal soft ray length	23.3	20.9
Pelvic spine length	12.8	14.6

*Plectranthias alcocki* can be distinguished from *P. intermedius* (Kotthaus, 1973) by having a slightly notched fin margin before soft-rayed part (*vs* fin margin distinctly notched before soft-rayed part); dorsal-fin rays X, 15 (*vs* X, 17); pectoral fins 14 rays (*vs* 14 or 15); greatest body depth 1.3–1.4 (*vs* 2.6) in head length; large eye, 3.9–4.3 in head length (*vs* 3.0); lateral-line with 28 pored scales (*vs* 31–34 in *P. intermedius*) (Heemstra and Randall, 2009). *Plectranthias alcocki* is differentiated from *P. inermis* Randall, 1980 in having X, 15 (*vs* X, 16 to 18) dorsal-fin rays, 4<sup>th</sup> dorsal spine longest (*vs* 3<sup>rd</sup> spine longest), pectoral-fin rays 14 (*vs* 13 rays), pelvic fin length 2 in head length (*vs* 1.6–1.8), head length 2.0–2.2 in standard length (*vs*

2.2–2.4), inter orbital length 10.8–11.5 in HL (vs 11–14) and serrated preopercle (smooth) (Heemstra and Randall, 2009).

*Plectranthias alcocki* is differentiated from *P. foresti* Fourmanoir, 1977 in having 14 pectoral–fin rays (13 rays in *P. foresti*), gill rakers 5 + 11 (4–5 + 11–13), body depth 2.9 (vs 2.6–2.8) in SL, fourth spine 2.6–2.8 in HL (vs 2.4–2.5), pectoral fin 2.6–2.9 in SL (2.5–2.7), pelvic fin length two in head length (1.7–1.85) (Randall, 1980). *Plectranthias alcocki* and *P. vexillarius* Randall, 1980 share the same complete lateral–line with 28 pored scales, margin of preopercle finely serrate with 33 serrae but *P. alcocki* can be distinguished from *P. vexillarius* by a large eye diameter 3.9–4.3 (vs 3.0–3.8 in head length) and presence of black blotch at base of fourth to eighth spines, one at base of the last three spines, and two at base of soft portion of fin vs 4 irregular rows of large brown blotches. *P. alcocki* is further distinguished from *P. vexillarius* in having the 4th dorsal spine longest (vs 3rd) and dorsal–fin rays X, 15 (vs X, 17) (Heemstra and Randall, 2009).

*Plectranthias winniensis* Tyler, 1966 differs from *Plectranthias alcocki* in certain meristic counts and morphometrics such as: dorsal fin rays (X, 15–17), deeply notched dorsal fins, pectoral rays 16–18, gill raker count 4–6 + 11–15, pelvic fin length 1.5–1.8 in SL, orbit diameter 2.7–3.0 in HL and its colour pattern (having a spot in the base of last three anal rays) and absence of cirri on dorsal–fin spines (Heemstra & Randall, 2009). *Plectranthias alcocki* and *P. nanus* Randall 1980 also share similar characters, including the same pectoral–fin ray and gill–raker counts, 1 row of large scales between 5<sup>th</sup> dorsal spine and lateral line, 4<sup>th</sup> spine longest in both species and maxilla reaching a vertical at rear edge of eye. They differ in dorsal soft–ray count (15 for *P. alcocki*, 16 for *P. nanus*), lateral line count (28 for *P. alcocki*, 16–22 for *P. nanus* and incomplete), antrorse–spine count (no antrorse spine in *P. alcocki*, 2 spines in *P. nanus*) and very large eye in *P. alcocki* (4.0–4.3 in HL), compared with *P. nanus* (3.4–4.0 in HL) (Randall, 1980; Heemstra and Randall, 2009).

Holotype. CMFRIGB.31.139.30.10, 72.2 mm SL, off Kollam, Kerala coast, India, southeastern Arabian Sea, 180–320 m depth, shrimp trawl, collected by K.K. Bineesh and K.V. Akhilesh, 22 August, 2012. Paratype. CMFRIGB.31.139.30.10.1, 63.7 mm SL. Same data as holotype.

### 6.3 *Liopropoma randalli*

The genus *Liopropoma* was proposed by Gill, 1861 for *Perca aberrans* Poey (1860) a species based on a single specimen from deepwater off Cuba. Members of this genus are small to medium-sized and colorful fishes, belonging to the tribe Liopropomini within the family Epinephelidae, which occur in tropical and subtropical waters of the Indo-Pacific and Western Atlantic (Baldwin and Johnson, 1993; Craig and Hastings, 2007). Since this review, another species, *Liopropoma dorsoluteum* Kon, Yoshino and Sakurai, 1999, has been described from the Indo-West Pacific off Japan and Taiwan. In their review of the Indo-West and Central Pacific species of *Liopropoma*, Randall and Taylor (1988) provided a detailed account the genus and of 18 species, which included 7 new species. In addition to these species, two nominal species are known from the Eastern Pacific and 6 are known from the Western Atlantic. Of the 27 nominal species of *Liopropoma*, 7 species are known to occur in the Indian Ocean for at least part of their range: *L. africanum* (Smith, 1954); *L. dorsoluteum*; *L. lunulatum* (Guichenot, 1863); *L. mitratum* Lubbock and Randall, 1978; *L. multilineatum* Randall and Taylor, 1988; *L. susumi* (Jordan and Seale, 1906); *L. tonstrinum* (Lubbock and Randall, 1978; Randall and Taylor, 1988; Khalaf and Zajonz, 2007).

Most species of *Liopropoma* are poorly represented in collections and as a result, a number of species are described based on only one or two specimens. Recent surveys of fish landing sites in south western India and southern Indonesia resulted in the collection of four specimens of an undescribed species of *Liopropoma*. These specimens were collected by gillnetter off the coast of Mangalore in depths of 170–260 m (n = 2) and from a fish landing site in Lombok, eastern Indonesia (n = 2). This new species of *Liopropoma* from the Indian Ocean is described herein.

The methods used in this work follow Randall and Taylor (1988) and descriptions of the measurements taken are provided by these authors. Lengths are given as standard length (SL) unless otherwise stated. The morphometric characters of the specimens were measured with a digital vernier caliper with an accuracy of 0.5 mm and expressed as percentage of standard length (SL) or head length (HL). Gill-raker counts were taken from the first gill arch of the right side. Vertebral and

caudal-fin ray counts were taken from radiographs of the type specimens. In the description, values in parentheses refer to data for the paratypes when different from the holotype.

***Liopropoma randalli* Akhilesh, Bineesh & White, 2012**

(Figure 6.5, 6.6; Table 6.3)

**Diagnosis.** Dorsal fin rays VIII, 12; anal fin rays III, 8; pectoral-fin rays 14 or 15; lateral line scales 46–49; dorsal fin continuous with eighth dorsal spine slightly longer than sixth and seventh spines; anterior nostril at front of snout; longest dorsal soft ray 2.14–2.33 in head length; 1<sup>st</sup> anal-fin spine 10.4–12.2 in head length, 2<sup>nd</sup> anal-fin spine 4.4–4.9 in head length; pelvic fin relatively short, 5.0–5.8 in SL; body depth 3.3–4.7 in SL; pinkish red with a blackish stripe extending from snout along midlateral body and onto centre of caudal-fin base, numerous blackish semicircular spots on back, lower sides and soft dorsal and caudal fins.

**Description.** Dorsal rays VIII, 12 (last ray divided to base); anal rays III, 8; pectoral rays 14 (14–15); pelvic rays I, 5; lateral line scales about 47 (47–49), plus at least 3 pored scales beyond end of hypural; scales above lateral line to origin of dorsal fin about 4; scales below lateral line to origin of anal fin about 18; circumpeduncular scales about 36 (some scales missing in some types, thus counts not possible on some types); gill rakers 6 (5 as rudiments) + 13 (11–13; 5 or 6 as rudiments); vertebrae 10 + 14. Body moderately elongate, depth at dorsal-fin origin 3.57 (3.34–4.73) in SL; body moderately compressed, width 1.88 (1.73–1.83) in body depth; head long, its length 2.57 (2.45–3.10) in SL; snout long, 4.10 (4.16–4.28) in head length; eyes moderate in size, orbit diameter 5.45 (4.68–6.08) in head length; interorbital space flat, least width 5.57 (5.52–5.73) in head length; caudal peduncle length 1.78 (1.62–1.81) in head length.

Mouth terminal, large, maxilla extending posteriorly to vertical through posterior third of eye, upper-jaw length 2.34 (2.29–2.40) in head length; mouth oblique, lower jaw projecting; teeth in jaws and on palate small, depressible inward, in villiform bands; bands of teeth broadest anteriorly in jaws with a maximum of about 10 irregular rows in upper jaw and about 8 in lower jaw; inner teeth progressively larger; villiform teeth on vomer in a chevron-shaped patch of about 4 or 5 rows, the inner teeth larger; teeth on palatines in a long narrow band with about

6 rows at widest; tongue slender, its tip rounded. Opercle with three flat spines; upper spine blunt, covered by a scale, more anterior to other two; middle spine vertical with and closer to lower spine than upper spine; preopercular margin broadly rounded at corner; posterior margin of preopercle very finely serrate (more so on lower portion); margin of corner and lower limb of preopercle thin and fleshy. Anterior nostril a thin membranous tube set directly in front of eye at edge of groove separating front of snout from upper lip; posterior nostril with a low fleshy rim, above centre of eye, separated from edge of orbit by a distance equal to about a third to half the space between nostrils; a large pore anteromedially from each posterior nostril and usually a pore anterodistally from each posterior nostril; a large pore medial to each anterior nostril at edge of groove separating front of snout from upper lip; a pair of relatively small pores usually present on interorbital space (on each side above front of pupil).

Lateral line strongly arched above pectoral region, 3 rows of scales between the highest point (below fifth dorsal-fin spine) and the lateral line. Head fully scaled except lips; maxilla with or without scales; about 10 diagonal rows of scales from edge of orbit to corner of preopercle (many scales missing in preserved specimens so difficult to make accurate count); small scales extending about one-third distance to margin of soft dorsal and anal fins and most length of caudal fin; paired fins with small scales basally. Dorsal fin continuous and deeply notched; dorsal-fin origin above seventh lateral-line scale; first dorsal-fin spine slender and short, 2.62 (2.42–3.18) in second spine, 11.00 (9.98–10.84) in head length; third dorsal spine longest, 3.72 (3.33–3.58) in head length; sixth dorsal spine shortest; eighth dorsal spine slightly longer than seventh spine; longest dorsal fin ray (seventh or eighth) 2.18 (2.15–2.32) in head length; origin of anal fin beneath base of third dorsal-fin soft ray; third anal-fin spine longer than second, 4.48 (3.75–4.49) in head length; longest anal soft ray (third) 2.18 (2.10–2.26) in head length; caudal fin emarginate, 1.78 (1.62–1.81) in head length, tips pointed; pectoral fins long, pointed, reaching to a vertical at anal-fin origin, 1.48 (1.31–1.64) in head length; origin of pelvic fins slightly anterior to base of pectoral fins, longest pelvic ray 2.22 (2.08–2.35) in head length.

**Coloration.** When fresh: ground colour of head and body pinkish red; a yellowish to dark greenish brown stripe extending from snout, through centre of eye to edge of

opercle, continuing as a blackish stripe slightly above the lateral midline of body to the base of caudal fin, extending a blackish blotch on basal third of central caudal fin; scattered dark brown to black semicircular spots present along back and on sides below dark stripe, several sometimes present on upper head and on opercle; a narrow yellow stripe below eye across distal part of maxilla to just posterior to eye (about horizontal when mouth closed); rim of orbit usually bright yellow; dorsal fin mostly pinkish with scattered small blackish spots on soft portion, and a yellowish bar on outer soft portion between about 4<sup>th</sup> and 6<sup>th</sup> soft rays; caudal fin pinkish red, upper and lower margins yellow, scattered small blackish spots centrally; anal fin pinkish with a broad yellow bar originating at base of first spine and extending to distal parts of 4<sup>th</sup> or 5<sup>th</sup> soft ray, without spots; pectoral fins pinkish red, without spots; pelvic fins pale pinkish, without spots. In preservative: head, body and fins pale; dark stripe on sides from snout to caudal fin and scattered dark spots on body and fins still distinct.

**Distribution.** This species known from off the southwest coast of India in the Arabian Sea, at depths of 170–260 m, and off the island of Lombok in eastern Indonesia.

**Etymology.** The species is named in honour of John E. Randall, in recognition of his contribution to the taxonomy of marine fishes and more specifically his contribution to the taxonomy of this genus of tropical fishes.

Proposed common name: Indian basslet

**Comparisons.** *Liopropoma randalli* can be distinguished from all other described species of *Liopropoma* by a combination of colour pattern, morphology and meristics. Amongst the Indo–West Pacific species of *Liopropoma*, *L. randalli* is readily distinguished from *L. africanum*, *L. collettei* Randall and Taylor, 1988, *L. flavidum* Randall and Taylor, 1988, *L. mitratum*, *L. multilineatum*, *L. pallidum* (Fowler, 1938), *L. susumi* and *L. tonstrinum* in having a continuous dorsal fin (although deeply notched) vs. a divided dorsal fin with seventh and eighth spines not joined to rest of dorsal fin by a membrane. *Liopropoma randalli* can be distinguished from *L. aurora* (Jordan and Evermann, 1903) and *L. latifasciatum* (Tanaka, 1922) in possessing 12 vs. 13 dorsal soft rays and 8 vs. 9 anal soft rays (Randall and Taylor, 1988). Similarly, *Liopropoma japonicum* (Döderlein and

Steindachner, 1883) is readily distinguished from *L. randalli* in possessing 10 vs. 8 anal-fin soft rays and 14 vs. 12 dorsal-fin soft rays (Randall and Taylor, 1988). *Liopropoma maculatum* (Steindachner and Döderlein, 1883) is also readily distinguished in having a much higher number of lateral line scales (61–66) compared to the new species (46–49). *Liopropoma erythraeum* Randall and Taylor, 1988 and *L. dorsoluteum* can be distinguished from *L. randalli* in having the anterior nostril about same distance from upper lip than from posterior nostril vs. at front of snout and in having the eighth spine shorter than the sixth and seventh vs. the eighth spine longer than the sixth and seventh spines (Randall and Taylor, 1988; Kon *et al.*, 1999). *Liopropoma incomptum* Randall and Taylor, 1988 and *L. aragai* Randall and Taylor, 1988 can be easily separated from the new species in colour pattern with the former two species lacking any dark markings on head, body or fins vs. a dark midlateral stripe and numerous semicircular dark spots. Similarly, *L. swalesi* (Fowler and Bean, 1930) also differs markedly in colour in having 6 or 7 dark stripes on sides and a prominent dark ocellus in soft portions of dorsal and anal fins vs. a single dark midlateral stripe and no ocelli on fins in *L. randalli*.

*Liopropoma randalli* is similar to *L. lunulatum*, a widely distributed Indo–West Pacific species, which also has blackish semicircular-shaped spots on body and sometimes fins. The new species differs from *L. lunulatum* in having a blackish midlateral stripe (vs. no dark stripes on sides) which extends from snout (vs. a yellow stripe from snout to opercle) to base of caudal fin; a narrower body (depth 3.34–3.57 vs. 2.85–3.3 in SL); and longest dorsal ray shorter (longest dorsal fin ray 2.14–2.33 vs. 1.85–2.1 in head length) (Randall and Taylor, 1988). These two species are also differ in structure of the CO1 gene with *L. lunulatum* specimens from French Polynesia (Moorea Biocode project) differing from *L. randalli* by 6.5% ([www.boldsystems.org](http://www.boldsystems.org)).

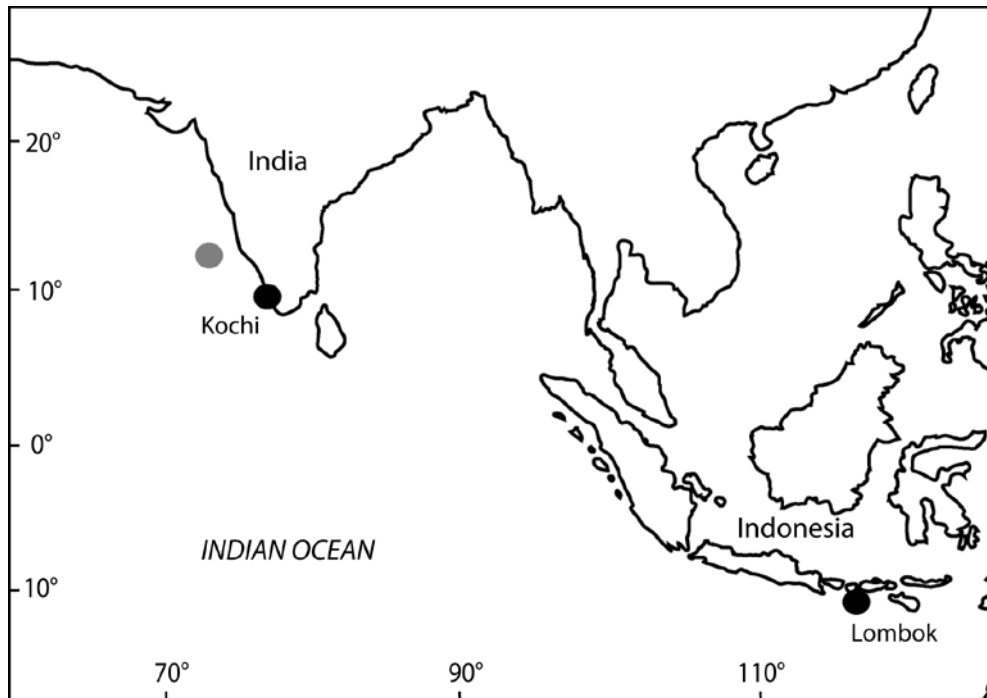
*Liopropoma randalli* is most similar to *L. lemniscatum* from the Northwest Pacific but is easily distinguished from this species in colour pattern with the new species possessing numerous blackish semicircular spots above and below a blackish midlateral stripe vs. a dark brownish midlateral stripe and dorsal midline and without small blackish spots on body or fins. The new species also differs from *L. lemniscatum* in the following characters: First anal-fin spine length 10.4–12.2 vs. 8.2–8.3 in head length; Second anal-fin spine length 4.4–4.9 vs. 3.8–4.0 in head



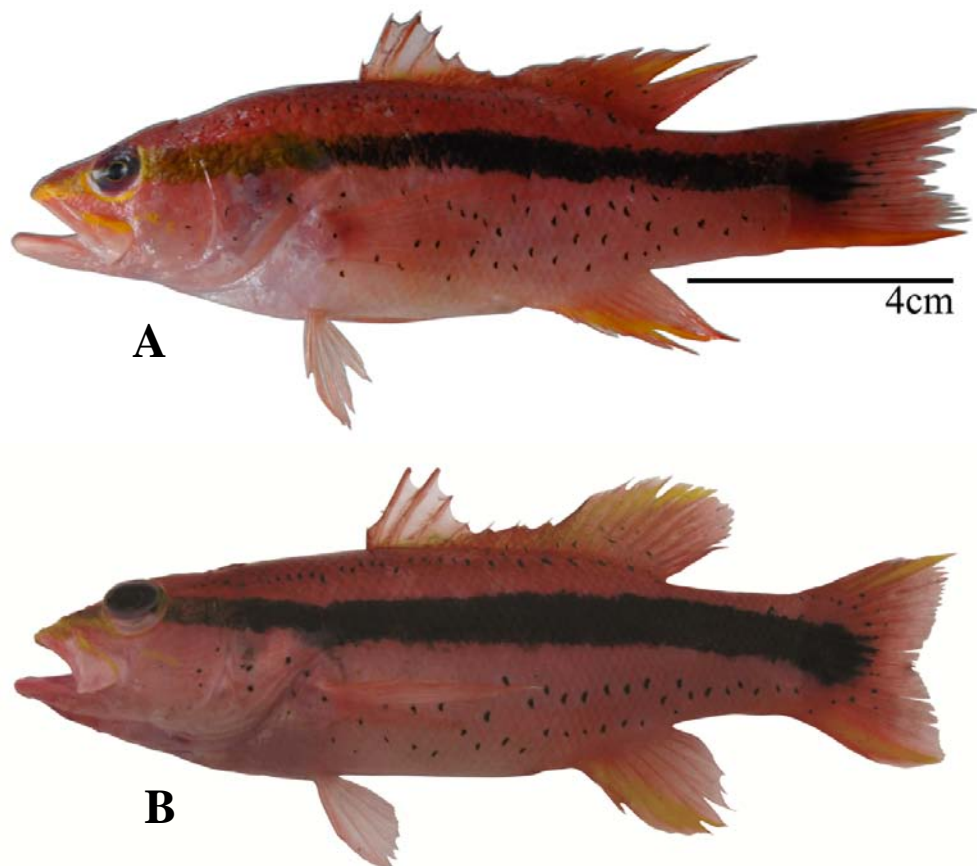
length; caudal fin length 3.8–4.5 vs. 3.7 in SL; and pelvic-fin length 5.0–5.8 vs. 4.7–5.0 in SL. A third *Liopropoma* specimen collected at the Tanjung Luar fish landing site in Lombok, Indonesia, was identified as *L. dorsoluteum*. This specimen is a new distributional record for this species which has previously only been recorded from Japan and Taiwan (Kon *et al.*, 1999).

Specimens examined, including types, are deposited in the collections of: CMFRI – Central Marine Fisheries Research Institute (CMFRI), Kochi, India; CSIRO – Australian National Fish Collection Hobart; KPM – Kanagawa Prefectural Museum of Natural History, Japan; MZB – Museum Zoologicum Bogoriense, Cibinong, Indonesia. Other material examined in this study: *Liopropoma dorsoluteum*: MZB (unreg.), 191 mm SL, Tanjung Luar fish landing site, Lombok, East Nusa Tenggara, Indonesia (08°45' S, 116°35' E), collected by W. White and Dharmadi, 04 Nov. 2010; *Liopropoma lemniscatum*: KPM–NI 18883, female, 16 km off from Kasari Bay, Amami-oshima I., Amami Is., Ryukyu Is., Japan, 340 m depth, 11<sup>th</sup> May 2007, collected by T. Hashimoto.

Holotype. CMFRI GB. 31. 139. 31. 1, 112 mm SL, 112 mm SL, Kochi Fisheries Harbor, Kerala State, gillnet off Mangalore, southwestern India, Arabian Sea, collected by K.V. Akhilesh and K.K. Bineesh, 12 Oct. 2010, NCBI GenBank Accession number: JF505293. Paratypes. 3 specimens: CMFRI GB. 31. 139. 31. 1, 132 mm SL, collected with holotype, NCBI GenBank Accession number: JF505292; CSIRO H 7218–02, 113 mm SL, Tanjung Luar fish landing site, Lombok, East Nusa Tenggara, Indonesia (08°45' S, 116°35' E), collected by W. White and Dharmadi, 26 Jan. 2011; MZB (unregistered), 92 mm SL, Tanjung Luar fish landing site, Lombok, East Nusa Tenggara, Indonesia (08°45' S, 116°35' E), collected by W. White and Dharmadi, 04 Nov. 2010.



**Figure 6.5.** Map showing the collection locations (black circles) for the four type specimens of *Liopropoma randalli*: The grey circle indicates the area where the type specimens from India were caught prior to landing.



**Figure 6.6.** Lateral view of *Liopropoma randalli*: A. holotype, 112 mm SL, Kochi, India (fresh); B. paratype CSIRO H 7218-02, 113 mm SL, east Lombok, Indonesia.

**Table 6.3.** Proportional measurements of the holotype (CMFRI GB. 31. 139. 31. 1) and three paratypes of *Liopropoma randalli* as percentage of standard length. Ranges for all types also provided.

Measurements	Holotype	Paratypes			All types	
		CMFRI GB. 31. 139. 31. 1.1	CSIRO 7218– 02	MZB unreg.	Min.	Max.
Total length TL (mm)	139	163	141	114	114	163
Standard length SL (mm)	112	132	113	92	92	132
Body depth	31.34	27.9	29.93	28.03	27.9	31.34
Body width	16.65	dam.	16.33	16.19	16.19	16.65
Head length	43.56	42.61	40.8	39.05	39.05	43.56
Snout length	10.63	9.96	9.67	9.39	9.39	10.63
Orbit diameter	7.99	7.01	8.39	8.34	7.01	8.39
Interorbital length	7.81	7.43	7.39	7	7	7.81
Upper-jaw length	18.57	17.77	17.06	17.07	17.06	18.57
Caudal peduncle depth	16.78	15.08	16.35	15.71	15.08	16.78
Caudal peduncle length	18.43	17.59	17.88	17.4	17.4	18.43
Predorsal length	45.54	44.17	44.29	42.57	42.57	45.54
Preanal length	69.96	68.77	71.7	69.59	68.77	71.7
Prepelvic length	39.84	38.5	37.39	38.35	37.39	39.84
Dorsal-fin base length	39.04	37.6	38.19	39.44	37.6	39.44
First dorsal spine length	3.96	4.27	3.76	3.75	3.75	4.27
Second dorsal spine length	10.38	10.32	11.97	11.48	10.32	11.97
Third dorsal spine length	11.7	11.9	12.23	11.72	11.7	12.23
Seventh dorsal spine length	5.52	6.98	3.6	4.26	3.6	6.98
Eighth dorsal spine length	6.29	7.74	4.79	4.37	4.37	7.74
First dorsal ray	11.17	11.57	10.24	9.96	9.96	11.57
Longest ray	19.99	18.39	18.95	18.19	18.19	19.99
Last dorsal ray	10.18	8.81	11.73	10.53	8.81	11.73
Anal-fin base length	13.68	13.95	13.9	14.67	13.68	14.67
First anal spine length	3.67	3.56	3.35	3.77	3.35	3.77
Second anal spine length	9.09	9.48	8.35	8.91	8.35	9.48
Third anal spine length	9.72	9.5	9.56	10.43	9.5	10.43
Longest anal ray	19.99	18.86	19.43	18.12	18.12	19.99
Caudal fin length	24.46	23.48	22.98	24.12	22.98	24.46
Pectoral fin length	29.42	25.97	26.16	29.87	25.97	29.87
Pelvic spine length	9.04	9.89	9.45	10.24	9.04	10.24
Pelvic fin length	19.63	18.16	17.68	18.8	17.68	19.63

#### 6.4 *Symphysanodon xanthopterygion*

The marine fish family Symphysanodontidae contains a single genus, *Symphysanodon*, and 11 previously described species (Anderson and Springer, 2005; Khalaf & Krupp, 2008; Quéro *et al.*, 2009). In addition, Anderson and Springer (2005) reported a species of *Symphysanodon*, as yet undescribed, that is known only from the stomach contents of a coelacanth (*Latimeria chalumnae*) caught in the Comoros in the southwestern Indian Ocean, Heemstra *et al.*, (2006) reported a similar species from the demersal habits of Ngazidja islands (Western Indian Ocean) and Campos *et al.*, (2009) reported two larval *Symphysanodon*, collected off southern Brazil, that may represent another undescribed species. Adult specimens of *Symphysanodon* are small to medium-sized fishes, occurring in depths of about 80 to 700 m in the Atlantic, Pacific, and Indian oceans. Four species of *Symphysanodon* have been described from the Indian Ocean (*sensu lato*) *S. andersoni* Kotthaus, 1974 (southwest of Socotra Island, near the entrance to the Gulf of Aden; also reported from the Gulf of Kutch, an inlet in the northeastern quadrant of the Arabian Sea on the west coast of India by Manilo & Bogorodsky, 2003); *S. rhax* Anderson and Springer, 2005 (off the Maldive Islands); *S. disii* Khalaf and Krupp, 2008 (Gulf of Aqaba); and *S. pitondelafournaisei* Quéro *et al.*, 2009 (off Reunion Island).

Methods used are those of Anderson (1970) and Anderson & Springer (2005), counting lateral-line scales on left side where possible. We used the notation of Ahlstrom *et al.*, (1976) to express the formula for the configuration of supraneural bones, anterior neural spines, and anterior dorsal pterygiophores. Institutional abbreviations are: GMBL-Grice Marine Biological Laboratory, College of Charleston, Charleston, South Carolina; UF-Florida Museum of Natural History, University of Florida, Gainesville; USNM-National Museum of Natural History, Smithsonian Institution, Washington, DC; ZMH-Zoologisches Museum der Universität Hamburg, Hamburg, Bundesrepublik Deutschland. DNR-Designated National Repository, Central Marine Fisheries Research Institute, Kochi, Kerala, India.

***Symphysanodon xanthopterygion* Anderson & Bineesh, 2011**

(Figure 6.7; Table 6.4)

**Diagnosis.** A species of *Symphysanodon* distinguishable from the other species of the genus by the following combination of characters. First caudal vertebra with parapophyses. Total gillrakers on first arch 38 to 42. Lateral–line scales 54 to 59. Sum of lateral–line scales plus total number of gillrakers on individual specimens 94 to 101. Length of head 33 to 37% SL. Depth of head 18 to 21% SL. Length of snout 5 to 6% SL. Depth of body 24 to 27% SL. Lower lobe of caudal fin bright yellow; bright yellow spot usually present on posterior part of opercle.

**Description.** Morphometric data appear in Table 6.4. Branchiostegals 7. Dorsal–fin rays IX, 10. Anal–fin rays III, 7. Pectoral–fin rays 16 to 18 (17). Pelvic–fin rays I, 5. Caudal–fin rays: principal 17 (9 + 8); branched 15 (8 + 7); procurrent 12 or 13 dorsally, 11 to 13 ventrally. Gillrakers on first arch 11 to 13 + 27 to 30 (29)—total 38 to 42. Tubed lateral–line scales 54 to 59 (56, 54). Sum of total number of gillrakers plus lateral–line scales, in individual specimens, 94 to 101 (98, 96). No spur on posteriormost ventral procurrent caudal–fin ray, but penultimate ventral procurrent caudal–fin ray shortened basally (see Johnson, 1975). Vertebrae 25 (10 precaudal + 15 caudal). Formula for configuration of supraneural bones, anterior neural spines, and anterior dorsal pterygiophores 0/0/0 + 2 + 1/1/1. First caudal vertebra with parapophyses. Neural spine of second preural centrum short. Autogenous haemal spine associated with second preural centrum. Parhypural autogenous, bearing a hypurapophysis. Hypurals 1 and 2 fused, hypurals 3 and 4 fused. Hypural 5 autogenous. Epurals 3. Epineurals associated with first 9 or 10 vertebrae. Pleural ribs on vertebrae 3 through 10. Trisegmental pterygiophores: 3 associated with dorsal fin, 2 or 3 with anal fin. Snout blunt. Anterior ends of premaxillae incised, forming a notch that receives anterior ends of dentaries. Dorsalmost margin of maxilla covered by very narrow suborbital with mouth closed. Mouth terminal, oblique; premaxillae protrusile; jaws about equal. Maxilla reaching posteriorly to vertical beyond middle of eye. Anterior and posterior nares fairly closely set on each side of snout. Pseudobranchiae present. Interorbital region flattened to very slightly convex. Opercle with two flattened spines. Both limbs of preopercle almost always without serrae, margins usually smooth or almost smooth, occasionally slightly roughened; angle of preopercle with short spine, spine–like process, or a few serrae or

occasionally smooth. Dorsal fin continuous and not incised at junction of spines and segmented rays. Scales ctenoid. Most of head, including maxillae, dentaries, lacrymals, dorsal and lateral aspects of snout, and interorbital region with scales. Branchiostegals and branchiostegal membranes without scales; most of gular region without scales, but with some scales anteriorly. Dorsal and anal fins without scales (except for some scales proximally on posteriormost soft rays), but with low scaly sheaths at their bases; pectoral and pelvic fins scaly basally; both lobes of caudal fin scaly. Large modified scales associated with pelvic fin, just dorsal to pelvic spine (axillary scales) and in ventral midline between the pelvic fins (interpelvic scales). Lateral line gently curved beneath dorsal fin. Caudal fin well forked.

Premaxilla with outer series of small conical teeth and inner band of extremely small teeth; anteriorly teeth in outer series considerably enlarged; premaxillary notch toothless. Dentary with series of small conical teeth extending from elevated posterodorsal surface of jaw almost to symphysis; numerous teeth at anterior end of jaw adjacent to symphysis and on elevated posterodorsal surface of jaw conspicuously enlarged; some enlarged teeth at anterior end of jaw exerted and reaching in advance of premaxillary notch when mouth closed; symphysis toothless. Vomer and palatines with extremely small teeth; vomerine tooth patch chevron shaped, without posterior prolongation; palatine teeth in narrow band. Endopterygoids without teeth. No evidence of teeth on tongue, but tongue with numerous papillae.

**Coloration:** Head mostly red orange, usually with bright yellow patch on posterior part of opercle; body red orange dorsally, pallid ventrally; iris of eye mainly yellow; dorsal fin yellow to orange; pectoral fin rosy; pelvic and anal fins pallid; upper lobe of caudal fin orange to red, lower lobe bright yellow.

**Comparisons.** *Symphysanodon xanthopterygion* can be distinguished from all other described species of *Symphysanodon* except *S. typus* by number of tubed scales in the lateral line—54 to 59 in *S. xanthopterygion* vs. 42 to 52 (60 or 61 in *S. andersoni*); the range of counts of lateral-line scales in *S. typus* (49 to 55) overlaps slightly the range for *S. xanthopterygion*. The range of sums of total numbers of first-arch gillrakers plus numbers of tubed lateral-line scales in individual specimens is 94 to 101 in *S. xanthopterygion*, distinguishing it from all other species except *S. typus* which, has a slightly overlapping range range of 86 to 94. The ranges

for a number of morphometric characters (in percentages of standard length) in *S. xanthopterygion* differ (or overlap only slightly) with those of *S. andersoni* and *S. typus*. The photograph included with the account of *S. typus* shows a fish with a yellow patch on the posterior part of the opercle, yellow lower caudal-fin lobe, and overall light rosy coloration (Kimura *et al.*, 2003). The coloration of *S. xanthopterygion* is similar, but the yellow on the lower caudal-fin lobe is brighter and the general body coloration is redder. *S. xanthopterygion* is fairly common in collections made off Quilon in 150 to 250 meters.



**Figure 6.7.** Paratype of *Symphysanodon xanthopterygion*, CMFRI/PFD/SYM/8.1, 127 mm SL; southeastern Arabian Sea, Kerala Coast, off Quilon, India.

**Etymology.** The name *xanthopterygion* is from the Greek—*xanthos* (yellow), *pterygion* (fin)—referring to the yellow coloration of the lower caudal-fin lobe. The name of the new species is a noun in apposition to the generic name *Symphysanodon*.

Proposed common name: Indian Bunquelovely, Indian slopefish

**Holotype:** USNM 400886, 141 mm SL, female, off Quilon, India, Malabar Coast, southeastern Arabian Sea, 09°05' N, 75°52' E; 240 meters; collected by K.K. Bineesh and K. V. Akhilish, 08 September 2010.

**Paratypes:** DNR GB. 31.146.1.2, DNR GB. 31.146.1.2.1, DNR GB. 31.146.1.2.2–3 specimens, 127–131 mm SL; GMBL 10–016, one specimen, 136 mm SL; USNM 400887, one specimen, 145 mm SL. CMFRI/PFD/SYM/8.1–8.8, 8 specimens, 119–146 mm SL; UF 180312, one specimen, 137 mm SL.

**Table 6.4.** Morphometric data on *Symphysanodon xanthopterygion*. Standard lengths in mm, other measurements in percentages of standard length; > = slightly damaged

Measurement	n	Range	Holotype
Standard length	15	119–146	141
Head, length	15	33.2–36.5	35
Head, depth	15	18.4–20.5	19.8
Snout, length	15	5.1–6.2	6
Fleshy orbit, diameter	15	8.1–9.6	9.6
Postorbital length of head	15	17.6–21.3	17.8
Suborbital width	15	0.24–0.87	0.57
Cheek, height	15	5.7–7.0	6.7
Maxilla width	15	4.0–5.6	4.2
Upper jaw, length	15	13.6–15.0	14.4
Lower jaw, length	15	14.0–15.2	14.8
Bony interorbital, width	15	6.1–7.9	7.1
Internarial distance	15	0.60–0.93	0.71
Predorsal–fin length	15	33.9–35.6	35.5
Body, depth	15	23.8–27.4	27
Caudal peduncle, depth	15	8.8–10.5	9.8
Caudal peduncle, length	15	4.0–26.6	24.3
Anal fin, length of base	15	3.2–16.0	14.9
Depressed anal fin, length	15	24.0–27.8	25.2
Pectoral fin, length	15	23.5–29.4	29.4
Pelvic fin, length	15	20.5–24.3	23.2
Upper caudal–fin lobe, length	13	29.1–47.3	ca. 39.3
Lower caudal–fin lobe, length	13	28.4–40.4	ca.36.4
First dorsal spine, length	15	4.3–6.3	6
Second dorsal spine, length	13	7.8–9.4	9.1
Third dorsal spine, length	15	9.8–11.6	10.7
Fourth dorsal spine, length	14	10.8–13.1	12.5
Last dorsal spine, length	15	11.1–13.0	11.7
Longest dorsal spine, length	15	11.4–13.5	12.7
First anal spine, length	15	3.8–5.9	4.2
Second anal spine, length	15	8.1–9.4	9.2
Third anal spine, length	15	9.8–11.6	11.5

### 6.5 *Opisthognathus pardus*

The ten species of *Opisthognathus* previously known from the western Indian Ocean, including the Red Sea, were newly described or reviewed by Smith-Vaniz (2009, 2010). A total of 39 Indo-West Pacific species of *Opisthognathus* are currently recognized as valid (Eschmeyer and Fong, 2012), with at least 19 others yet to be described. In addition to the new species described herein, only four other species of



*Opistognathus* are known from India or Sri Lanka: *Opistognathus nigromarginatus* Rüppell, 1830, *O. rosenbergii* Bleeker, 1856, *O. variabilis* Smith–Vaniz, 2009 and *O. macrolepis* Peters, 1866.

The Indian Ocean record of *Opistognathus macrolepis* is based on a color photograph of a 63 mm SL specimen (ZSI F-10576/2) collected off the Kalpakkam coast near Chennai (Tamil Nadu). This jawfish was previously known only from the type locality (Bangkok), the Gulf of Thailand where described by Wongratana (1975) as *Opistognathus rex*, and the Gulf of Carpentaria where described by Whitley (1966) as *Merogymnoides carpentariae*.

Methods of counts and measurements follow Smith–Vaniz (2009, 2010). Infraorbital bones and the upper and lower jaws on the right side of the holotype were removed, cleared and stained and drawn with the aid of a camera lucid. Position of the fifth cranial nerve was determined by dissection prior to clearing and staining. Abbreviations for institutional depositories are Central Marine Fisheries Research Institute (CMFRI) and Zoological Survey of India (ZSI), Kolkata.

***Opistognathus pardus* Smith-Vaniz, Bineesh & Akhilesh, 2012**

(Figure 6.8)

**Diagnosis.** A species of *Opistognathus* with the following combination of characters: rigid maxilla without flexible lamina posteriorly; head mostly covered with small, irregular-shaped dark spots (head without such spots except in the Australian species *O. darwinensis* Macleay, 1878); dorsal fin XI, 11; total gill rakers 40–41; outermost segmented pelvic-fin ray tightly bound to adjacent ray, with interradiation membrane not incised distally (interradiation membrane deeply incised in all other Indo-Pacific species except *O. muscatensis* Boulenger, 1887).



**Figure 6.8.** *Opistognathus pardus*, CMFRI GB.31.104.1.2, off Kollam, Kerala.

**Description.** (When bilateral counts vary, those from the right side are given in parentheses). Dorsal-fin rays XI, 11. Anal-fin rays II, 11. Pectoral-fin rays 22 (23). Caudal fin: procurrent rays 3+3, segmented ray 8+8, middle 12 branch; hypural 5 present. Vertebrae: 10 precaudal+16 caudal, last rib on vertebra 3. A single supraneural bone inserted between neural spines 1–2. Gill rakers 15 (16) + 25.

Scales absent on head and body anterior to vertical from 3rd dorsal-fin spine, including area above and below lateral line; pectoral-fin base with a few scales or scale pockets and belly completely scaly. Body with about 42 or (44) oblique scale rows. Lateral-line ends below vertical from 2nd segmented dorsal-fin ray. Lateral-line pores arranged in single series along embedded lateral-line tubes. Cephalic sensory pores relatively sparse and absent from nape; all dentary and preopercular pore positions with relatively large, single pores. Two relatively large infraorbital pores present posteriolaterally on the right side of the holotype could not be detected on left side.

Anterior nostril about mid-way between posterior nostril and dorsal margin of upper lip, consisting of short simple tube that when depressed does not reach posterior nostril; height of anterior nostril shorter than maximum diameter of posterior nostril. Dorsal fin moderately low, gradually increasing in height to about middle of spinous dorsal fin; profile with only slight increase in height at origin of segmented rays. Dorsal-fin spines relatively slender and straight without flexible tips; all except 1<sup>st</sup> and 2<sup>nd</sup> segmented dorsal- and 1st anal-fin rays branched distally. Outermost segmented pelvic-fin ray tightly bound to adjacent ray, interradiar membrane not incised distally. Posterior margin of preopercle with indistinct free margin. No papillae on inner surface of lips. Fifth cranial nerve passes under A1 $\beta$  section of adductor mandibulae.

Upper jaw extends about 1.0 eye diameters behind posterior margin of orbit, widest at end and slightly rounded, without a flexible lamina posteriorly (Fig. 4); supramaxilla relatively large and terminaly positioned. Premaxilla with an outer row of moderate conical teeth; anteriorly outer teeth with irregular inner series of smaller conical teeth and a wide inner band of much smaller teeth which is bordered by an inner horizontal row of 5–6 teeth at least as large as those in the outer row. Dentary with an outer row of conical teeth which become larger posteriorly; anteriorly an inner band of much smaller teeth, those on the margin largest; posteriorly band

becomes a single inner row of 5–6 teeth as large as the adjacent outer row teeth. Vomerine teeth absent. Infraorbital bones tubular with wide openings for sensory canals (Fig. 4), 3rd infraorbital relatively robust and without a suborbital shelf.

Measurements of the 98.8 mm SL male holotype, as percent of SL: predorsal length 30.1; preanal length 60.2; dorsal–fin base 64.2; anal–fin base 30.8; pelvic fin length 15.7; caudal fin length 24.9; depth at anal–fin origin 21.3; caudal peduncle depth 12.2; head length 33.8; postorbital–head length 22.3; upper jaw length 20.3; postorbital–jaw length 9.3; orbit diameter 9.4. As percent of head length: postorbital–head length 65.9; upper jaw length 60.2; postorbital–jaw length 27.5; orbit diameter 27.9.

**Color in life** (Fig. 6.8). The most distinctive aspect of the color pattern is a series of small, irregular, dark brown spots, on a light tan background, which completely cover the head except for the venter, upper jaw and lower half of the opercle; throat and gill membranes orange–yellow; many scales missing on body but those remaining suggest the body was uniform brown, becoming paler ventrally; dorsal fin background color mostly dusky orange–brown becoming darker distally, except middle of soft dorsal fin brown with broad yellow distal margin; spinous dorsal fin with narrow pale margin and three gray–blue markings, each with narrow dark margins; very narrow diagonal stripe extends from near tip of 1st spine to anterior base of 3<sup>rd</sup> spine; ocellus with dusky center on basal third of fin between spines 5–7 and on basal half of fin between spines 9–11; smaller ocellus also present on middle of soft portion of dorsal fin; anal fin yellow–orange; pectoral fin tan and caudal fin brown with posterior margin yellow–orange, narrower ventrally.

**Color pattern after preservation.** Similar to above with head spots the most conspicuous markings and ocelli on dorsal fin less apparent; inside of mouth and lining of upper jaw and adjacent membranes pale.

**Comparisons.** *Opistognathus pardus* can be distinguished from all other species of the genus by its unique color pattern, consisting of a heavily spotted head and the spinous dorsal fin with a very narrow, pale diagonal stripe anteriorly followed by two ocelli. It and *O. muscatensis* are the only Indo–Pacific species that have the two outermost segmented pelvic fin rays tightly bound to each other without the interradiation membrane incised distally. In addition, other species with a rigid maxilla,

without a flexible lamina posteriorly, typically have more segmented dorsal–fin rays (12–13) or fewer total gill rakers (19–37) or both. *Opistognathus macrolepis* also differs in having a uniform brown body, dorsal and anal fins with a dark stripe, 29–33 total gill rakers and the outer premaxillary and dentary teeth relatively elongate with blunt tips.

**Etymology.** From the Greek Pardos (leopard), in reference to the distinctive pattern of head spots. The name should be treated as an appositional noun.

Proposed common name for this species is Leopard jawfish.

**Holotype.** CMFRI GB.31.104.1.2, male 98.8 mm SL, off Quilon, Kerala, SW coast of India, trawled in 110–220 m, obtained from local fisherman at Sakthikulangara Fisheries Harbour (Quilon) 27 August 2010.

### 6.6 *Dipturus* sp. A

Skates (order Rajiformes, family Rajidae) are an extremely diverse group of fishes, characterized by a high morphological conservatism (McEachran and Dunn, 1998). *Dipturus* Rafinesque, 1810 is the second most speciose genus within Rajidae, with approximately 44 described species (Ebert and Compagno, 2007; Last *et al.*, 2008). *Dipturus* species are medium to large sized skates, most exceed 100 cm TL and some reach up to 250 cm TL (Seret, 1989). Members of the genus *Dipturus* exhibit long rostral cartilage (length more than 60% of dorsal head length), greatly depressed and laterally expanded mesocondyle, and large in size when adult (total length greater than 55 cm) according to Ishihara (1987). The interrelationship of the genus *Dipturus* to other closely related skate genera of the subfamily Rajini is largely unresolved. A wide–ranging genus in cool temperate to tropical waters, found mostly on continental shelves and slopes (Ebert and Compagno, 2007). The genus is most abundant in the Indo–Pacific region and Atlantic Ocean (Last *et al.*, 2008), but poorly represented in the northern Indian Ocean.

The skates are poorly known in the Eastern Arabian Sea, especially the deepsea forms. In Indian waters eight species of skates are reported to occur but the status and identification of several species are considered questionable and requires confirmation (Akhilesh *et al.*, 2014). Presently, the only member of the genus *Dipturus* recognized as occurring in Indian waters is *D. johannisdavisi* (Alcock, 1899). This species is easily distinguished from other regional skates by the presence

of an elongated, acutely pointed snout, single nuchal thorn, and the absence of dorsal median thorns (Alcock, 1899). During fish landing site surveys along the west coast of India, on 25<sup>th</sup> December 2008 an unusual skate exhibiting characteristics of the genus *Dipturus* distinct from all congeners in several characters was observed at Cochin Fisheries Harbor, Kerala, southwest coast of India. Herein we described these specimens as a new species.

Morphometric measurements follow a modification of Hubbs and Ishiyama (1968) and Last *et al.*, (2008), and meristics include tooth, spiral valve counts. The specimens were deposited at CMFRI and NBFGR, Kochi, India.

***Dipturus* sp. A**

(Figure 6.9–6.12. Table 6.5)

Arabian skate

**Diagnosis.** A large species of *Dipturus*, with the following combination of characters: one median dorsal thorn row on disc. Five rows on tail. Pre orbital snout length 20.8% TL, pre oral snout length 20.8% TL, Inter orbital distance 2 in orbit length, pre orbital disc width 310 mm (27.4% TL) Posterior edge of nasal curtain fringed. Dorsal and caudal fins with small spinicules. Tail gradually tapering posteriorly. Interdorsal space shorter than base of first dorsal fin. Dorsal and ventral surface of disc generally prickly rough except for smooth area near the rostrum. Dorsal rostrum with a band of developed prickles and that on anterior of snout denser, anterior most dorsal and ventral tip of rostrum devoid of prickles. Interorbital and branchial space prickly with few developed dermal denticles. Intestinal spiral valve with 11 turns. Tooth with 33/33-4/5. Pelvic fin radials 1 +23.

**Description.** Disc quadrangular and relatively broad, 1.2 times as wide as long in holotype, width 73.8% of total length (TL), maximum disc width at 64.3% disc length (DL) and 38.5% TL; disc with angular to narrowly rounded apices, anterior weakly concave and posterior broadly convex; disc margin anterior to spiracles weakly convex with short concavity near snout tip, weakly concave from spiracles to apices of pectoral fins; apices rounded; strongly convex posterior margin of disc; free rear tip broadly rounded. Head very long, length (ventral) 2.8 in TL, 1.7 in DL. Snout very well produced, narrowly pointed, preorbital snout length 7.3 times eye length, 3.8 times interorbital space, 34.8% DL; preoral length 2.2 times internarial

distance, 26.7% DW. Snout tip pointed, lacking distal process or filament. Snout angle 76.3°. Eyes smaller than spiracle, eye length 2 times interorbital space. Spiracle large, length 1.1 times eye length, opening sub-rhomboidal in shape. Distance between spiracles 1.2 times distance between orbits. Nostril sub-circular to oval; anterior nasal flap forming an open, posterolaterally directed tube, mostly unobscured by nasal curtain; posterior lobes very well developed, meeting medially to form nasal curtain, produced posterolaterally, distal ends sub-rectangular with fringe on the posterior margin in holotype; internarial distance 2.1 times distance between first gill slits, 1.2 times distance between fifth gill slits. mouth width 1.1 times internarial distance; lateral teeth obscured by posterior nasal flaps; total tooth counts in upper jaw 33 arranged in 4 rows and in lower jaw 33 arranged in 4 rows; teeth arranged in quincunx, blunt and flat in females, tooth shape unknown in adult males.

Pelvic fins large, anterior lobes long, well-developed, with a deeply incised lateral margin and elongated posterior lobes; anterior lobe length 11.1% TL, with a narrowly rounded to bluntly pointed tip; posterior lobe 11.9 times anterior lobe length, lateral margins convex, inner margin weakly convex, tips rounded distally. Pelvic fin radials 1 + 23. Tail rather slender, not strongly depressed, not tapering distally from base to first dorsal fin; width at insertions of pelvic fins 2.0 times width at first dorsal fin origin; tail moderately long, length 43.8% TL; tail width 1.1 times height at pelvic fin insertion, width at first dorsal fin origin 1.1 times height; lateral tail fold present but not well developed (clearly visible from below first dorsal extending to tail tip and broadest at below and behind second dorsal fins. Dorsal fins medium-sized, first dorsal fin height 2.0 times base length; dorsal fins similarly sized: first dorsal fin taller than second dorsal fin but with same base lengths; dorsal fins strongly raked with long bases; anterior margins convex; first dorsal fin apices rounded, second dorsal fin apices angular; inner margins posterodorsally directed; dorsal fins separated, with interdorsal distance 57.9% first dorsal fin base length.

Dorsal surface with rostral, orbital, postorbital, nuchal thorns, median and tail thorns present. Orbital thorns 21–24, regularly spaced, arranged in semi-circle from preorbit to anterior margin of spiracle; equal in size, thorns well developed and posteriorly directed. Postorbital thorns (spiracular) 16–19 are immediately posterior to last orbital thorn. Nuchal thorns probable variable in number, holotype had 7

thorns with expanded bases. Midback thorns 13 arranged in a single median row and directed posterolaterally. Disc posterior free rear area between posterior and inner margins with small patch of thornlets; Tail thorns of holotype arranged in 5 rows (2 irregular); median thorn row larger than others; thorns well developed, strongly recurved, posteriorly directed; with some staggering; thorns well developed and equal in size between rows, strongly recurved. Predorsal thorns 45 (18 small) in holotype and beginning around the pectoral fin insertion. Interdorsal thorns of 4, arrangement on holotype 2 larger and 2 smaller thorns. Intestinal spiral valve 11 turns.

**Coloration.** Dorsal surface uniformly dark brown, with dark grey ventral surface. Rostral sides very pale. Holotype with a small dark blotches on rostrum (dorsal) and dorsal and ventral side. Ventral disc with small black pores. Denticles along the ventral anterior margin whitish.

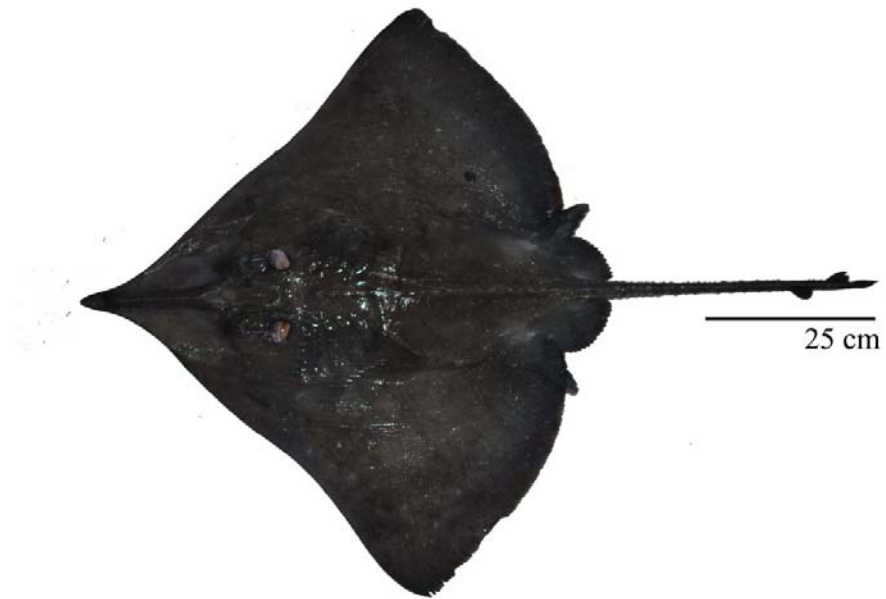
**Barcode sequence.** A 630 base pair amplicon from the 5' region of the mitochondrial COI gene was bidirectionally sequenced for eight specimens. Specimens of *Dipturus* sp. A, *Dipturus* cf. *johannisdavisii*, *Dipturus* sp. B and *Okamejei powelli* were barcoded. The COI sequences of three species of *Dipturus* and one species of *Okamejei powelli* showed a clear cut barcode split congruent with morphological diversity.

**Size.** Maximum total length is 113 cm TL for an adult female and the only known specimen of this species to date.

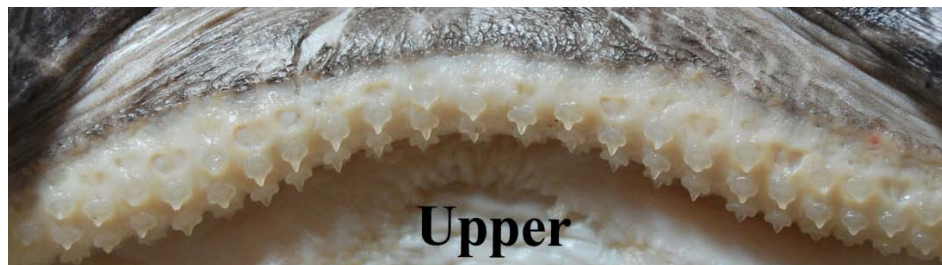
**Distribution.** Presently known from continental slope off southwest coast of India (Eastern Arabian Sea) at depths of 400 to 550 m and off Chennai, east coast of India (Bay of Bengal) at depths of 500 to 600 m.

Proposed common name is Arabian skate.

**Remarks.** *Dipturus johannisdavisii* (Alcock, 1899) a small sized *Dipturus* species (max: 230 mm DW) is the only valid *Dipturus* species occurring in Indian waters, which is clearly distinct from *Dipturus* sp. A in its size and other features like the presence of single nuchal thorn, no median dorsal spines, and a single tail thorn row. Alcock (1899) previously suggested the possibility of a large undescribed species of *Dipturus* off the Kerala coast at a depth of 1483 m.



**Figure 6.9.** Dorsal view of *Dipturus* sp. A (CMFRI GA. 4.11.2.2)



**Figure 6.10.** Upper teeth structure of *Dipturus* sp. A (CMFRI GA. 4.11.2.2)



**Figure 6.11.** Lower teeth structure of *Dipturus* sp. A (CMFRI GA. 4.11.2.2)



**Figure 6.12.** Lateral view -dorsal and caudalfins of *Dipturus* sp. A (CMFRI GA. 4.11.2.2,)



**Table 6.5.** Morphometric data for the specimen of *Dipturus* sp. A Measurements expressed as percentage of total length.

Measurements	GA.4.11.22,female
Total length (mm)	1130
Disc width	73.8
Disc length (direct)	59.8
Snout to maximum width	38.5
Snout length (preorbital direct)	20.8
Snout to spiracle (Direct)	25.8
Head (ventral length)	36.3
Head (dorsal length)	27.4
Orbit diameter	2.8
Orbit and spiracle length	5.4
Spiracle length (main aperture)	3.2
Distance between orbits	5.5
Distance between spiracles	6.4
Snout to cloaca (1st hemal spine)	58
Cloaca to first dorsal	29.2
Cloaca to second dorsal	34.2
Cloaca to caudal-fin tip distance	42.5
Cloaca to caudal origin	38.3
Ventral snout length (pre upper jaw)	19.7
Pre oral snout length	20.8
Prenasal length	18.6
Mouth width	8.4
Distance between nostrils	8.9
Nasal curtain length	4.1
anterior nasal curtain base width	1.5
Nasal curtain (total width)	9.7
Nasal curtain (min. width)	9
Snout to first gill	30.8
Width of first gill opening	2.8
Width of third gill opening	3
Width of fifth gill opening	2
Distance between first gill openings	18.9
Distance between third gill openings	14.9
Distance between fifth gill openings	11.1
Length of anterior pelvic lobe	11.1
Length of posterior pelvic lobe	11.9
Pelvic base width	7.6
Tail at axil of pelvic fins (width)	2.6
Tail at axil of pelvic fins (height)	2.4
Tail at midlength (width)	1.2
Tail at midlength (height)	1.4
Tail at D1 origin (width)	1.3
Tail at D1 origin	1.2
D1 base length	3.3
D1 height	1.6
D1 origin to caudal-fin tip	13.1
D2 origin to caudal-fin tip	7.8
D2 base length	3.6
D2 height	1.7

Interdorsal distance	1.9
Caudal-fin length	4.1
Caudal-fin base length	4.1
Caudal fin height	0.5
Cloaca to pelvic-clasper insertion	3
Snout to origin of first dorsal	87.3
Snout to origin of second dorsal	92.4
Tail length (haemal spine - tail tip)	42.3

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**Chapter 7**

**Summary**

## Chapter 7

### Summary

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The deep-sea fishes are those living at depths greater than 200 m and exhibit diverse adaptations to live in the deep-sea habitat. They use many adaptations to live in the extreme habitat and these ecological characteristics of deep-sea fishes can make them vulnerable to fishing pressure. These species often have a slow growth rate, high longevity, low fecundity and hence low productivity. Recently, many of the deep-sea fishes are getting a good market price and the quantities of these groups landed have also increased. Despite the rich diversity and new fishing grounds for targeted shrimp resources, works on taxonomy are limited. There have been very few recent attempts to resolve the confusions especially in the deep-sea species, using molecular tools on Indian deep-sea fishes. Hence, the present study was undertaken to (i) generate reference molecular signatures of deep-sea fish species found on the southern coast of India using partial sequences of two mitochondrial genes - 16S rRNA and COI (ii) analyse the genetic divergence within and between species to resolve taxonomic ambiguity, if any, and to describe any new deep-sea fishes encountered during the study.

- ❖ Altogether 120 species of deep-sea fishes from 63 families were collected from the southern coast of India. Field identification and nomenclature of the fish species collected were followed based on the morphological and meristic characters described by Alcock, Smith's Sea fishes, and other published papers. The identification of deep-sea chondrichthyans species was based on Alcock (1899), Misra (1969), Talwar and Kacker (1984), Compagno (1984), Last and Stevens (1994), Carpenter and Niem (1999), and Compagno *et al.*, (2005).
- ❖ Fresh gills and muscle tissue samples (100 mg approximately) were collected from the specimens before formalin fixation and were used for DNA extraction following the standard salt extraction protocol and DNeasy (Qiagen) DNA extraction kit. Two DNA fragments were amplified, representing two partial gene regions such as 16S rRNA and COI by employing specific universal primers.

- ❖ A total of 94 bidirectional partial sequences of mitochondrial 16S rRNA (525-591 bp) and 178 sequences of COI (640-672 bp) genes was generated from deep-sea chondrichthyan species belonging to 38 species and 28 genera.
- ❖ A total of 120 bidirectional partial sequences of mitochondrial 16S rRNA (608-624 bp) and 426 sequences of COI (588-675 bp) genes was generated for deep-sea teleost fishes belonging to 82 species and 62 genera.
- ❖ The estimates of genetic divergence with all the two genes were sufficient enough to discriminate all the individuals of different species collected during the present study. The mean pairwise K2P genetic distance values among species based on 16S rRNA ranged from 1.8% to a maximum of 24%. With COI, the maximum inter-specific K2P distance was 32% and minimum 3%.
- ❖ Two species, *Odontanthias perumali* and *Lophiodes triradiatus* are resurrected by examining fresh materials. A taxonomic re-evaluation of the status of the *Holanthias perumali* Talwar, 1976 combining meristic, morphological and molecular data (COI) reveal that *Holanthias perumali* is very distinct species from *Odontanthias rhodopeplus* (Gunther, 1872).
- ❖ Five species of deep-sea fishes, *Chelidoperca investigatoris*, *Chelidoperca occipitalis*, *Chlorophthalmus corniger*, *Sphenanthias whiteheadi* and *Rhinobatos variegatus* were redescribed using freshly collected materials.
- ❖ A very rare bandfish, *Sphenanthias whiteheadi*, is re-discovered and described from the southwest and southeast coasts of India for the first time after its original description and the rarity of the fish is challenged. A mitochondrial COI barcode sequence was generated for the specimen.
- ❖ The Stripenose Guitarfish, *Rhinobatos variegatus* is one of the least known guitar fish species of the world known only from a single female specimen collected from the Gulf of Mannar (Bay of Bengal), Tuticorin. Present study, redescribed *Rhinobatos variegatus*, based on the specimens collected from the southern coasts of India.
- ❖ Several authors considered *Chlorophthalmus bicornis* is a valid species but this study confirm that *C. bicornis* is a junior synonym of *C. corniger*. *Chlorophthalmus corniger* is redescribed on the basis of recently collected

specimens. The species is redefined as a species of *Chlorophthalmus* with the lower jaw terminating in a distinctly projecting horizontal plate with strong, spine-like processes directed forward from the plate's corners; body silvery grey, with numerous minute black spots and traces of broad darker crossbars; base of anterior dorsal fin spines and distal parts of dorsal fins black; adipose fin tiny with numerous black spots; caudal fin black; 3.5 scales above lateral line; three rows of cheek scales; head very large, 34.3–40.1% standard length (SL); eye large, 29.8–40.8% head length (HL); pectoral fin long, extending to beyond dorsal fin base, 21.7–26.2% SL. *C. bicornis* is a junior synonym of *C. corniger* based on the examination of the type series of both species. It is confined to the northern half of the Indian Ocean, reliably recorded from Somalia and the Gulf of Aden to southern Java, Indonesia, at depth 200-500 m. A lectotype and three paralectotypes were designated for *C. corniger*.

- ❖ *Chelidoperca investigatoris* (Alcock, 1890) was described from two specimens collected off the Ganjam coast, Bay of Bengal and *Chelidoperca occipitalis* Kotthaus, 1973 was described from a single specimen collected off the Socotra Islands, Arabian Sea. In the present study, more additional specimens of these two species were collected from off Kollam, southwest coast of India. For *C. occipitalis* report from southwestern India forms a considerable extension of its known distribution range. *Chelidoperca investigatoris* and *C. occipitalis* are redescribed based on these fresh specimens.
- ❖ A new species of serranid fish, *Chelidoperca maculicauda* are described based on three specimens, (123-129 mm SL), recently collected from the Arabian Sea, off Quilon, Kerala, India. The combination of caudal fin shape and a unique colour pattern of five red bars on a pinkish body and pale yellow fins with a bright red margin on the anal fin, a small grey spot distally on the dorsal half and bluish white spots on the ventral half of the caudal fin, distinguishes the new species from other congeners. Other distinguishing characters include: head length 40.3 (42.3-42.6)% SL; orbital length 9.3 (8.9- 9.1) in SL; 2.5-3 scales above lateral line to dorsal origin; serrae on margin of preopercle 40-46. Lateral-line scales 42; dorsal fin

continuous, with ninth dorsal spine shorter than tenth spine; longest dorsal soft ray (7<sup>th</sup> or 8<sup>th</sup>) 2.4 (2.3-2.4) in head length.

- ❖ *Symphysanodon xanthopterygion*, new species, reported herein from 15 specimens collected near Quilon, India, off the Kerala coast in the southeastern Arabian Sea, becomes the twelfth described species in the genus. The following characters in combination allow the separation of *Symphysanodon xanthopterygion* from its congeners: parapophyses present on first caudal vertebra, total number of gillrakers on first arch 38 to 42, tubed lateral-line scales 54 to 59, sum of lateral-line scales plus total number of gillrakers in individual specimens 94 to 101, length of head 33 to 37% SL, depth of head 18 to 21% SL, length of snout 5 to 6 % SL, depth of body 24 to 27 % SL, lower lobe of caudal fin bright yellow .
- ❖ A new epinephelid fish, *Liopropoma randalli* is described based on two specimens gillnetted off the southwestern coast of India (off Mangalore, Arabian Sea) and landed at Kochi (Kerala). It differs from all other species in the genus in its striking colour pattern, a broad black band from behind the eye to the caudal peduncle, semicircular dark-brown to black spots that cover the pink to reddish body, and a combination of the following characters: 46 to 47 lateral line scales and 5–6 + 12–13 (the last 5 as rudiments) gill rakers on the first arch, longest dorsal spine (third) which is 3.6–3.7 HL; shorter pelvic fins 2.3–2.5 in HL and snout length 9.96 to 10.63 % of SL.
- ❖ A new species of Jawfish, *Opistognathus pardus*, is described based on a single specimen, 98.8 mm SL, recently collected from the Western Indian Ocean off Quilon (Kerala), India. The combination of a rigid maxilla without flexible lamina posteriorly, a unique colour pattern in which most of the head is covered with small, irregular-shaped, dark spots, dorsal-fin rays XI, 11, and the outermost segmented pelvic-fin ray tightly bound to adjacent ray, with the inter radial membrane not incised distally distinguishes the new species from other congeners.
- ❖ A new species of anthiine fish, *Plectranthias alcocki* is described and illustrated based on two specimens, (63.7– 72.5 mm SL), recently collected from deep-waters of the Arabian Sea, off Kollam, Kerala, India. The

following combination of characters distinguishes it from all other congeners: Dorsal-fin rays X, 15; anal-fin rays III, 7; pectoral-fin rays 14, all unbranched; pelvic-fin rays I, 5; lateral-line complete, the pored lateral-line scales 28; scales above lateral line to origin of dorsal fin 1; scales dorsally on head extending to posterior nostrils; no scales on maxilla or chin; orbital length 8.6 in SL; margin of preopercle finely serrate, the serrae 33 (28), ventral edge without antrorse spines; dorsal fin continuous and notched; first anal-fin spine 4.9 (5.6) in HL, second anal-fin spine 2.2 (2.6) in HL; pelvic fins relatively short, 4.0–4.3 in SL; the dorsal fin with a black blotch at base of fourth to eighth spines, one at base of the last three spines, and two at base of soft portion of fin, the dark pigment extending onto adjacent body.

- ❖ A new longnosed skate, *Dipturus pillai* sp. nov., is described based on seven specimens, collected off southwest coast of India (Arabian Sea) and off Chennai (Bay of Bengal) at 400-600 m depth. This can be separated from other species of *Dipturus* from the Arabian Sea based on size, colour, characters like disc width 73.8%TL; disc length (direct) 59.8 %TL and characters like; single median thorn row on disc, 5 thorn rows on tail (2 irregular); nuchal thorns separated from the median dorsal thorns by a small gap, presence of orbital and spiracular thorns, absence of scapular thorn.
- ❖ In this study, 38 deep-sea chondrichthyan species from Indian waters were barcoded for a 655 bp region of the mitochondrial gene, cytochrome c oxidase subunit 1 (COI). 178 specimens from 36 species and 28 genera were assessed. Generally five specimens were barcoded per species, but numbers ranged from one to thirteen. The average Kimura 2 parameter (K2P) distance separating individuals within species was 0.32%, and the average distance separating species within genera 6.73%. Seven taxa, from the genera *Apristurus*, *Iago*, *Squalus*, *Torpedo*, *Narcine*, and *Dipturus*, were suggested as putative new species that requiring formal species description. The present study attests to the ability of DNA barcoding to accurately identify sharks, rays and their products from Indian waters.
- ❖ Altogether, 82 deep-sea teleost species of 43 families from Indian waters were barcoded for a 655 bp region of the mitochondrial gene, cytochrome c oxidase subunit 1 (COI). The collection comprises 18 fish species that were not previously reported from the Indian waters. Eight of these taxa are



confirmed as putative new species and six as new records for Indian waters. The overall mean distance of individuals among the deep-sea teleost fishes was 23.3%.

- ❖ Thirty five individuals of six priacanthids species were sampled from different localities along the coast of India covering the Arabian Sea and Bay of Bengal. The partial sequence of 16S rRNA and cytochrome c oxidase subunit I (COI) genes were analysed for species identification and phylogenetic relationship among the Indian priacanthids (*Priacanthus hamrur*, *P. prolixus*, *P. blochii*, *P. sagittarius*, *Cookeolus japonicas* and *Pristigenys refulgens*). The genetic distance of intraspecies ranged from 0.000 to 0.002, while it varied from 0.049 to 0.158 for interspecies based on 16S sequences. Using COI data analysis, the genetic distance of intraspecies ranged from 0.000 to 0.005, while it varied from 0.057 to 0.162 for interspecies. In the current GenBank, *Priacanthus prolixus* had been considered as *P. hamrur* by mistake. Here we observed cryptic speciation for the species *Heteropriacanthus cruentatus*. Partial sequences of 16S rRNA and COI genes provided phylogenetic information to distinguish the thirteen species of Priacanthids indicating the usefulness of molecular markers in species identifications.
- ❖ The partial sequence of 16S rRNA and cytochrome C oxidase subunit I (COI) genes of the mitogenomes were analysed for species identification among the genus *Chelidoperca* (*Chelidoperca investigatoris*, *C. occipitalis* and *C. maculicauda*). Sequence analysis of COI gene and 16S gene very clearly indicated that all the 11 fish specimens fell into three distinct groups, which are genetically distant from each other and exhibited identical phylogenetic reservation.
- ❖ Sixty five individuals of eight myctophid species were sampled from Off Tuticorin and Off Kollam covering the Arabian Sea and Bay of Bengal. The partial sequence of 16S rRNA and cytochrome c oxidase subunit I (COI) genes were analysed for species identification and phylogenetic relationship among the Indian myctophids (*Diaphus watasei*, *Diaphus garmani*, *Diaphus* sp. A, *D. thiollieri*, *Myctophum spinosum*, *Myctophum obtusirostre*, *Myctophum* sp. A and *Myctophum* sp. B). The genetic distance of intraspecies ranged from 0.000 to 0.004, while it varied from 0.058 to 0.19

for interspecies based on 16S sequences. Using COI data analysis, the genetic distance of intraspecies ranged from 0.000 to 0.004, while it varied from 0.036 to 0.136 for interspecies. Partial sequences of 16S rRNA and COI genes provided phylogenetic information to distinguish the nine species of myctophids indicating the usefulness of molecular markers in species identifications.

- ❖ The present study shows that both 16S rRNA and COI genes have been useful in resolving taxonomic ambiguities, resurrecting species, and identifying new species from other congeners. Further research focused on deep water species collection, cataloguing and taxonomic identity, coupled with molecular markers will be essential for deep-sea fisheries management and conservation. The present study adds a large amount of sequence data that include both COI and 16S rRNA for less studied or sampled region. The present study discovered eight new deep-sea fish species only from the southern coast of India.
- ❖ Additional sampling in the area, including unexplored Andaman waters and deeper sampling (below 1000 m) promises new additional discoveries. Further, the development of a comprehensive DNA barcode library of the deep-sea fish resources would help to find out new fish species and supports species description and also to understand the genetic variability within or between the species. Molecular taxonomy act as a powerful tool for deep-sea fish species identification and will contribute much for future taxonomic research efforts on deep-water fishes.

**Chapter 8**

**References**

## Chapter 8

## References

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## ***Publications***

## *List of Publications*

1. **Bineesh, K.K.**, Akhilesh, K.V., Abdussamad, E.M. and Pillai, N.G.K. (2013). *Chelidoperca maculicauda*, a new species of perchlet (Teleostei: Serranidae) from the Arabian Sea. *Aqua, International Journal of Ichthyology*, 19 (2): 71-78.
2. **Bineesh, K. K.**, Akhilesh, K. V., Gopalakrishnan, A., & Jena, J. K. (2014). *Plectranthias alcocki*, a new anthiine fish species (Perciformes: Serranidae) from the Arabian Sea, off southwest India. *Zootaxa*, 3785 (3), 490-496.
3. **Bineesh, K. K.**, Akhilesh, K. V., Gomon, M. F., Abdussamad, E. M., Pillai, N. G. K., & Gopalakrishnan, A. (2014). Redescription of *Chlorophthalmus corniger*, a senior synonym of *Chlorophthalmus bicornis* (Family: Chlorophthalmidae). *Journal of fish biology*, 84(2), 513-522.
4. **Bineesh, K.K.**, Sajeela, K.A, Akhilesh, K.V, Abdussamad, E.M. and Pillai, N.G.K. (2011). Rediscovery and description *Sphenanthias whiteheadi* Talwar, 1973 with DNA barcodes from the southern coasts of India. *Zootaxa*, 3098: 64–68.
5. **Bineesh, K.K.**, Sebastine, M, Akhilesh, K.V. and Pillai, N.G.K. (2010). First record of the Garman's lanternfish *Diaphus garmani* (Family: Myctophidae) from Indian waters. *Journal of the Marine Biological Association of India*, 52 (1): 109-112.
6. **Bineesh, K.K.**, Akhilesh, K.V, Shanis, C.P.R, Abdussamad, E.M and Pillai N.G.K. (2012). First report of longfin escolar, *Scombrolabrax heterolepis* (Perciformes: Scombrolabracidae) from Indian waters. *Marine Biodiversity Records*, Marine Biological Association of the United Kingdom. 5, e77, 1-3.
7. Akhilesh, K. V., **Bineesh, K. K.**, Gopalakrishnan, A., Jena, J. K., Basheer, V. S., & Pillai, N. G. K. (2014). Checklist of Chondrichthyans in Indian waters. *Journal of the Marine Biological Association of India*, 56(2), 109-120.
8. **Bineesh, K. K.**, Akhilesh, K. V., Abdussamad, Pillai, N. G. K., Ralf Thiel., J. K. Jena & A. Gopalakrishnan (2014). Redescriptions of *Chelidoperca investigatoris* (Alcock, 1890) and *Chelidoperca occipitalis* Kotthaus, 1973 (Perciformes: Serranidae) from the south-west coast of India. *Indian Journal of Fisheries*, 61(4): 117-122.
9. Hsuan-Ching Ho, **Bineesh, K. K.**, & Akhilesh, K. V. (2014). Rediscovery of *Lophiodes triradiatus* (Lloyd, 1909), a senior synonym of *L. infrabrunneus* Smith and Radcliffe (Lophiiformes: Lophiidae). *Zootaxa*, 3786(5), 587-592.
10. Akhilesh, K.V, **Bineesh, K.K.** and White, W.T. (2012). *Liopropoma randalli*, a new serranid (Teleostei: Perciformes) fish from the Indian Ocean. *Zootaxa*. 3439: 43–50.

11. William. D. Anderson, JR and **Bineesh, K.K.** (2011). A new species of the perciform fish genus *Symphysanodon* (Symphysanodontidae) from the Arabian Sea off the southwestern coast of India. *Zootaxa*. 2966: pp. 31–36.
12. William, F, Smith-Vaniz, **Bineesh, K.K.** and Akhilesh, K.V. (2012). *Opistognathus pardus*, a new species of jawfish (Teleostei: Opistognathidae) from the Western Indian Ocean. *Zootaxa*, 3523: 20–24.
13. Akhilesh, K.V, Hashim, M, **Bineesh, K.K.**, Shanis, C.P.R. and Ganga, U. (2010). New distributional records of deep-sea sharks from Indian waters. *Journal of the Marine Biological Association of India*, 52 (1): 29-34.
14. **Bineesh, K.K.**, Mohitha, C, Vineesh, N, Basheer, V.S, Pillai, N.G.K, Joselet, M, Jena, J.K. and Gopalakrishnan, A. (2014). Molecular identification of three deep-sea fishes of the genus *Chelidoperca* (Perciformes, Serranidae) from Indian waters. *Indian Journal of Fisheries*, 62(4) : 104-108
15. **Bineesh, K.K.**, Gopalakrishnan, A, Jena, J.K, Basheer, V.S, Mohitha, C, Vineesh, N, Joselet, M and Pillai, N.G.K. (2014). Molecular identification of Bigeyes (Perciformes, Priacanthidae) from Indian waters. *Mitochondrial DNA* Early Online 1-5
16. **Bineesh, K. K.**, A. Gopalakrishnan, K. V. Akhilesh, K. A. Sajeela, E. M. Abdussamad, N.G.K. Pillai, V.S. Basheer, J.K. Jena and Robert D. Ward (2016). DNA barcoding reveals species composition of sharks and rays in the Indian commercial fishery. *Mitochondrial DNA Online first*
17. Hsuan-Ching Ho, Rajeesh Kumar, M and **K. K. Bineesh** (2015). *Chuanax multilepis* sp. nov., a new species of *Chaunax* (Lophiiformes: Chaunacidae) from southern India. *Zootaxa*, 4103 (2): 130-136.



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## ***Plectranthias alcocki*, a new anthiine fish species (Perciformes: Serranidae) from the Arabian Sea, off southwest India**

K. K. BINEESH<sup>1</sup>, K. V. AKHILESH<sup>2</sup>, A. GOPALAKRISHNAN<sup>2</sup> & J. K. JENA<sup>3</sup>

<sup>1</sup>National Bureau of Fish Genetic Resources, Central Marine Fisheries Research Institute campus, P.B.No.1603, Ernakulam North, P.O., Kochi-682 018, Kerala, India. E-mail: kkbineesh@gmail.com

<sup>2</sup>Central Marine Fisheries Research Institute, P.B.No.1603, Ernakulam North, P.O., Kochi-682 018, Kerala, India. E-mail: agopalkochi@gmail.com; akhikv@gmail.com

<sup>3</sup>National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow - 226 002 India. E-mail: jkjena2@rediffmail.com

### **Abstract**

A new species of anthiine fish, *Plectranthias alcocki* n. sp. is described and illustrated based on two specimens, (63.7–72.5 mm SL), recently collected from deep-waters of the Arabian Sea, off Kollam, Kerala, India. The following combination of characters distinguishes it from all other congeners: Dorsal-fin rays X, 15; anal-fin rays III, 7; pectoral-fin rays 14, all unbranched; pelvic-fin rays I, 5; lateral-line complete, the pored lateral-line scales 28; scales above lateral line to origin of dorsal fin 1; scales dorsally on head extending to posterior nostrils; no scales on maxilla or chin; gill rakers 5 + 11 (2 + 7 developed); circumpeduncular scales 10; fourth dorsal spine longest, 2.8 (2.6) in head length (HL), longest dorsal-fin soft ray (second) 2.4 (2.7) in head length; body depth 34.4 (35)% SL; head length 46 (49.8)% SL; orbital length 8.6 in SL; margin of preopercle finely serrate, the serrae 33 (28), ventral edge without antrorse spines; dorsal fin continuous and notched; first anal-fin spine 4.9 (5.6) in HL, second anal-fin spine 2.2 (2.6) in HL; pelvic fins relatively short, 4.0–4.3 in SL; the dorsal fin with a black blotch at base of fourth to eighth spines, one at base of the last three spines, and two at base of soft portion of fin, the dark pigment extending onto adjacent body.

**Key words:** *Plectranthias alcocki*, new species, Serranidae, Arabian Sea, India

### **Introduction**

The serranid fish genus *Plectranthias* (Serranidae: Anthiinae) was established by Bleeker (1873) for *Plectropoma anthioides* Günther, 1872, and contains small benthic species found in tropical and subtropical seas on coral or rocky reefs at depths of 20 to 300 m, hence not often caught in trawls. They are poorly represented in museum collections and nearly half of the valid species are known from only one or two specimens (Randall, 1980; Heemstra & Randall, 2009). Recently, Wu *et al.* (2011) described two new species based on single specimens from Taiwan.

Randall (1980) recognized 30 species in an early revision of the genus. Forty-eight valid species of *Plectranthias* are currently known (Eschmeyer & Fong, 2013), of which only 13 occur in the Indian Ocean for at least part of their range (Heemstra & Randall, 2009). The most recently described species is *P. flammeus* Williams *et al.*, (2013) from the Marquesas Islands, French Polynesia. Only one species, *Plectranthias intermedius* (Kotthaus, 1973), is known from the Arabian Sea (Manilo & Bogorodsky, 2003). The purpose of this paper is to describe the second Arabian Sea species, *Plectranthias alcocki*, currently known only from off Kollam, southwest coast of India.

## *Chelidoperca maculicauda*, a new species of perchlet (Teleostei: Serranidae) from the Arabian Sea

K. K. Bineesh<sup>1</sup>, K. V. Akhilesh, E. M. Abdussamad and N. G. K. Pillai

Central Marine Fisheries Research Institute, P. B. No. 1603, Ernakulam North P.O., Cochin – 683 018, Kerala, India. <sup>1</sup>Corresponding author: email: kkbineesh@gmail.com

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### Abstract

A new species of serranid fish, *Chelidoperca maculicauda* n. sp. is described based on three specimens, (123-129 mm SL), recently collected from the Arabian Sea, off Quilon, Kerala, India. The combination of caudal fin shape and a unique color pattern of five red bars on a pinkish body and pale yellow fins with a bright red margin on the anal fin, a small grey spot distally on the dorsal half and bluish white spots on ventral half of the caudal fin, distinguishes the new species from other congeners. Other distinguishing characters include: fourth dorsal spine longest 2.8 (3) in head length; body depth 23.3 (22.8-24.5) % SL (standard length), 4.3 (4.1-4.4) in SL; head length 40.3 (42.3-42.6) % SL; orbital length 9.3 (8.9-9.1) in SL; 2.5-3 scales above lateral line to dorsal origin; serrae on margin of preopercle 40-46. Lateral-line scales 42; dorsal fin continuous, with ninth dorsal spine shorter than tenth spine; longest dorsal soft ray (7<sup>th</sup> or 8<sup>th</sup>) 2.4 (2.3-2.4) in head length.

### Zusammenfassung

Beschrieben wird eine neue Art der Sägebarsche: *Chelidoperca maculicauda* n. sp. auf der Grundlage von drei Exemplaren (123 - 129 mm SL), die kürzlich im Arabischen Meer vor Quilon, Kerala, Indien, gefangen wurden. Diese neue Art lässt sich von anderen Angehörigen der Gattung durch die Kombination folgender Merkmale unterscheiden: Schwanzflossenform, einprägsames Farbmuster mit fünf roten Streifen auf leicht rosafarbenem Rumpf, blassgelbe Flossen mit hellrotem Rand an der Afterflosse, ein kleiner grauer Fleck distal auf der dorsalen Hälfte und bläulich weiße Flecken auf der ventralen Hälfte der Schwanzflosse. Zu weiteren Unterscheidungsmerkmalen gehören: vierter Rückenflossenstrahl am längsten (2,8-(3-)fache Kopflänge); Körpertiefe 23,3 (22,8-24,5) % der Standardlänge SL, 4,3 (4,1-4,4) Anteil an SL; Kopflänge 40,3 (42,3-42,6) % von SL; Augenhöhlenlänge 9,3 (8,9-9,1) Anteil an SL; 2,5 bis 3 Schuppen oberhalb der Seitenlinie bis zum Ansatz der Rückenflosse; 40 - 46 Sägezähnen am Rand des Präoperculum. Außerdem 42 Seitenlinienschuppen; durchgehende Rückenflosse, wobei der neunte Flossenstrahl kürzer ist als der zehnte; längster Rückenweichflossenstrahl (der 7. oder 8.) 2,4-(2,3 bis 2,4-)fache Kopflänge.

### Résumé

Une nouvelle espèce de Serranidé, *Chelidoperca maculicauda* n. sp. est décrite sur base de trois spécimens (123-129 mm de LS), collectés récemment dans la mer d'Arabie, au large de Quilon, de Kerala, Inde. La nouvelle espèce se distingue d'autres congénères par la combinaison de la forme de la caudale et d'un patron de coloration unique de cinq barres rouges sur un corps rosâtre et de nageoires jaune pâle avec un large liseré rouge sur l'anale, une petite tache grise distalement sur la moitié de la dorsale et de taches d'un blanc bleuâtre sur la moitié ventrale de la caudale. Autres traits distinctifs: la quatrième épine dorsale est la plus longue, 2,8 (3) de la longueur de la tête; hauteur du corps 23,3 (22,8-24,5) % de la LS, 4,3 (4,1-4,4) de LS, longueur de la tête 40,3 (42,3- 42,6) % de la LS; longueur orbitale 9,3 (8,9-9,1) en LS; 2,5 -3 écailles au-dessus de la ligne latérale jusqu'à la base de la dorsale; des serrae sur le bord du préopercule 40-46. Écailles de la ligne latérale 42; une nageoire dorsale continue, avec la neuvième épine dorsale plus courte que la dixième; le plus long rayon mou dorsal (le 7<sup>e</sup> ou le 8<sup>e</sup>) 2,4 (2,3-2,4) de la longueur de la tête.

### Sommario

Una nuova specie di pesci serranidi, *Chelidoperca maculicauda* n. sp., è descritta sulla base di tre esemplari (123-129 mm SL), recentemente raccolti dal Mare Arabico, al largo di Quilon, Kerala, India. La combinazione di caratteri, quali la forma della pinna caudale e una livrea originale di cinque barre rosse su un corpo rosa pallido e pinne gialle con un margine rosso brillante sulla pinna anale, una macchia grigia piccola distalmente nella metà dorsale e bluastre macchie bianche sulla metà ventrale della pinna caudale, distingue la nuova specie da altri congeneri. Altri caratteri distintivi sono: spina dorsale più lunga la quarta (2.8 (3) volte nella lunghezza della testa), profondità del corpo 23.3 (22.8-24.5)% SL (lunghezza standard), 4.3 (4.1-4.4), in SL, lunghezza della testa 40.3 (42.3-42.6)% SL; lunghezza dell'orbitale 9.3 (8.9-9.1) in SL; 2.5-3 scaglie sopra la linea laterale all'origine della dorsale; margine del preopercolo con 40-46 dentelli. Scaglie in linea laterale 42; pinna dorsale continua, con nona spina dorsale più corta della decima; raggio più lungo della dorsale molle (7° o 8°) 2.4 (2.3-2.4) nella lunghezza della testa.

## Redescription of *Chlorophthalmus corniger*, a senior synonym of *Chlorophthalmus bicornis* (Family: Chlorophthalmidae)

K. K. BINEESH\*†, K. V. AKHILESH\*, M. F. GOMON‡, E. M. ABDUSSAMAD\*,  
N. G. K. PILLAI\* AND A. GOPALAKRISHNAN§

\*Central Marine Fisheries Research Institute, P. B. No.1603, Ernakulam North, P. O., Kochi-682 018, Kerala, India, ‡Ichthyology, Sciences Department, Museum Victoria, GPO Box 666, Melbourne, VIC 3001, Australia and §National Bureau of Fish Genetic Resources, CMFRI campus, P. B. No.1603, Ernakulam North, P. O., Kochi-682 018, Kerala, India

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*Chlorophthalmus corniger* is redescribed on the basis of recently collected specimens. The species is redefined as a species of *Chlorophthalmus* with the lower jaw terminating in a distinctly projecting horizontal plate with strong, spine-like processes directed forward from the plate's corners; body silvery grey, with numerous minute black spots and traces of broad darker crossbars; base of anterior dorsal fin spines and distal parts of dorsal fins black; adipose fin tiny with numerous black spots; caudal fin black; 3.5 scales above lateral line; three rows of cheek scales; head very large, 34.3–40.1% standard length ( $L_S$ ); eye large, 29.8–40.8% head length ( $L_H$ ); pectoral fin long, extending to beyond dorsal fin base, 21.7–26.2%  $L_S$ . *Chlorophthalmus bicornis* is a junior synonym of *C. corniger* based on the examination of the type series of both species. It is confined to the northern half of the Indian Ocean, reliably recorded from Somalia and the Gulf of Aden to southern Java, Indonesia, at depths between 200 and 500 m. A lectotype and three paralectotypes were designated for *C. corniger*. DNA barcodes for Indian species of *Chlorophthalmus* were generated.

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Key words: DNA barcodes; India; Indian Ocean.

### INTRODUCTION

The greeneyes of the family Chlorophthalmidae (Aulopiformes) are known from tropical and subtropical seas worldwide at upper continental slope depths. Eschmeyer & Fong (2013) list 21 nominal species in the family, two of which are referable to the Atlantic genus *Parasudis* and another three that have been synonymized with the type species of *Chlorophthalmus*, shortnose greeneye *Chlorophthalmus agassizi* Bonaparte 1840. Of the remaining 16 species of *Chlorophthalmus* currently regarded as valid, 11 have been reported from the Indo-western and central Pacific Ocean. More than half of these have been reported in publications as occurring in the Indian Ocean. Bottom trawls operated by fishery oceanographic R.V. *Sagar Sampada* from 1983 to 1991 in the south-eastern Arabian Sea have revealed the existence of rich

†Author to whom correspondence should be addressed. Tel.: +919946770190; email: kkbineesh@gmail.com



## Redescription of *Sphenanthias whiteheadi* Talwar (Perciformes: Cepolidae) with DNA barcodes from the southern coasts of India

K.K. BINEESH<sup>1,3</sup>, K. A. SAJEELA<sup>2</sup>, K.V. AKHILESH<sup>1</sup>, N. G. K. PILLAI<sup>1</sup> & E.M. ABDUSSAMAD<sup>1</sup>

<sup>1</sup>Central Marine Fisheries Research Institute (CMFRI), P. B. No. 1603, Ernakulam North P.O., Kochi - 683 018, Kerala, India

<sup>2</sup>National Bureau of Fish Genetic Resources (NBFGR), Cochin Unit, CMFRI Campus, P.B.No.1603, Ernakulam North, P.O., Kochi-682 018, Kerala, India

<sup>3</sup>Corresponding author. E-mail: kkbineesh@gmail.com

### Abstract

A very rare bandfish, *Sphenanthias whiteheadi* Talwar 1973, is re-discovered and described from the southwest and south-east coasts of India for the first time after its original description and the rarity of the fish is challenged. A mitochondrial COI barcode sequence was generated for the specimen.

**Key words:** *Sphenanthias whiteheadi*, Cepolidae, India

### Introduction

The bandfishes of the family Cepolidae (Perciformes) are known from all tropical and subtropical waters and comprise 22 valid species in 4 genera worldwide (Eschmeyer & Fong, 2011). Members of the genus *Sphenanthias* can be differentiated from the similar *Owstonia* in having lateral lines separate and not forming loops in front of dorsal fins (Smith Vaniz, 2001; Liao *et al.*, 2009). The genus *Sphenanthias* is represented by only one valid species in the Arabian Sea (Manilo & Bogorodsky, 2003), *Sphenanthias whiteheadi* described by Talwar from four specimens collected from southwest coast of India off Quilon at 300 m (Talwar, 1973). *Sphenanthias simoterus* Smith 1968, *Owstonia weberi* (Gilchrist, 1922), and *Owstonia totomiensis* Tanaka 1908 are listed as occurring in this area but the validity of these occurrences are questionable since no detailed description was given in publications discussing their occurrence. After the original description of *S. whiteheadi* there were no reports of this species from the Indian coasts. The present paper confirms the validity and status of *S. whiteheadi* Talwar 1973 and provides a short re-description of the species.

### Material and methods

Specimens of *Sphenanthias whiteheadi* were collected from the southwest coast of India near Quilon (Kerala) and the southeast coast of India near Tuticorin (Tamilnadu) in December 2009 from commercial deep-sea shrimp trawler bycatch landings operated at 220 to 350 m depths (Fig. 1). Identification of the species was based on Talwar (1973). Measurements were made to the nearest 0.1 mm using dial calipers. All measurements are expressed as percentage of standard length (SL). The specimens of *S. whiteheadi* were deposited in the Designated National Repository (DNR), Central Marine Fisheries Research Institute, Kochi, Kerala, India (Accession number: GB.31.31.4.1).

DNA analysis was carried out to generate the DNA barcode for the species. Tissue samples collected from one specimen were preserved in 95% Ethanol and used for DNA extraction and sequencing. Total DNA was extracted by standard protocols (Miller *et al.*, 1988). A partial sequence of the mitochondrial COI gene was PCR amplified using primer Fish F1 (5' - TCA ACC AAC CAC AAA GAC ATT GGC AC - 3') and Fish R1 (5' -TAG ACT TCT



## Short Communication

# First record of the Garman's lanternfish *Diaphus garmani* (Family: *Myctophidae*) from Indian waters

\*K. K. Bineesh, Manju Sebastine, K. V. Akhilesh and N. G. K. Pillai

Central Marine Fisheries Research Institute, P. B. No. 1603, Ernakulam North P. O., Cochin-682 018, Kerala, India. \*E-mail: bineeshmarine@yahoo.com

### Abstract

The myctophid *Diaphus garmani* is recorded for the first time from Indian waters. Three specimens (54-59 mm standard length) were collected from deep sea shrimp trawlers off Quilon, southwest coast of India, between 80°-110° N and 74°-76° E, at depths from 250 to 450 m.

**Keywords:** *Diaphus garmani*, first record, southwest coast of India, Myctophidae

### Introduction

Lanternfish of the family Myctophidae are found in all the oceans, with 32 genera and 240 species (Nelson, 2006). An important characteristic of myctophids is the presence of luminescent organs called photophores along the ventral body surface and head. For species identification, different patterns of photophores have been used along with meristic data. These fishes have species-specific diel vertical migration patterns (Watanabe *et al.*, 1999). Like many other mesopelagic fishes, myctophids are an important constituent of the diet of commercially important oceanic fishes and marine mammals (Jackson *et al.*, 1998).

Investigations on the myctophid fauna of the western and northern Arabian Sea were carried out by R/V Dr. *Fridtjof Nansen* during 1975-1981 and 1983-84 (Gunnar *et al.*, 1999). Studies on distribution and abundance of Myctophidae in the EEZ of India were carried out by FORV *Sagar Sampada* during 1985-1986 (Mini and James, 1990). About 55 species of myctophids are known from the Arabian Sea including its southern part of the Indian Ocean (Nafpaktitis, 1978). Karuppasamy *et al.*, (2006) reported 27 species of myctophids from the Indian EEZ. Somvanshi *et al.* (2009) reported 5 species of myctophids from the southwest coast of India.

*Diaphus* is the most speciose of the myctophid genera, with 70-75 known species (Nafpaktitis *et al.*, 1995). The members of this genus can be assigned

to two distinct groups on the basis of the presence or absence of a suborbital (So) luminous organ and an inner series of broad-based, forward-hooked teeth on the posterior part of the premaxilla (Nafpaktitis, 1978). *Diaphus garmani* is a small diaphid fish, which attains a maximum length of about 60 mm.

### Material and Methods

Three specimens of *D. garmani* (Fig. 1) were collected in April 2009 from a commercial deep-sea shrimp trawler which operated from 250 to 450 m depth in the outer shelf of southwest coast of India between 8° N - 11° N and 74° E - 76° E. Identification was based on the luminous organs on the head, number of fin rays, gill rakers and morphometric characters (Nafpaktitis, 1978). Photophore nomenclature follows Hulley (1984). The specimens of *D. garmani* were deposited in the National Biodiversity Referral Museum, CMFRI, India under the accession Number GB.27.1.5.25.



Fig. 1. *Diaphus garmani*, 54 mm SL



# Checklist of Chondrichthyans in Indian waters

Akhilesh K. V<sup>\*1</sup>, K. K. Bineesh<sup>2</sup>, A. Gopalakrishnan<sup>1</sup>, J. K. Jena<sup>3</sup>, V. S. Basheer<sup>2</sup> and N. G. K. Pillai<sup>1</sup>

<sup>1</sup>Central Marine Fisheries Research Institute, P.B.No.1603, Ernakulam North, P.O., Kochi-682 018, Kerala, India.

<sup>2</sup>National Bureau of Fish Genetic Resources, CMFRI campus, P.B.No.1603, Ernakulam North, P.O., Kochi-682 018, Kerala, India

<sup>3</sup>National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow - 226 002, U.P., India.

\*Correspondence e-mail: [akhikv@gmail.com](mailto:akhikv@gmail.com)

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Original Article

## Abstract

Conservation, management and sustainable utilisation of biological resources depend on the accurate identification of exploited taxa, which emphasises the need for systematic taxonomic research. Chondrichthyans (sharks, rays, skates and chimaeras) are considered to be one of the most vulnerable exploited marine resources, however, the basic taxonomic study of these groups in Indian waters needs improvement to achieve better management for their sustainable exploitation. We discuss issues concerning chondrichthyan taxonomic research in India and provide an extended, updated checklist of chondrichthyans listed/reported from Indian waters, together with comments on their occurrence.

**Keywords:** *Chondrichthyans, checklist, taxonomy, status, India, diversity, management, conservation.*

## Introduction

India has many different climatic, ecological and biogeographical zones, and diverse faunal and floral groups in its ecosystems. Conservation and management of this diversity is important to maintain the equilibrium of ecosystems and for their potential human usage. Conservation, management

and sustainable utilisation depend on the quantitative and qualitative assessment of biodiversity, taxonomic identity and understanding the taxa of concern (Narendran, 2001; Agnarsson and Kuntner, 2007; Prathapan *et al.*, 2009).

Large amounts of research funding and effort have been invested to provide an inventory of the biodiversity of India. However, our current taxonomic and systematic knowledge on certain groups are inadequate, scattered and mostly unorganised (Narendran, 2001; Hariharan and Balaji, 2002; Kumaran, 2002; Aravind *et al.*, 2004; Das *et al.*, 2006; James, 2010; Vishwanath and Linthoingambi, 2010; Wafar *et al.*, 2011). Understanding the fauna and its diversity in specific habitats/ecosystems/regions of the country, with their distribution patterns and phylogeography, is an important baseline for future studies and for the formulation of conservation and management plans.

Chondrichthyans include all cartilaginous fish species commonly called sharks, rays, skates and chimaeras. They are widely distributed in all the world's oceans, but are most diverse in the tropical and subtropical Indo-Pacific Ocean (Bonfil, 2002). Chondrichthyans are one of the most vulnerable groups due to their biological characteristics. Global concern over these apex predators is increasing as

# First report of longfin escolar, *Scombrolabrax heterolepis* (Perciformes: Scombrolabracidae) from Indian waters

K.K. BINEESH, K.V. AKHILESH, C.P.R. SHANIS, E.M. ABDUSSAMAD AND N.G.K. PILLAI

Central Marine Fisheries Research Institute (CMFRI), Pelagic Fisheries Division, Post Box No. 1603, Ernakulam North P.O., Kochi-682 018, India

*The present paper reports the first record of occurrence of longfin escolar Scombrolabrax heterolepis in the Indian waters. A single specimen measuring 188.5 mm standard length was collected from a commercial deep-sea shrimp trawl by-catch operated at 220 to 350 m depths in the Arabian Sea off Trivandrum during October 2010. The specimen is described and figured.*

**Keywords:** *Scombrolabrax heterolepis*, distribution extension, south-western India, Arabian Sea

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## INTRODUCTION

The taxonomy of deep-sea fishes of India was pioneered by the outstanding works of A.W. Alcock, based on the samples collected during the voyage of the Indian marine survey steamer, HMS 'Investigator'. Alcock's publications (1889–1907) are the major detailed work on the deep-sea fauna of Indian waters. Recent studies on deep-sea fishes from the Indian exclusive economic zone has resulted in the description of many new species and new records from Indian waters (Oommen, 1978; Sajeevan *et al.*, 2009; Akhilesh *et al.*, 2010; Bineesh *et al.*, 2010; Kurup *et al.*, 2010; Anderson & Bineesh, 2011).

The longfin escolar, *Scombrolabrax heterolepis* Roule, 1921 (Scombrolabracidae) originally described from south of Madeira, eastern Atlantic is a monotypic member of the suborder Scombrolabracoidae (Nelson, 2006). Also known as the black mackerel, *S. heterolepis* is a widespread but uncommon deep-sea fish known from tropical and subtropical areas of the Pacific, Indian and Atlantic Oceans, but not known from the eastern Pacific and south-eastern Atlantic. *Scombrolabrax heterolepis* inhabits continental shelves and slopes at depths between 130 and 1374 m (Higgins *et al.*, 1970; Filho *et al.*, 2010). To date no specimens have ever been reported from Indian waters. This paper confirms the presence of *S. heterolepis* in Indian waters.

One specimen of *S. heterolepis* (188.5 mm standard length (SL)) was collected on 16 October 2010 from a commercial deep-sea shrimp trawler operated at 220 to 350 m depths in the Arabian Sea off Trivandrum (Figures 1 & 2). The specimen was deposited in the National Marine Biodiversity Referral Museum at Central Marine Fisheries

Research Institute (CMFRI), Cochin, India under the Accession Number GB.43.6.16.10. The morphometric and meristic characters of the specimen agree well with data from previous studies (McEachran & Fechhelm, 2005; Filho *et al.*, 2010) (Table 1). This is the first record from the west coast of India and confirms the widespread distribution of this species.

## DIAGNOSIS

Dorsal fin spines and rays XII + I, 14; anal fin spines and rays III, 16; pectoral fin rays 19; lateral line scales 47; lower limb of first gill arch with 5 well-developed gill rakers, about 10 clusters of minute spines on upper limb. Body moderately elongate and compressed; head large, 34.6% of SL; the interorbital region flat, 8.5% of SL; very large eye, 10.3% of SL, its diameter almost as long as the conical snout, snout 3.5 in head length; large terminal mouth, the upper jaw protractile, the lower projecting slightly beyond the upper; teeth in upper jaw in a row of small to moderate, compressed canines, with three very large, stout canines in front; teeth of lower jaw larger without large canines; two large nasal openings each side of snout. Two dorsal fins, base of first dorsal fin about twice as long as base of second dorsal fin; origin of first dorsal fin slightly posterior to pectoral-fin base. Caudal fin forked and moderately small. Very long pectoral fins, 31.9% of SL, reaching a vertical at anal-fin origin. Pelvic fins originating below origin of pectoral fins; lateral line running closely to dorsal base, ending slightly before end of second dorsal fin. The morphometric characteristics of the present specimen match with the representatives described by previous studies except anal finbase length, which was less (16.2% of SL in the present study) than that (19–22% of SL) reported by Filho *et al.* (2010).

### Corresponding author:

K.K. Bineesh

Email: kkbineesh@gmail.com



## Note

# Redescriptions of *Chelidoperca investigatoris* (Alcock, 1890) and *Chelidoperca occipitalis* Kotthaus, 1973 (Perciformes: Serranidae) from the south-west coast of India

K. K. BINEESH<sup>1</sup>, K. V. AKHILESH<sup>2</sup>, E. M. ABDUSSAMAD<sup>2</sup>, N. G. K. PILLAI<sup>2</sup>, RALF THIEL<sup>3</sup>  
J. K. JENA<sup>4</sup> AND A. GOPALAKRISHNAN<sup>2</sup>

<sup>1</sup>National Bureau of Fish Genetic Resources (NBFGR) Cochin Unit, CMFRI Campus, P. B.No.1603, Ernakulam North, P.O., Kochi - 682 018, Kerala, India

<sup>2</sup>Central Marine Fisheries Research Institute, P.B. No. 1603, Ernakulam North, P.O., Kochi - 682 018, Kerala, India

<sup>3</sup>Biocenter Grindel und Zoological Museum, University of Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

<sup>4</sup>National Bureau of Fish Genetic Resources, Canal Ring Road, P. O. Dilkusha, Lucknow - 226 002 Uttar Pradesh, India

e-mail: kkbineesh@gmail.com

## ABSTRACT

*Chelidoperca investigatoris* (Alcock, 1890) was described based on two specimens collected off the Madras coast (Tamil Nadu), Bay of Bengal and *Chelidoperca occipitalis* Kotthaus, 1973 was described from a single specimen collected off the Socotra Islands, Arabian Sea. In 2009-2010, many additional specimens of these two species were collected from off Kollam (Kerala), south-west coast of India. For *C. occipitalis* report from south-western India forms a considerable extension of its known distribution range. *Chelidoperca investigatoris* and *C. occipitalis* are redescribed based on these new specimens.

Keywords: *Chelidoperca investigatoris*, *Chelidoperca occipitalis*, India, Perchlets, Redescription

The serranid fish genus *Chelidoperca*, proposed by Boulenger (1895) for *Centropristis hirundinaceus* Valenciennes, 1831, comprises seven species; *Chelidoperca hirundinacea* (Valenciennes, 1831), *C. pleurospilus* (Günther, 1880), *C. lecroimi* Fourmanoir, 1982, *C. investigatoris* (Alcock, 1890), *C. margaritifera* Weber, 1913, *C. occipitalis* Kotthaus, 1973 and *C. maculicauda* Bineesh and Akhilesh, 2013 (Bineesh *et al.*, 2013; Eschmeyer and Fong, 2014).

Members of the *Chelidoperca* genus are usually found on continental shelf and slope muddy bottoms in the Indo-West Pacific (Nelson, 2006; Bineesh *et al.*, 2013). Three species of *Chelidoperca*, namely *C. investigatoris*, *C. occipitalis* and *C. maculicauda* are known from the Arabian Sea (Baranes and Golani, 1993; Manilo and Bogorodsky, 2003; Jayaprakash *et al.*, 2006; Sajeevan *et al.*, 2009; Bineesh *et al.*, 2013). However, *C. investigatoris* and *C. maculicauda* are the only valid species of the genus known from the Indian Exclusive

Economic Zone (Bineesh *et al.*, 2013). This study provides new report of *Chelidoperca occipitalis* from southern India and provides a redescription of *Chelidoperca occipitalis* and *C. investigatoris* based on recently collected materials from Arabian Sea off south-west coast of India.

During weekly observations of fish landings along the south-west coast of India, specimens of *C. investigatoris* and *C. occipitalis* were collected from bycatch landings of commercial deep sea shrimp trawls operated in the Arabian Sea, off Kollam during 2009-2010. These were landed at Sakthikulangara Fisheries Harbour, Kollam (Quilon), Kerala. Species were identified following Alcock (1890), Kotthaus (1973), Senou (2002) and Park *et al.* (2007). Morphometric measurements of formalin (5%) preserved specimens were taken following Hubbs and Lagler (1964). The specimens are deposited in the fish collection of the Designated National Repository (DNR) at Central Marine Fisheries Research Institute (CMFRI), Cochin, Kerala, India. Institutional abbreviations are:





## Rediscovery of *Lophiodes triradiatus* (Lloyd, 1909), a senior synonym of *L. infrabrunneus* Smith and Radcliffe (Lophiiformes: Lophiidae)

HSUAN-CHING HO<sup>1,2,5</sup>, K. K. BINEESH<sup>3</sup> & K.V. AKHILESH<sup>4</sup>

<sup>1</sup>National Museum of Marine Biology & Aquarium, Pingtung, Taiwan

<sup>2</sup>Institute of Marine Biodiversity & Evolutionary Biology, National Dong Hwa University, Pingtung, Taiwan.

E-mail: [ogcoho@gmail.com](mailto:ogcoho@gmail.com)

<sup>3</sup>Kochi Unit, National Bureau of Fish Genetic Resources, Kerala, India

<sup>4</sup>Cochin University of Science and Technology, Kochi, Kerala, India

<sup>5</sup>Corresponding author

### Abstract

Examination of the holotype and three recently collected additional specimens from the Indian Ocean has revealed that *Lophius triradiatus* Lloyd, 1909 (now under *Lophiodes*) is a valid species and a senior synonym of *Lophiodes infrabrunneus* Smith & Radcliffe, 1912 and *Lophiodes abdituspinus* Ni, Wu & Li, 1990. A detailed description of the additional specimens is provided.

**Key words:** Pisces, taxonomy, *Lophius triradiatus*, *Lophiodes infrabrunneus*, Indian Ocean

### Introduction

Lloyd (1909a) described *Lophius triradiatus* based on a single specimen (ZSI 878/1, 55 mm TL; Fig. 1A) collected from the Laccadive Sea (off Kerala coast, 549 m), India. The species is characterized by having three free dorsal-fin spines in the cephalic position only, lacking the post-cephalic dorsal-fin spines. Caruso (1981) reassigned the species to *Lophiodes*, but designated it a *nomen dubium* due to the very poor condition of the holotype (Fig. 1B). Smith & Radcliffe in Radcliffe (1912) described *Lophiodes infrabrunneus* based on specimens collected from the Philippines that also have only three dorsal-fin spines. Caruso (1981) recognized it as a valid species and diagnosed the species by all three dorsal-fin spines relatively short and other characters.

Ni *et al.* (1990) described a third species with three dorsal-fin spines, *Lophiodes abdituspinus*, from the South China Sea. Ho *et al.* (2009) redescribed *Lophiodes infrabrunneus* and placed *Lophiodes abdituspinus* as its junior synonym.

Although there was a suspicion that *Lophiodes triradiatus* and *L. infrabrunneus* might be conspecific, the validity of *L. triradiatus* was still unknown. Because they were not able to examine the holotype or any additional specimen from India, Ho *et al.* (2009: 67) followed Caruso's (1981) opinion and "redescribed *Lophiodes infrabrunneus* Smith & Radcliffe 1912 rather than resurrecting *L. triradiatus*."

Recently, three specimens lacking post-cephalic dorsal-fin spines collected from near the type locality were found in Indian and South African collections. These specimens are similar to the specimens of *Lophiodes infrabrunneus* examined by Ho *et al.* (2009). We also examined the holotype of *Lophius triradiatus* and determined that it is a valid species of *Lophiodes*, and that *L. infrabrunneus* and *L. abdituspinus* Ni, Wu & Li, 1990 are junior synonyms.

Hence, we resurrect *Lophius triradiatus* Lloyd, 1909 (now under *Lophiodes*) and synonymize two junior synonyms under the Articles 23.1 and 23.2 of the International Code of Zoological Nomenclature (ICZN, 2013, online version). A detailed description of the recently collected specimens is provided.



## Article

### *Liopropoma randalli*, a new serranid (Teleostei: Perciformes) fish from the Indian Ocean

K.V. AKHILESH<sup>1,3</sup>, K.K. BINEESH<sup>1</sup> & WILLIAM T. WHITE<sup>2</sup>

<sup>1</sup>Central Marine Fisheries Research Institute (CMFRI), P. B. No. 1603, Ernakulam North P. O., Cochin-683 018, Kerala, India.

<sup>2</sup>CSIRO Marine and Atmospheric Research, GPO Box 1538, Hobart, Tasmania 7001, Australia

<sup>3</sup>Corresponding author. E-mail: akhikv@gmail.com

#### Abstract

A new serranid fish, *Liopropoma randalli* n. sp. is described from four specimens collected from the Indian Ocean off southwestern India and eastern Indonesia. It differs from all other species in the genus in its striking color pattern, a broad black band from behind the eye to the caudal peduncle, semicircular dark-brown to black spots that cover the pink to reddish body, and a combination of the following characters: 46 to 49 lateral line scales; 1–2 (4 as rudiments) + 12–13 (4–5 as rudiments) gill rakers on the first arch (total 17–19); longest dorsal soft ray 2.1–2.3 in head length; 1<sup>st</sup> anal-fin spine 10.4–12.2 in head length; 2<sup>nd</sup> anal-fin spine 4.4–4.9 in head length; pelvic fin relatively short, 5.1–5.7 in SL; and body depth 3.2–3.6 in SL.

**Keywords:** *Liopropoma randalli*, Serranidae, Perciformes, Indian Ocean, India, Indonesia

#### Introduction

The genus *Liopropoma* was proposed by Gill 1861 for *Perca aberrans* Poey (1860) a species based on a single specimen from deepwater off Cuba. Members of this genus are small to medium-sized and colorful fishes, belonging to the tribe Liopropomini within the serranid subfamily Epinephelinae, which occur in tropical and subtropical waters of the Indo-Pacific and Western Atlantic (Randall & Taylor, 1988). In their review of the Indo-West and Central Pacific species of *Liopropoma*, Randall & Taylor (1988) provided a detailed account of the genus and of 18 species, which included seven new species. Since this review, another species, *Liopropoma dorsoluteum* Kon, Yoshino & Sakurai, 1999, has been described from the Indo-West Pacific off Japan and Taiwan. In addition to these species, two species are known from the Eastern Pacific and six are known from the Western Atlantic (Eschmeyer & Fong, 2011).

Of the 27 nominal species of *Liopropoma*, seven species are known to occur in the Indian Ocean for at least part of their range: *L. africanum* (Smith, 1954); *L. dorsoluteum* Kon, Yoshino & Sakurai, 1999 (based on Indonesian record referred to later in this paper); *L. lunulatum* (Guichenot, 1863); *L. mitratum* Lubbock & Randall, 1978; *L. multilineatum* Randall & Taylor, 1988; *L. susumi* (Jordan & Seale, 1906); *L. tonstrinum* Randall & Taylor, 1988 (Lubbock & Randall, 1978; Randall & Taylor, 1988; Khalaf & Zajonz, 2007; CMFRI, 2009).

Most species of *Liopropoma* are poorly represented in collections and as a result, a number of species are described based on only one or two specimens. Recent surveys of fish landing sites in southwestern India and southern Indonesia resulted in the collection of four specimens of an undescribed species of *Liopropoma*. These specimens were collected by gillnet off the coast of Mangalore in depths of 170–260 m (n = 2) and from a fish landing site in Lombok, eastern Indonesia (n = 2). This new species of *Liopropoma* from the Indian Ocean is described herein.



## A new species of the perciform fish genus *Symphysanodon* (Symphysanodontidae) from the Arabian Sea off the southwestern coast of India

WILLIAM D. ANDERSON, JR.<sup>1</sup> & K. K. BINEESH<sup>2</sup>

<sup>1</sup>Grice Marine Biological Laboratory, College of Charleston, 205 Fort Johnson, Charleston, South Carolina 29412-9110, USA.  
E-mail: andersonwd@cofc.edu

<sup>2</sup>Pelagic Fisheries Division, Central Marine Fisheries Research Institute, Cochin – 18, India. E-mail: kkbineesh@gmail.com

### Abstract

*Symphysanodon xanthopterygion*, new species, reported herein from 15 specimens collected near Quilon, India, off the Kerala Coast in the southeastern Arabian Sea, becomes the twelfth described species in the genus. The following characters in combination distinguish *S. xanthopterygion* from its congeners: parapophyses present on first caudal vertebra, total number of gillrakers on first arch 38 to 42, tubed lateral-line scales 54 to 59, sum of lateral-line scales plus total number of gillrakers in individual specimens 94 to 101, head length 33 to 37% SL, head depth 18 to 21% SL, snout length 5 to 6% SL, body depth 24 to 27% SL, lower caudal-fin lobe bright yellow.

**Key words:** *Symphysanodon xanthopterygion*, Arabian Sea, India, Kerala Coast, Quilon

### Introduction

The marine fish family Symphysanodontidae contains a single genus, *Symphysanodon*, and 11 previously described species (Anderson and Springer, 2005; Khalaf and Krupp, 2008; Quéro *et al.*, 2009). In addition, McCosker (1979) and Anderson and Springer (2005) reported a species of *Symphysanodon*, as yet undescribed, that was obtained from the stomach of a coelacanth (*Latimeria chalumnae*) caught in the Comoros in the southwestern Indian Ocean. Later Heemstra *et al.* (2006) mentioned an undescribed species of *Symphysanodon* from the Comoros that may be conspecific with the species reported from the coelacanth stomach. Also, Campos *et al.* (2009) reported two larval *Symphysanodon*, collected off southern Brazil, that may represent another undescribed species.

*Symphysanodon* (with adults reaching less than 175 mm SL) occurs in depths of about 80 to 700 m in the Atlantic, Pacific, and Indian oceans. Four species of *Symphysanodon* have been described from the Indian Ocean (*sensu lato*)—*S. andersoni* Kotthaus, 1974 (southwest of Socotra Island, near the entrance to the Gulf of Aden; also reported from the Gulf of Kutch, an inlet in the northeastern quadrant of the Arabian Sea on the west coast of India by Manilo and Bogorodsky, 2003); *S. rhax* Anderson and Springer, 2005 (off the Maldivian Islands); *S. disii* Khalaf and Krupp, 2008 (Gulf of Aqaba); and *S. pitondelafournaisei* Quéro *et al.*, 2009 (off Reunion Island). Herein we describe *S. xanthopterygion* based on 15 specimens collected in the Arabian Sea off Quilon, India.

### Methods and abbreviations

Methods used are those of Anderson (1970) and Anderson and Springer (2005), counting lateral-line scales on left side where possible. Institutional abbreviations are: CAS—California Academy of Sciences, San Francisco; CMFRI—Central Marine Fisheries Research Institute, Cochin, Kerala, India; DNR—Designated National Repository, CMFRI, Cochin, Kerala, India; GMBL—Grice Marine Biological Laboratory, College of Charleston, Charleston, South Carolina; UF—Florida Museum of Natural History, University of Florida, Gainesville;





# Article

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## ***Opistognathus pardus*, a new species of jawfish (Teleostei: Opistognathidae) from the Western Indian Ocean**

WILLIAM F. SMITH-VANIZ<sup>1</sup>, K. K. BINEESH<sup>2</sup> & K. V. AKHILESH<sup>2</sup>

<sup>1</sup>Research Associate, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611-7800, USA.

E-mail: smithvaniz@gmail.com

<sup>2</sup>Central Marine Fisheries Research Institute, P. B. No. 1603, Ernakulam North P.O., Cochin - 683 018, Kerala, India.

### **Abstract**

A new species of jawfish, *Opistognathus pardus*, is described based on a single specimen, 98.8 mm SL, recently collected from the Western Indian Ocean off Quilon (Kerala), India. The combination of a rigid maxilla without flexible lamina posteriorly, a unique color pattern in which most of the head is covered with small, irregular-shaped, dark spots, dorsal-fin rays XI, 11, and the outermost segmented pelvic-fin ray tightly bound to adjacent ray, with the interradiation membrane not incised distally distinguishes the new species from other congeners. This is the fourth species of *Opistognathus* known from the coast of India or Sri Lanka. A range extension for *O. macrolepis* is also reported.

**Key words:** Jawfish, *Opistognathus*, new species, India

### **Introduction**

The purpose of this paper is to describe a new species of the jawfish genus *Opistognathus* Cuvier recently collected off Quilon (Kerala), India. The ten species of *Opistognathus* previously known from the western Indian Ocean, including the Red Sea, were newly described or reviewed by Smith-Vaniz (2009, 2010). A total of 39 Indo-West Pacific species of *Opistognathus* are currently recognized as valid (Eschmeyer, 2012), with at least 19 others yet to be described. In addition to the new species described herein, only four other species of *Opistognathus* are known from India or Sri Lanka: *Opistognathus nigromarginatus* Rüppell 1830, *O. rosenbergii* Bleeker 1856, *O. variabilis* Smith-Vaniz 2009, and *O. macrolepis* Peters 1866.

The Indian Ocean record of *Opistognathus macrolepis* is based on a color photograph examined by the first author of a 63 mm SL specimen (ZSI F-10576/2) collected off the Kalpakkam coast near Chennai (Tamilnadu). This jawfish was previously known only from the type locality (Bangkok), the Gulf of Thailand where described by Wongratana (1975) as *Opistognathus rex*, and the Gulf of Carpentaria where described by Whitley (1966) as *Merogymnoides carpentariae*. A full description of the Indian Ocean specimen is in preparation by Sudipta Biswas and others.

### **Materials and method**

Methods of counts and measurements follow Smith-Vaniz (2009, 2010). Infraorbital bones and the upper and lower jaws on the right side of the holotype were removed, cleared and stained and drawn with the aid of a camera lucid. Position of the fifth cranial nerve was determined by dissection prior to clearing and staining. Abbreviations for institutional depositories are Marine Biodiversity Museum at Central Marine Fisheries Research Institute, Kochi, India (CMFRI) and Zoological Survey of India, Kolkata (ZSI).



## New distributional records of deep-sea sharks from Indian waters

\*K. V. Akhilesh, M. Hashim, K. K. Bineesh, C. P. R. Shanis and U. Ganga

Central Marine Fisheries Research Institute, PB No. 1603, Cochin-682 018,  
Kerala, India. \*E-mail: akhikv@gmail.com

### Abstract

This paper reports the first documented record of three deepwater sharks from Indian waters *i.e.*, *Hexanchus griseus* (Hexanchidae), *Deania profundorum* (Centrophoridae), pygmy false catshark (undescribed) (Pseudotriakidae) and presents a taxonomic account of smooth lanternshark, *Etmopterus pusillus* (Etmopteridae) and leafscale gulper shark, *Centrophorus squamosus* (Centrophoridae), caught by hooks & line units operated in the Arabian Sea, west coast of India and landed at Cochin Fisheries Harbour (Kerala), southwest coast of India.

**Keywords:** Deep-sea sharks, new reports, Arabian Sea, Indian EEZ

### Introduction

The Arabian Sea with its unique ecological features such as position between two land masses, presence of islands, features like oxygen minimum zone (OMZ), circulation pattern, currents, influence of monsoon and high saline water intrusion from Persian Gulf and Red Sea etc. supports a very diverse ichthyofauna. Reports on the diversity of deep-sea fish fauna especially that on deep-sea chondrichthyans from Indian waters are very few. Raje *et al.* (2007) listed 47 species of sharks in commercial landings along the Indian coast mainly from catches made within 100 m depths. However elasmobranchs are also known from deeper waters and probably many species, which are not yet recorded, occur in the unexploited/underexploited deep waters of the Indian EEZ.

The targeted deep-sea shark fishery in Indian waters, especially along the southwest and southeast coasts of India started lately after 2002 by the multiday shark fishermen of Thoothoor (Tamilnadu). The fishery targets gulper sharks (Centrophoridae) but many other deep-sea chondrichthyans occur as by catch, which were dominated by bramble shark, *Echinorhinus brucus* and chimaera, *Neoharriotta pinnata* besides several small sized deep-sea sharks, skates and rays which are often discarded. Cochin

Fisheries Harbour (Kerala), is a major fishing base where chondrichthyans which are caught along the entire west coast of India by multiday deep-sea trawlers, longlines and hooks & line units are landed throughout the year. The species described in this communication were captured by hooks & line units specifically targeting for deep-sea sharks operated off southwest coast of India at depths beyond 250 m. Deep-sea sharks, *Hexanchus griseus* (Hexanchidae), *Deania profundorum* (Centrophoridae) and pygmy false catshark (undescribed) (Pseudotriakidae) represent new species records from the Indian EEZ. In this paper these species are described and the occurrence of *Etmopterus pusillus* and *Centrophorus squamosus* off southwest coast of India is confirmed.

### Material and Methods

During weekly observations of fish landings at Cochin Fisheries Harbour (CFH), Cochin, southwest coast of India, specimens of *Hexanchus griseus*, *Centrophorus squamosus*, *Deania profundorum*, *Etmopterus pusillus* and pygmy false catshark (undescribed) were collected from the deep-sea hooks & line landings operated in the Arabian Sea during April 2008. Species identification was based on Compagno (1984), Smith and Heemstra (1986), Shirai and Tachikawa (1993) and Compagno *et al.*



## Note

# Molecular identification of three deepsea fish species of the genus *Chelidoperca* (Perciformes: Serranidae) from Indian waters

K. K. BINEESH<sup>1</sup>, C. MOHITHA<sup>1</sup>, N. VINEESH<sup>1</sup>, V. S. BASHEER<sup>1</sup>, M. JOSELET<sup>2</sup>  
N. K. G. PILLAI<sup>3</sup>, J. K. JENA<sup>4</sup> AND A. GOPALAKRISHNAN<sup>3</sup>

<sup>1</sup>Peninsular and Marine Fish Genetic Resources Centre, ICAR-National Bureau of Fish Genetic Resources  
ICAR- Central Marine Fisheries Research Institute Campus, Kochi - 682 018, Kerala, India

<sup>2</sup>Nirmalagiri college, Nirmalagiri P. O., Kannur - 670 701, Kerala, India

<sup>3</sup>ICAR-Central Marine Fisheries Research Institute, P. B. No. 1603, Ernakulam North P. O., Kochi - 682 018  
Kerala, India

<sup>4</sup>ICAR-National Bureau of Fish Genetic Resources, Canal Ring Road, P. O., Dilkusha, Lucknow - 226 002  
Uttar Pradesh, India

e-mail: kkbineesh@gmail.com

## ABSTRACT

The deepwater basslets of the genus *Chelidoperca* has eight nominal species and these are relatively small fishes caught in trawl nets operated at depths greater than 100 m. Mitochondrial cytochrome c oxidase subunit 1 (COI) and 16S rRNA gene sequence variation among three species under the genus *Chelidoperca* viz., *C. investigatoris*, *C. occipitalis* and *C. maculicauda* from Indian waters and their phylogenetic relationship with other representatives from same genus was studied. Fifteen individuals of *Chelidoperca* were sampled from different localities in the east and west coasts of India and further, four COI sequences from GenBank were used to reconstruct the phylogenetic relationship in the genus *Chelidoperca*. Based on COI sequence data analysis, the intraspecies genetic distance ranged from 0.000 to 0.005 while interspecies distance varied from 0.073 to 0.194. With respect to 16S rRNA sequences, the intraspecies genetic distance ranged from 0.000 to 0.002, while interspecies genetic distance varied from 0.062 to 0.118. The mean genetic difference observed between *C. investigatoris* and the other species used in this study was 11.53%. Results of the study revealed that the genus *Chelidoperca* is monophyletic.

Keywords: *Chelidoperca*, Deepsea fishes, Mitochondrial COI gene, 16S rRNA

The family Serranidae comprises carnivorous marine fishes inhabiting tropical and subtropical waters. Members of the genus *Chelidoperca* are usually found in muddy continental shelf and slope bottoms in the Indo-West Pacific (Bineesh *et al.*, 2013). These fishes are relatively small, usually less than 200 mm in length and are generally caught in trawl nets at depths greater than 100 m. Hence, only little information is available on these perchlets and are poorly represented in the museum collections worldwide (Williams and Carpenter, 2015). The genus *Chelidoperca* has eight valid species: *Chelidoperca hirundinacea* (Valenciennes, 1831); *C. investigatoris* (Alcock, 1890); *C. lecromi* Fourmanoir, 1982; *C. margaritifera* Weber, 1913; *C. occipitalis* Kotthaus, 1973; *C. pleurospilus* (Gunther, 1880); *C. maculicauda* Bineesh & Akhilesh, 2013 and *C. santosi* Williams & Carpenter, 2015 (Eschmeyer and Fong, 2015). Three species of *Chelidoperca*, namely *C. investigatoris*, *C. occipitalis* and *C. maculicauda* are reported from Indian waters (Bineesh *et al.*, 2013). Targeted trawl fishery for deepsea shrimp is being carried out at three major harbours in India at Saktikulangara,

Thoothukudi and Chennai. Deepsea shrimp trawl bycatch has been estimated to comprise >40 species (Pillai *et al.*, 2009; Akhilesh *et al.*, 2011). *Chelidoperca* spp. are landed as bycatch in deepsea shrimp trawlers and sold in the local market (Bineesh *et al.*, 2014; Shanis *et al.*, 2014).

Accurate identification of morphologically similar species is essential for sustainable management of the fishery. Species identification using traditional morphology based methods sometime result in misidentification due to the morphological plasticity and overlapping characters. In such cases, DNA based molecular markers have proven to resolve taxonomic ambiguity to a great extent (Hebert *et al.*, 2003). Presently, DNA based species identification has become very popular due to its ease of use, application to all life stages including eggs and larvae and even cooked food items. In the present study, mitochondrial COI and 16S rRNA gene sequences of the three species of *Chelidoperca* viz., *C. investigatoris*, *C. occipitalis* and *C. maculicauda* were analysed to assess the genetic relatedness and phylogenetic relationship.

## SHORT COMMUNICATION

**Molecular identification of Bigeyes (Perciformes, Priacanthidae) from Indian waters**Bineesh K. K<sup>1</sup>, Gopalakrishnan A<sup>2</sup>, Jena J. K<sup>3</sup>, Basheer V. S<sup>1</sup>, Mohitha C<sup>1</sup>, Vineesh N<sup>1</sup>, Joselet M<sup>4</sup>, and N. G. K. Pillai<sup>2</sup><sup>1</sup>National Bureau of Fish Genetic Resources, Central Marine Fisheries Research Institute Campus, Kochi, Kerala, India, <sup>2</sup>Central Marine Fisheries Research Institute, Kochi, Kerala, India, <sup>3</sup>National Bureau of Fish Genetic Resources, Dilkusha, Lucknow, India, and <sup>4</sup>Nirmalagiri College, Kannur, Kerala, India**Abstract**

Thirty-five individuals of six priacanthid fish species were sampled from different localities along the coast of India covering the Arabian Sea and Bay of Bengal. The partial sequence of 16S rRNA and cytochrome oxidase subunit I (COI) genes were analyzed for species identification and phylogenetic relationship among the Indian priacanthids (*Priacanthus hamrur*, *P. prolixus*, *P. blochii*, *P. sagittarius*, *Cookeolus japonicus*, and *Pristigenys refulgens*). The intraspecific genetic distance ranged from 0.000 to 0.002, while distances varied from 0.008 to 0.157 interspecies based on 16S sequences. Using COI data analysis, the intraspecific genetic distance ranged from 0.000 to 0.005, while interspecies distances varied from 0.009 to 0.108. Several sequences labeled *Priacanthus hamrur* in GenBank are shown to be *P. prolixus*. We also observed cryptic speciation in *Heteropriacanthus cruentatus*. Partial sequences of 16S rRNA and COI genes provided phylogenetic information to distinguish thirteen species of priacanthids, indicating the usefulness of molecular markers in species identification.

**Keywords**

Bigeyes, COI, DNA barcoding, Indian waters, 16S rRNA

**History**Received 23 June 2015  
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Published online 9 December 2015**Introduction**

The Bigeyes of family Priacanthidae are marine percoid fishes, with four genera and 19 species. They comprise relatively small epibenthic predatory fishes occurring in rocky and coral habitats in Indo-Pacific region with 15 species and four species in the Atlantic (Iwatsuki et al., 2012; Starnes, 1988). The bigeyes are characterized by extremely large eyes, rough scales, bright red coloration, and deep, laterally compressed oval to moderately elongate bodies. They occur as solitary or in small aggregations, but species like *P. hamrur* form large aggregations. Exploratory surveys conducted along the Indian EEZ have revealed higher concentration of priacanthids along the West Coast than the East Coast (James & Pillai, 1989; Sivakami et al., 1998). Bigeyes are concentrated in 40–100 m depth range along the Southwest Coast and 100–200 m depth range along the Northwest coast (Bande et al., 1989; Sivakami et al., 1998).

Priacanthids are generally identified using morphological, meristic, and anatomical characters (Starnes, 1988). But due to morphological similarity and overlapping meristic ranges taxonomic ambiguities exist in several species. Synonym citations in database like FishBase indicate the possibility of taxonomic ambiguity in genera like *Heteropriacanthus* and *Pristigenys*. Most of the species in the family are widely distributed with geographic variations, suggesting the possibility of cryptic species and possible undescribed species.

Accurate identification of exploited fishes is very important in case of morphological similarity, for fisheries management and

population studies. Molecular methods in the form of DNA barcodes have been used to differentiate species and identifying cryptic species (Hebert et al., 2003). Mitochondrial DNA genes have been extensively used in fish phylogenetics, since they are highly conserved compared to nuclear DNA, resulting in the accumulation of differences between species (Timm et al., 2008). The 16S rRNA and COI genes have been useful in resolving taxonomic ambiguities, resurrecting species, and identifying market mislabeling in fishes (Iwatsuki, 2013; Keskin & Atar, 2012; Ward et al., 2005).

In this study, the sequences of the COI and 16S rRNA genes were generated for different species of priacanthids along the Indian Coast. In combination with the retrieved sequence data from GenBank, the cryptic species (speciation without an obvious morphological signature) in the genus *Priacanthus* may facilitate further investigations to find out the accurate species diversity and distributions.

**Materials and methods**

Six species of priacanthids from three genera were collected and the voucher specimens were kept in the National referral museum of the Central Marine Fisheries Research Institute (CMFRI), Kochi and Zoological Survey of India (ZSI), Kozhikode, India. Tissue samples were collected from fragments of white muscle or gill tissues for DNA extraction following the protocol of Miller et al. (1988). The concentration of isolated DNA was checked using the UV spectrophotometer.

The mitochondrial 16S rRNA and COI genes were amplified in 25 µl reaction volume containing 1 × assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 1.5 mM MgCl<sub>2</sub> (Genei, Bangalore, India), 5 pmoles of each primer, 200 µM of each dNTP (Genei, Bangalore, India), 1.5 U *Taq* DNA



RESEARCH ARTICLE

## DNA barcoding reveals species composition of sharks and rays in the Indian commercial fishery

K. K. Bineesh<sup>a</sup>, A. Gopalakrishnan<sup>b</sup>, K. V. Akhilesh<sup>b</sup>, K. A. Sajeela<sup>a</sup>, E. M. Abdussamad<sup>b</sup>, N. G. K. Pillai<sup>b</sup>, V. S. Basheer<sup>a</sup>, J. K. Jena<sup>c</sup> and Robert D. Ward<sup>d</sup>

<sup>a</sup>National Bureau of Fish Genetic Resources, Central Marine Fisheries Research Institute Campus Ernakulam North, Kochi, Kerala, India; <sup>b</sup>Central Marine Fisheries Research Institute, Ernakulam North, Kochi, Kerala, India; <sup>c</sup>National Bureau of Fish Genetic Resources, Dilkusha, Lucknow, India; <sup>d</sup>CSIRO National Research Collections Australia, Hobart, Tasmania, Australia

### ABSTRACT

DNA barcoding was successfully used for the accurate identification of chondrichthyans in the Indian commercial marine fishery. About 528 specimens of 111 chondrichthyan species and 34 families, collected from the Indian EEZ, were barcoded for a 655 bp region of the mitochondrial gene cytochrome c oxidase subunit 1 (COI). Generally, five specimens per species were barcoded, but numbers ranged from 2 to 13. The average Kimura 2 parameter (K2P) distance separating individuals within species was 0.32%, and the average distance separating species within genera was 6.73%. Ten species were suggested as putative new species requiring formal descriptions. Based on the morphology and molecular support, 11 elasmobranch species were confirmed first records for Indian waters. The present study confirms the ability of DNA barcoding for the accurate identification of sharks, rays, and their products from Indian waters.

### ARTICLE HISTORY

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### KEYWORDS

Barcoding; chondrichthyans; identification; COI; Indian Ocean; taxonomy

### Introduction

Chondrichthyans (chimaeras, sharks, rays, and skates) are widely distributed in all the oceans, but are most diverse in the tropical and subtropical Indo-Pacific (Bonfil, 2002). Chondrichthyans are exploited in commercial, artisanal, and recreational fishing activities but the major catch occurs as bycatch in the commercial fishery. They are highly vulnerable to over exploitation and habitat degradation due to their K-selected life history (Dulvy et al., 2014; Stevens et al., 2000). Overfishing and bycatch have significantly reduced many populations of these apex predators (Baum et al., 2005; Graham et al., 2010; Robbins et al., 2006). Concerns over the impact of fishing on elasmobranch population are being raised at international levels and many programs are being initiated to recover and protect this group through sustainable management plans (Dulvy et al., 2014; Ward-Paige et al., 2012). Accurate species identification is critical to the design of fishery conservation and management plans. (FAO, 1997; Last, 2007; White & Last, 2012). However, field identification of several closely related sharks (including carcharhinid, centrophorid, and triakid sharks) and batoids (Whiptail stingrays and skates) are often difficult (Tillett et al., 2012; Verissimo et al., 2014), and can lead to erroneous species compositions and diversity in catch reports (Camhi et al., 2009).

India is one of the leading chondrichthyan fishing nations for past several years (FAO, 2013), in 2013, the estimated landing of 46,471 tonnes (sharks 45.5%, rays 49.5%, and guitarfishes 5%)

accounting for 1.23% of its total marine fish landings (CMFRI, 2013). However, these catch data largely include the easily identifiable species and others will be often put in group names only (sharks, rays or *Carcharhinus* spp, *Himantura* spp., etc.). Despite the rich diversity and long history of the elasmobranch fishery, only a few detailed studies have been undertaken on the taxonomy and diversity of this group in India. For a long period, this important group was neglected by researchers due to impediments including taxonomic problems and large specimen sizes and costs. Nevertheless, elasmobranchs found in Indian waters have been catalogued by several researchers (Day, 1889; Misra, 1952, 1969; Raje et al., 2007; Talwar & Kacker, 1984). In recent years, several species have been added to the list (Akhilesh et al., 2010, 2013a,b; Bineesh et al., 2014, Babu et al., 2011; Soundararajan & Roy 2004; Sutaria et al., 2015). An updated and extended checklist of 227 chondrichthyan species reported/listed as occurring in Indian waters, put together by Akhilesh et al. (2014), suggested that 27 species (12%) had questionable status in India and another 41 species (18%) required additional confirmation with regard to their occurrence suggested there is an urgent need for a re-assessment of chondrichthyan diversity in Indian waters. However, it is possible that the actual species diversity occurring in the fishery or in Indian seas could have been underestimated, for many reasons: an extended long coastline, diverse habitat, a large number of widely distributed landing centers, varied operational depths and regions, limited chondrichthyan exploratory surveys, and low numbers of well-trained observers





## ***Chaunax multilepis* sp. nov., a new species of *Chaunax* (Lophiiformes: Chaunacidae) from the northern Indian Ocean**

HSUAN-CHING HO<sup>1,2\*</sup>, RAJEESH KUMAR MELEPPURA<sup>3</sup> & K. K. BINEESH<sup>4</sup>

<sup>1</sup>National Museum of Marine Biology & Aquarium, Pingtung, Taiwan

<sup>2</sup>Institute of Marine Biology, National Dong Hwa University, Pingtung, Taiwan

<sup>3</sup>Centre for Marine Living Resources and Ecology, Ministry of Earth Sciences, Kochi, Kerala, India

<sup>4</sup>ICAR-National Bureau of Fish Genetic Resources, Peninsular and Marine Fish Genetic Resources Centre, CMFRI Campus, Kochi, Kerala, India

<sup>5</sup>Correspondent author. E-mail: [ogcoho@gmail.com](mailto:ogcoho@gmail.com)

### **Abstract**

A new species of *Chaunax* is described on the basis of eight type and five non-type specimens. This species belongs to the *Chaunax abei* species group and can be distinguished from congeners in the group by having a continuous tooth patch on the vomer, not divided into two patches, and four or five neuromasts in the lower preopercular series. It can be further separated by the following combination of characters: large green spots on dorsal surface; simple spinules on dorsal surface; 12 pectoral-fin rays; 13–16 neuromasts in pectoral series; 30–37 neuromasts in lateral-line proper; typically four neuromasts on caudal-fin base; typically 7 neuromasts in mandible; typically 12 gill rakers on second gill arch; gill chamber and buccal cavity pale; and peritoneum black.

**Key words:** Pisces, Teleostei, *Chaunax multilepis*, new species, India

### **Introduction**

The first *Chaunax* species recorded from India was *Chaunax pictus* (Lowe, 1846), more than a century ago (Alcock, 1899). However, the species has been subsequently shown to be restricted to Atlantic waters (Caruso, 1989). Lloyd (1909) described a second species, *Chaunax apus*, collected from the Bay of Bengal, and he stated that this species is commonly collected in that region. Le Danios (1979) erroneously treated *Chaunax apus* as a junior synonym of *Chaunax endeavouri* Whitley, 1929, which was described subsequent to *C. apus*. Caruso (1989) recognized *C. apus* as an *nomen dubium* in *Chaunax*. Le Danios (1979) described *Chaunax umbrinus flammeus* (= *Chaunax flammeus*) based on one specimen collected from off Madagascar. Smith in Smith & Heemstra (1986) recorded *C. penicillatus* McCulloch, 1915 and *C. pictus* from South Africa, the latter most likely a misidentification. Ho & Last (2013) recognized six species of *Chaunax* from the Indian Ocean, including two new species from the eastern and western Indian Ocean, respectively. They also suggested that *C. apus* should be treated as a valid species.

During our recent examination of specimens of *Chaunax* collected from Indian waters, two different forms were typically found in collections, one with a uniformly pink color (uniformly creamy white in preservation) and one with many green spots on the body (turned into gray or brown spots in preservation).

Comparing these specimens with the original description and the holotype, we recognized the pink form as *C. apus*, based on the similar morphology and lateral-line neuromast counts, body proportions, and squamation. Moreover, Lloyd (1909) did not mention any spots on the body surface, an obvious character that could not be overlooked, even in most long-preserved specimens.

The green-spotted specimens lack cirri on the dorsal surface of head and appear to belong in the *Chaunax abei* species groups (*sensu* Ho *et al.*, 2013). However, there are several diagnostic characters that distinguish these specimens from all known species in this group, so these specimens are recognized as a new species. A formal description is provided herein.

## ***Appendices***

**Appendix I:** Details of deep-sea Chondrichthyan species DNA barcoded

Order	Family	Common name	Species	
Chimaeriformes	Rhinochimaeridae	Sicklefin Chimaera	<i>Neoharriotta pinnata</i> (Schnakenbeck, 1931)	
	Chimaeridae	African Chimaera	<i>Hydrolagus africanus</i> (Gilchrist, 1922)	
Hexanchiformes	Hexanchidae		<i>Hexanchus griseus</i> (Bonnaterre, 1788)	
			<i>Heptranchias perlo</i> (Bonnaterre, 1788)	
Echinorhiniformes	Echinorhinidae	Bramble Shark	<i>Echinorhinus brucus</i> (Bonnaterre, 1788)	
Lamniformes	Odontaspidae	Bigeye Sand Tiger	<i>Odontaspis noronhai</i> (Maul, 1955)	
		Lamnidae	Shortfin Mako	<i>Isurus oxyrinchus</i> Rafinesque, 1810
			Longfin Mako	<i>Isurus paucus</i> Guitart, 1966
Carcharhiniformes	Pseudocarchariidae		<i>Pseudocarcharias kamoharai</i> (Matsubara, 1936)	
	Scyliorhinidae		<i>Apristurus</i> sp. A	
			<i>Apristurus</i> sp. B	
			<i>Apristurus</i> sp. C	
			<i>Apristurus</i> sp. D	
		Indian swellshark	<i>Cephaloscyllium silasi</i> (Talwar, 1974)	
		Quagga Catshark	<i>Halaelurus quagga</i> (Alcock, 1899)	
		Bristly catshark	<i>Bythaelurus hispidus</i> (Alcock, 1891)	
	Pygmy ribbontail catshark	<i>Eridacnis radcliffei</i> Smith, 1913		
Squaliformes	Proscylliidae		<i>Iago</i> sp. A	
	Triakidae		<i>Iago</i> sp. B	
	Squalidae		<i>Squalus</i> sp. A	
	Centrophoridae		<i>Centrophorus atromarginatus</i> Garman, 1913	
			<i>Centrophorus granulosus</i> (Bloch & Schneider, 1801)	



			<i>Centrophorus squamosus</i> (Bonnaterre, 1788)
			<i>Centrophorus cf. zeehaani</i> White, Ebert & Compagno 2008
			<i>Deania profundorum</i> (Smith & Radcliffe, 1912)
	Etmopteridae	Smooth Lanternshark	<i>Etmopterus pusillus</i> (Lowe, 1839)
	Somniosidae	Longnose Velvet Dogfish	<i>Centroselachus crepidater</i> (Bocage & Capello, 1864)
			<i>Zameus squamulosus</i> (Günther, 1877)
Torpediniformes	Torpedinidae		<i>Torpedo</i> sp. A
	Narcinidae		<i>Benthobatis moresbyi</i> Alcock, 1898
			<i>Narcine</i> sp. A
Rajiformes	Rhinobatidae		<i>Rhinobatos variegatus</i> Nair & Lal Mohan, 1973
	Rajidae		<i>Dipturus</i> sp. A
			<i>Dipturus</i> sp. B
			<i>Dipturus cf. johannisdavisi</i>
			<i>Okamejei powelli</i> (Alcock, 1898)
Myliobatiformes	Plesiobatidae		<i>Plesiobatis daviesi</i> (Wallace, 1967)
	Dasyatidae		<i>Pteroplatytrygon violacea</i> (Bonaparte, 1832)

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## Appendix II:

List of deep-sea Chondrichthyan with COI GenBank accession numbers

Species	Accession No.
<i>Neoharriotta pinnata</i>	HM239670, KJ749670-KJ749676
<i>Hydrolagus africanus</i>	KF899529, KF899530, KF899531, KJ749662
<i>Hexanchus griseus</i>	HM239677, KF899461-KF899464
<i>Heptranchias perlo</i>	HM239668, KF899456-KF899460
<i>Echinorhinus brucus</i>	HM467790, KJ749661
<i>Odontaspis noronhai</i>	KF899559-KF899561
<i>Isurus oxyrinchus</i>	KF899536-KF899541
<i>Isurus paucus</i>	KF899542-KF899544
<i>Pseudocarcharias kamoharai</i>	KF899532-KF899535
<i>Apristurus</i> sp. A	KF899717-KF899721
<i>Apristurus</i> sp. B	KT766183-KT766185
<i>Apristurus</i> sp. C	KT766186-KT766187
<i>Apristurus</i> sp. D	KT766188-KT766190
<i>Cephaloscyllium silasi</i>	HM467791, KF899707-KF899711
<i>Halaelurus quagga</i>	HQ839734, JF260984, KF899712-KF899716
<i>Bythaelurus hispidus</i>	HM239667, KF899701-KF899706
<i>Eridacnis radcliffei</i>	KF899421-KF899425
<i>Iago</i> sp. A	KF899737-KF899742
<i>Iago</i> sp. B	KF899365, KJ749663-KJ749669
<i>Squalus</i> sp. A	KF899758-KF899763
<i>Centrophorus granulosus</i>	KF899391-KF899393
<i>Centrophorus squamosus</i>	KF899383-KF899386
<i>Centrophorus atromarginatus</i>	KF899387-KF899390

<i>Centrophorus zeehaani</i>	KF899394-KF899399
<i>Deania profundorum</i>	KF899378-KF899382
<i>Etmopterus pusillus</i>	KF899426-KF899428
<i>Centroselachus crepidater</i>	KF899400, KF899401
<i>Zameus squamulosus</i>	KF899769-KF899772
<i>Torpedo</i> sp. A	KF899725-KF899729
<i>Benthobatis moresbyi</i>	KJ768659-KJ768663
<i>Narcine</i> sp. A	KF899601-KF899605
<i>Rhinobatos variegatus</i>	HM467794, KF899673-KF899678
<i>Dipturus</i> sp. B	KF899416-KF899420
<i>Dipturus</i> cf. <i>johannisdavisi</i>	KF899412-KF899415
<i>Dipturus</i> sp. A	KF899402-KF899411
<i>Okamejei powelli</i>	KF899614-KF899619
<i>Plesiobatis daviesi</i>	HM467801, KF899645-KF899649
<i>Pteroplatytrygon violacea</i>	HM239671, KF899654-KF899658

List of deep-sea Chondrichthyan with 16S rRNA GenBank accession numbers

Species	Accession number
<i>Etmopterus pusillus</i>	KR149144-KR149148
<i>Echinorhinus brucus</i>	KR149149-KR149154
<i>Neoharriotta pinnata</i>	KR149155-KR149158
<i>Hexanchus griseus</i>	KR149159-KR149161
<i>Squalus</i> sp. A	KR149162-KR149165
<i>Heptranchias perlo</i>	KR149166-KR149169
<i>Pteroplatytrygon violacea</i>	KR149170-KR149172
<i>Rhinobatos variegatus</i>	KR149173-KR149176

<i>Torpedo</i> sp. A	KR149177-KR149179
<i>Centrophorus atromarginatus</i>	KR149180-KR149186
<i>Deania profundorum</i>	KR149187-KR149191
<i>Centrophorus zeehaani</i>	KR149192-KR149195
<i>Dipturus</i> sp. A	KR149196-KR149203
<i>Dipturus</i> sp. B	KR149204-KR149207
<i>Dipturus</i> cf. <i>gigas</i>	KR149208-KR149213
<i>Okamejei powelli</i>	KR149214-KR149219
<i>Cephaloscyllium silasi</i>	KR149220-KR149223
<i>Apristurus</i> sp. A	KR149224-KR149228
<i>Bythaelurus hispidus</i>	KR149229-KR149232
<i>Halaaelurus quagga</i>	KR149233-KR149236

**Appendix III.** List of primer pairs used to amplify mtDNA regions evaluated for the study

mtDNA Genes	Primers (Forward/ Reverse)	Primer Sequence (5'-3')	Melting Temp. (T <sub>m</sub> )	Annealing Temp. (T <sub>a</sub> )	Reference
<b>16SrRNA</b>					
16Sar-5' rRNA	L 2510 (F)	CGC CTG TTT ATC AAA AAC AT	63°C	58°C	Palumbi <i>et al.</i> , 1991
16Sbr-3' rRNA	H3080 (R)	CCG GTC TGA ACT CAG ATC ACG T	63°C		
<b>COI</b>					
COI 1-5'	WARD-1(F1)	TCA ACC AAC CAC AAA GAC ATT GGC AC	58°C	50°C	Ward <i>et al.</i> , 2005
COI 1-3'	WARD-1(R1)	TAGACTTCTGGGTGGCCAAAGAATCA	58°C		
COI 2-5'	WARD-2(F1)	TCGACTAATCATAAAGATATGGGCAC	58°C		
COI 2-3'	WARD-2(R1)	ACTTCAGGGTGACCGAAGAATCAGAA	58°C		

**Appendix IV.** PCR conditions used in the study

<b>DNA Region</b>	<b>Reaction Mix (25 µl)</b>	<b>Thermal Cycler Profile</b>	<b>Product Size</b>
16SrRNA	20 ng of template DNA, 1x assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0), 1.5 mM MgCl <sub>2</sub> (Genei, Bangalore, India), 5 pmoles of each primer, 200 µM of each dNTP (Genei, Bangalore, India), 1.5 U <i>Taq</i> DNA polymerase	95 °C - 5 min 29X 94 °C - 45 sec 58 °C - 30 sec 72 °C - 45 sec 72 °C - 5 min 4 °C - hold	520-570 bp
COI	20 ng of template DNA, 1x assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0), 1.5 mM MgCl <sub>2</sub> (Genei, Bangalore, India), 5 pmoles of each primer, 200 µM of each dNTP (Genei, Bangalore, India), 1.5 U <i>Taq</i> DNA polymerase	95 °C - 5 min 29X 94 °C - 45 sec 50 °C - 30 sec 72 °C - 45 sec 72 °C - 5 min 4 °C - hold	630-678 bp

## Appendix V: Genomic DNA isolation

Total DNA was extracted from the tissue samples following the procedure of Miller *et al.* (1988) with minor modifications. Average five samples for each species were selected for isolating the total DNA.

### Reagents required:

### Stock solutions:

#### 1. 0.5M Tris Cl (pH-8.0)

Tris base - 3.028 g

Distilled water - 40 ml

Adjust pH to 8.0 using HCl.

Make up the volume to 50 ml, autoclave and store at 4°C.

#### 3. 10mM Tris Cl (pH-7.5)

Tris base - 0.030 g

Distilled water - 20 ml

Adjust pH to 7.5 using HCl.

Make up the volume to 25 ml, autoclaved and stored at 4°C.

#### 2. 0.5M EDTA (pH-8.0)

EDTA - 9.31 g

Distilled water - 40 ml

Adjust pH to 8.0 using NaOH.

Make up the volume to 50 ml, autoclaved and stored at 4 °C.

#### 4. RNAase buffer

10mM Tris Cl (pH 7.5)- 10 µl

15mM NaCl - 30 µl

Distilled water - 960 µl

Autoclaved and stored at 4 °C.

## Working Solutions

### 1. Solution 1:

Tris-HCl (pH8.0) - 50 mM

EDTA (pH8.0) - 20 mM

SDS - 2 %

Prepared in double distilled water. Autoclave and store at 4°C

### 3. Proteinase K

Proteinase K – 20 mg/ml

Prepared in autoclave double distilled water and store at -20°C.

### 5. RNAase

RNAase - 10 mg/ml of RNAase buffer (autoclaved)

### 2. Solution 2:

NaCl solution (saturated) - (6 M)

Prepared in double distilled water.

Autoclave and store at 4°C

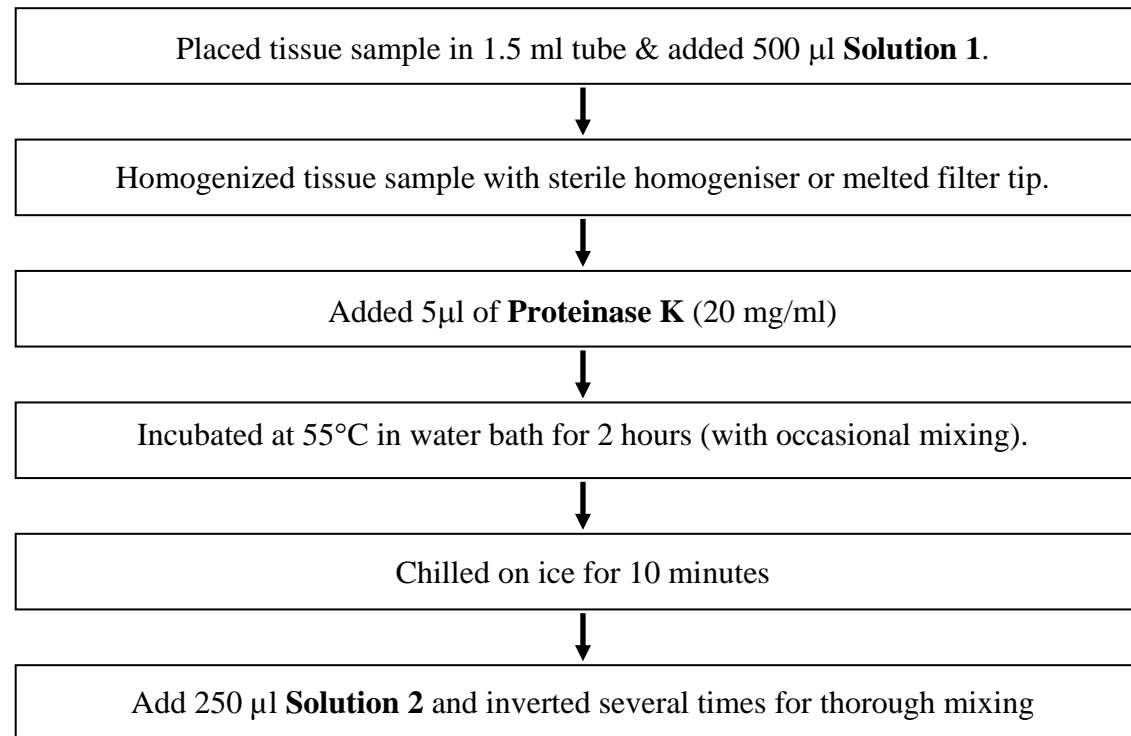
### 4. TE buffer

Tris Cl (pH-8.0) - 10 mM

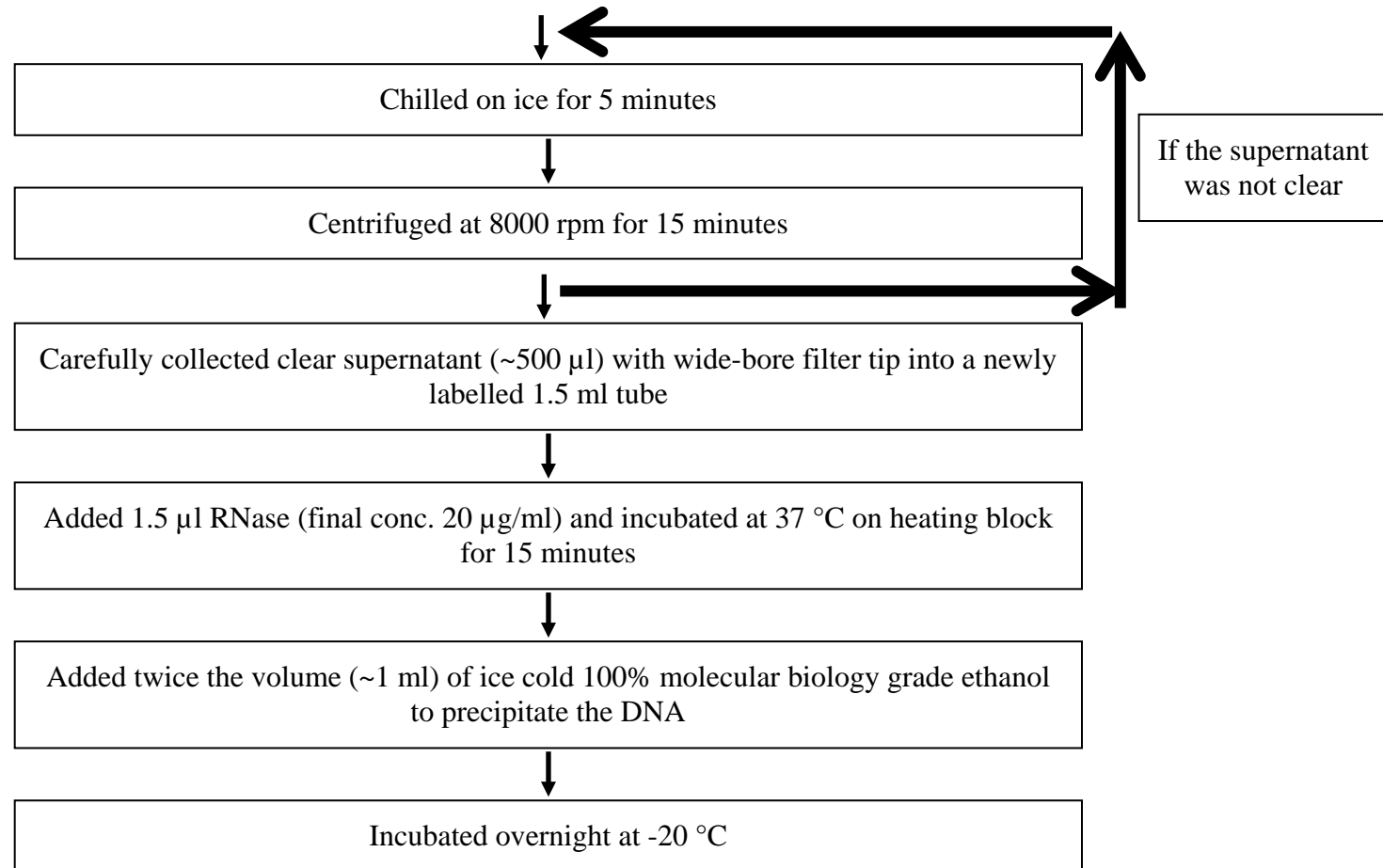
EDTA (pH-8.0) - 1 mM

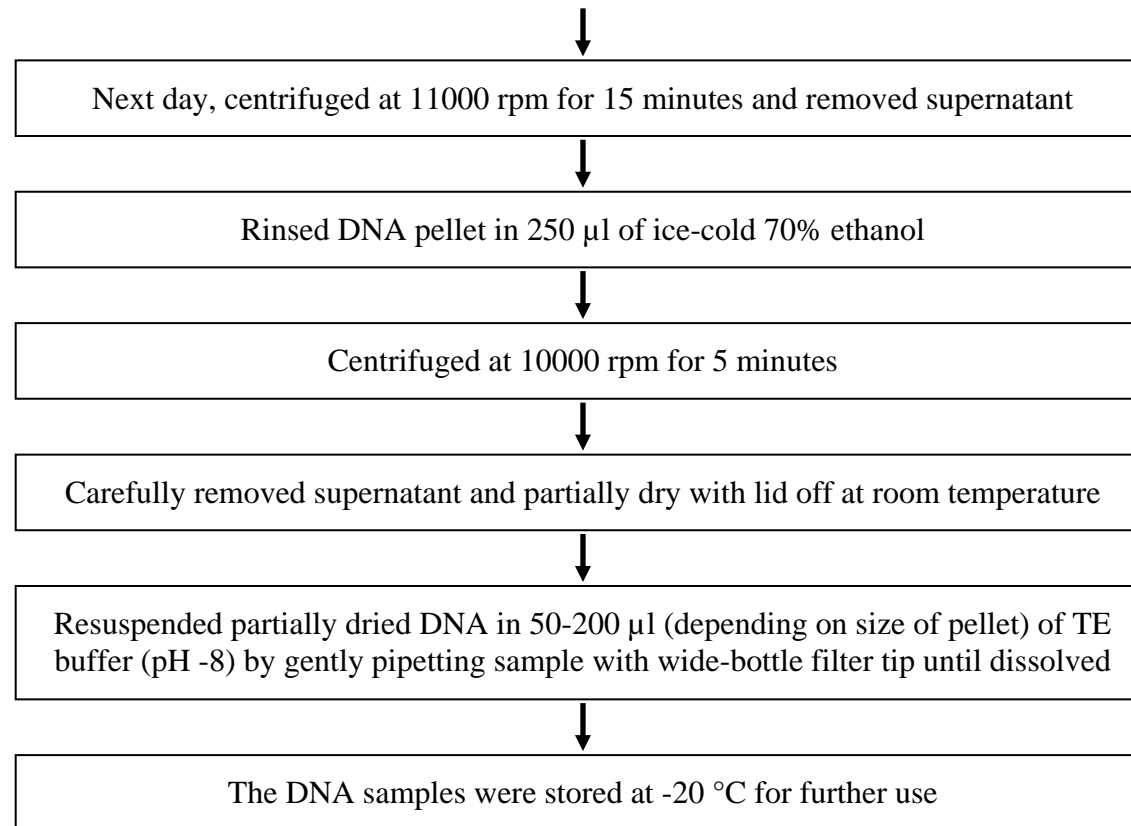
Prepared in double distilled water. Autoclave and store at 4°C

**Total DNA isolation protocol:** Tissue stored in alcohol was washed with TRIS buffer (pH 8.0) by spinning and then followed below listed steps.









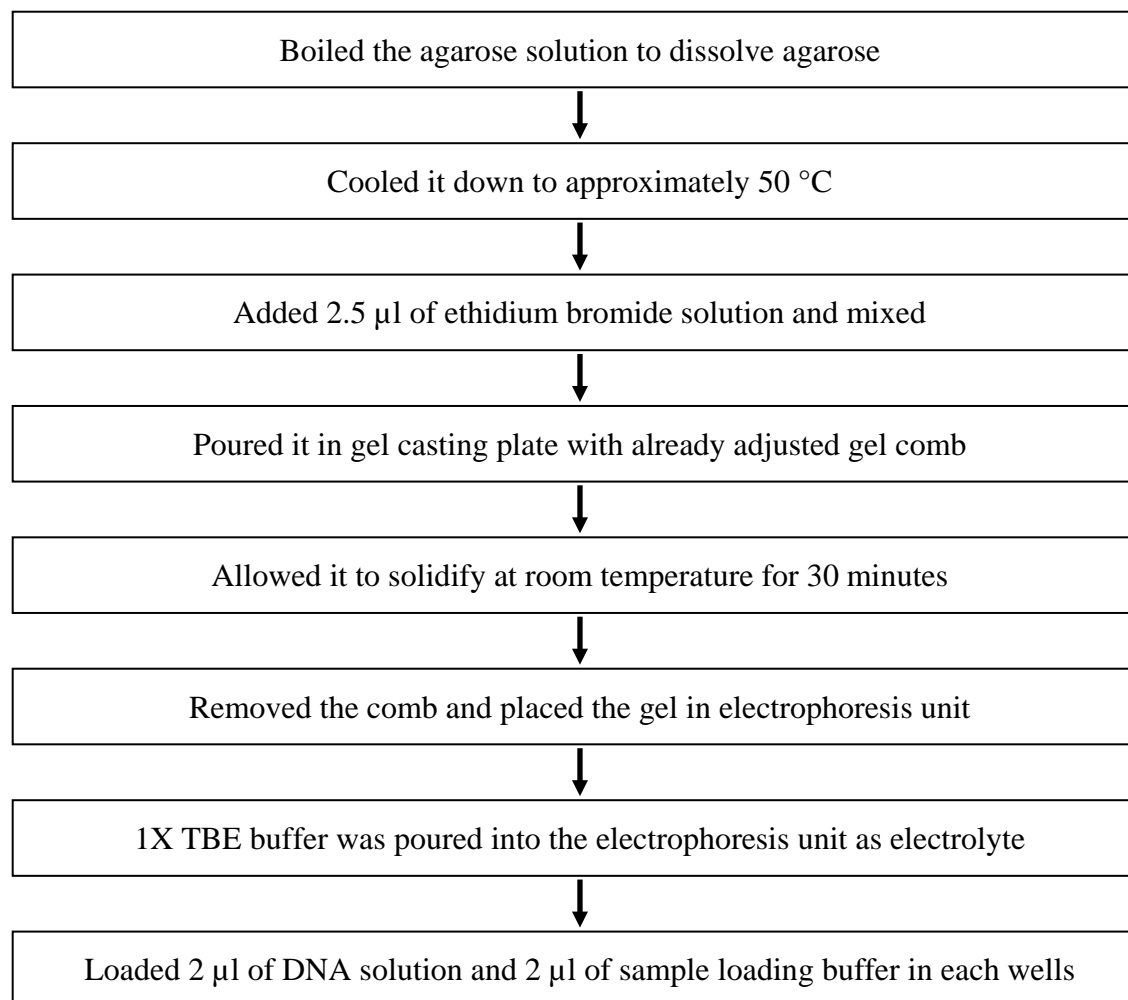
## Appendix VI: Agarose electrophoresis and visualization of bands

The extracted DNA was checked through 0.7% agarose gel (10 x 4 cm) electrophoresis with ethidium bromide incorporated in 1X TBE buffer.

### Reagents required

1. **TBE buffer 10X (pH-8.0)**
  - Tris base - 10.8 g
  - Boric acid - 5.5 g
  - EDTA - 0.75 g
  - Make up the solution to 100 ml with double distilled water.
  - Autoclaved and stored at 4 °C
2. **Gel loading buffer**
  - Bromophenol blue - 0.5%
  - Glycerol (mol. grade) - 30%
  - Prepared in 1X TBE
  - Store at 4 °C.
3. **Agarose solution (0.7%)**
  - Agarose - 0.21 g
  - 10X TBE - 3 ml
  - Distilled Water - 27 ml
4. **1X TBE buffer**
  - 10X TBE - 10 ml
  - Distilled Water - 90 ml
5. **Ethidium bromide solution**
  - Ethidium bromide - 10 mg
  - Distilled water - 2 ml

**Protocol:** Arranged the gel casting unit according to instructions of manufacturer.





Electrophoresis was done at constant voltage (80 V) for 1 hour



After electrophoresis the gel was observed in ultraviolet light and documented using documentation system Image Master VDS (Pharmacia Biotech)

**Appendix VII:** List of deep-sea fish species DNA barcoded

Order	Family	Species
Perciformes	Serranidae	<i>Hyorthodus octofasciatus</i> (Griffin, 1926)
		<i>Liopropoma randalli</i> Akhilesh, Bineesh & White, 2012
		<i>Sacura boulengeri</i> (Heemstra, 1973)
		<i>Odontanthias perumali</i> Talwar, 1976
		<i>Chelidoperca occipitalis</i> Kotthaus, 1973
		<i>Chelidoperca maculicauda</i> Bineesh & Akhilesh, 2013
		<i>Chelidoperca investigatoris</i> (Alcock, 1890)
		<i>Bembrops caudimacula</i> Steindachner, 1876
		<i>Symphysanodon xanthopterygion</i> Anderson & Bineesh, 2011
		<i>Histiopterus typus</i> Temminck & Schlegel, 1844
	Percophidae	<i>Bathyclupea hoskynii</i> Alcock, 1891
	Symphysanodontidae	<i>Erythrocles acarina</i> Kotthaus, 1974
	Pentacerotidae	<i>Priacanthus sagittarius</i> Starnes, 1988
	Bathyclupeidae	<i>Priacanthus blochii</i> Bleeker, 1853
	Emmelichthyidae	<i>Priacanthus hamrur</i> (Forsskål, 1775)
	Priacanthidae	<i>Priacanthus prolixus</i> Starnes, 1988
		<i>Pristigenys refulgens</i> (Valenciennes, 1862)
		<i>Cookeolus japonicus</i> (Cuvier, 1829)
	Nemipteridae	<i>Parascolopsis boesemani</i> (Rao & Rao, 1981)
		<i>Parascolopsis eriomma</i> (Jordan & Richardson, 1909)
		<i>Parascolopsis aspinosa</i> (Rao & Rao, 1981)
Trichiuridae	<i>Trichiurus auriga</i> Klunzinger, 1884	
	<i>Aphanopus intermedius</i> Parin, 1983	
Gobiidae	<i>Obliquogobius cometes</i> (Alcock, 1890)	
Acropomatidae	<i>Acropoma</i> sp. A	
Gempylidae	<i>Promethichthys prometheus</i> (Cuvier, 1832)	
	<i>Rexea bengalensis</i> (Alcock, 1894)	
	<i>Neoepinnula orientalis</i> (Gilchrist & von Bonde, 1924)	

		<i>Lepidocybium flavobrunneum</i> (Smith, 1843)
	Centrolophidae	<i>Psenopsis cyanea</i> (Alcock, 1890)
	Ariommatidae	<i>Ariomma indicum</i> (Day, 1871)
	Nomeidae	<i>Psenes cyanophrys</i> Valenciennes, 1833
		<i>Psenes arafurensis</i> Günther, 1889
		<i>Cubiceps</i> sp
		<i>Cubiceps whiteleggii</i> (Waite, 1894)
	Bramidae	<i>Brama dussumieri</i> Cuvier, 1831
	Cepolidae	<i>Sphenanthias whiteheadi</i> Talwar, 1973
		<i>Acanthocephala indica</i> (Day, 1888)
Polymixiiformes	Polymixiidae	<i>Polymixia</i> sp. A
Notacanthiformes	Notacanthidae	<i>Notacanthus</i> sp. A
		<i>Notacanthus indicus</i> Lloyd, 1909
Aulopiformes	Chlorophthalmidae	<i>Chlorophthalmus acutifrons</i> Hiyama, 1940
		<i>Chlorophthalmus corniger</i> Alcock, 1894
	Synodontidae	<i>Saurida</i> cf. <i>micropectoralis</i> Shindo & Yamada, 1972
		<i>Saurida tumbil</i> (Bloch, 1795)
		<i>Saurida longimanus</i> Norman, 1939
		<i>Saurida</i> sp. B
		<i>Saurida undosquamis</i> (Richardson, 1848)
		<i>Saurida</i> sp. A
Zeiformes	Parazenidae	<i>Cyttopsis rosea</i> (Lowe, 1843)
Zeiformes	Zeidae	<i>Zenopsis conchifer</i> (Lowe, 1852)
Ophidiiformes	Ophidiidae	<i>Neobythites steatiticus</i> Alcock, 1894
Lophiiformes	Diceratiidae	<i>Bufoceratias thele</i> (Uwate, 1979)
		<i>Bufoceratias</i> sp
	Chaunacidae	<i>Chaunax</i> sp. A
	Lophiidae	<i>Lophiodes lugubris</i> (Alcock, 1894)
		<i>Lophius indicus</i> Alcock, 1889
Scorpaeniformes	Scorpaenidae	<i>Ebosia falcata</i> Eschmeyer & Rama Rao, 1978
		<i>Pontinus nigerimum</i> Eschmeyer, 1983
	Tetrarogidae	<i>Snyderina guentheri</i> (Boulenger, 1889)

	Peristediidae	<i>Satyrichthys adeni</i> (Lloyd, 1907)
	Triglidae	<i>Lepidotrigla</i> cf. <i>omanensis</i> Regan, 1905
	Setarchidae	<i>Setarches guentheri</i> Johnson, 1862 <i>Setarches</i> sp. A
Beryciformes	Trachichthyidae	<i>Gephyroberyx darwinii</i> (Johnson, 1866) <i>Hoplostethus</i> sp
	Berycidae	<i>Beryx mollis</i> Abe, 1959
Osmeriformes	Holocentridae	<i>Ostichthys kaianus</i> (Günther, 1880)
	Platyroctidae	<i>Normichthys yahganorum</i> Lavenberg, 1965
	Alepocephalidae	<i>Alepocephalus bicolor</i> Alcock, 1891
	Argentinidae	<i>Glossanodon</i> sp. A
Myctophiformes	Myctophidae	<i>Benthoosema fibulatum</i> (Gilbert & Cramer, 1897) <i>Benthoosema pterotum</i> (Alcock, 1890) <i>Myctophum spinosum</i> (Steindachner, 1867) <i>Myctophum</i> sp. A <i>Diaphus watasei</i> Jordan & Starks, 1904 <i>Diaphus garmani</i> Gilbert, 1906 <i>Diaphus thiollierei</i> Fowler, 1934 <i>Diaphus</i> sp. A
Anguilliformes	Nemichthyidae	<i>Nemichthys acanthonotus</i> Alcock, 1894
Pleuronectiformes	Bothidae	<i>Laeops macrophthalmus</i> (Alcock, 1889) <i>Chascanopsetta lugubris</i> Alcock, 1894
	Samaridae	<i>Samaris</i> sp
	Cynoglossidae	<i>Cynoglossus carpenteri</i> Alcock, 1889

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**Appendix VIII:** List of fish species with COI and 16S rRNA GenBank accession number

Species	Accession No
<i>Hyporthodus octofasciatus</i> (Griffin, 1926)	KP244566-KP244571
<i>Liopropoma randalli</i> Akhilesh, Bineesh & White, 2012	KF814979-KF814982
<i>Sacura boulengeri</i> (Heemstra, 1973)	KR105842-KR105845
<i>Odontanthias perumali</i> Talwar, 1976	KR105805-KR105809
<i>Chelidoperca occipitalis</i> Kotthaus, 1973	JX185304, JX185306, JX185311, JX185313
<i>Chelidoperca maculicauda</i> Bineesh & Akhilesh, 2013	JX185308-JX185310
<i>Chelidoperca investigatoris</i> (Alcock, 1890)	KP009557-KP009559, JX185312, JX185310
<i>Bembrops caudimacula</i> Steindachner, 1876	KP244495-KP244503
<i>Symphysanodon xanthopterygion</i> Anderson & Bineesh, 2011	KR105909-KR105913
<i>Histiopterus typus</i> Temminck & Schlegel, 1844	KP244559-KP244565
<i>Bathyclupea hoskynii</i> Alcock, 1891	KP244492-KP244494
<i>Erythrocles acarina</i> Kotthaus, 1974	KP244547-KP244552
<i>Priacanthus sagittarius</i> Starnes, 1988	KF815027-KF815032
<i>Priacanthus blochii</i> Bleeker, 1853	KF815022-KF815026
<i>Priacanthus hamrur</i> (Forsskål, 1775)	KF815033-KF815037
<i>Priacanthus prolixus</i> Starnes, 1988	KF815011-KF815021
<i>Pristigenys refulgens</i> (Valenciennes, 1862)	KF815038-KF815041
<i>Cookeolus japonicus</i> (Cuvier, 1829)	KF815042-KF815045
<i>Parascolopsis boesemani</i> (Rao & Rao, 1981)	KR105824-KR105828
<i>Parascolopsis eriomma</i> (Jordan & Richardson, 1909)	KR105820-KR105823
<i>Parascolopsis aspinosa</i> (Rao & Rao, 1981)	KR105815-KR105819

<i>Trichiurus auriga</i> Klunzinger, 1884	KR105923-KR105930
<i>Acropoma</i> sp. A	KR231789-KR231789
<i>Aphanopus intermedius</i> Parin, 1983	KP244485-KP244486
<i>Obliquogobius cometes</i> (Alcock, 1890)	KP244597-KP244603
<i>Promethichthys prometheus</i> (Cuvier, 1832)	KP244604-KP244610
<i>Neopinnula orientalis</i> (Gilchrist & von Bonde, 1924)	KP244591-KP244596
<i>Lepidocybium flavobrunneum</i> (Smith, 1843)	KP244579-KP244580
<i>Psenopsis cyanea</i> (Alcock, 1890)	KR105836-KR105841
<i>Ariomma indicum</i> (Day, 1871)	KP244487-KP244491
<i>Psenes cyanophrys</i> Valenciennes, 1833	KJ020210-KJ020214
<i>Psenes arafurensis</i> Günther, 1889	KJ020215-KJ020217
<i>Cubiceps</i> sp	KR231797-KR231801
<i>Cubiceps whiteleggii</i> (Waite, 1894)	KP244519-KP244524
<i>Brama dussumieri</i> Cuvier, 1831	KJ020208-KJ020209
<i>Sphenanthias whiteheadi</i> Talwar, 1973	JN704806
<i>Acanthocephala indica</i> (Day, 1888)	KP244472-KP244478
<i>Notacanthus</i> sp. A	KR105803-KR105804
<i>Notacanthus indicus</i> Lloyd, 1909	KR105800-KR105802
<i>Chlorophthalmus acutifrons</i> Hiyama, 1940	JX944228-JX944233
<i>Chlorophthalmus corniger</i> Alcock, 1894	JX944224-JX944227
<i>Saurida</i> cf. <i>micropectoralis</i> Shindo & Yamada, 1972	KR105884-KR105891
<i>Saurida tumbil</i> (Bloch, 1795)	KR105892-KR105898
<i>Saurida longimanus</i> Norman, 1939	KR105853-KR105862
<i>Saurida</i> sp. B	KR105899-KR105903
<i>Saurida undosquamis</i> (Richardson, 1848)	KR105863-KR105877

<i>Saurida</i> sp. A	KR105878-KR105883
<i>Cyttopsis rosea</i> (Lowe, 1843)	KP244533-KP244539
<i>Zenopsis conchifer</i> (Lowe, 1852)	KR105931-KR105937
<i>Neobythites steatiticus</i> Alcock, 1894	KP244588-KP244590
<i>Bufoceratias thele</i> (Uwate, 1979)	KP244512
<i>Bufoceratias</i> sp	KP244513
<i>Chaunax multilepis</i> Ho, Meleppura & Bineesh 2016	KR231793-KR231796
<i>Lophiodes lugubris</i> (Alcock, 1894)	KP244581-KP244582
<i>Lophius indicus</i> Alcock, 1889	KP244583-KP244585
<i>Ebosia falcata</i> Eschmeyer & Rama Rao, 1978	KP244540-KP244546
<i>Pontinus nigerimum</i> Eschmeyer, 1983	KR105829-KR105835
<i>Snyderina guentheri</i> (Boulenger, 1989)	KR231819-KR231827
<i>Satyrichthys adeni</i> (Lloyd, 1907)	KR105846-KR105850
<i>Lepidotrigla cf. omanensis</i> Regan, 1905	KR231809-KR231810
<i>Setarches guentheri</i> Johnson, 1862	KR105907-KR105908
<i>Gephyroberyx darwinii</i> (Johnson, 1866)	KP244553-KP244558
<i>Haplostethus</i> sp	KR231806-KR231808
<i>Beryx mollis</i> Abe, 1959	KP244504-KP244511
<i>Ostichthys kaianus</i> (Günther, 1880)	KR105810-KR105814
<i>Normichthys yahganorum</i> Lavenberg, 1965	KR105797-KR105799
<i>Alepocephalus bicolor</i> Alcock, 1891	KP244479-KP244484
<i>Benthoosema fibulatum</i> (Gilbert & Cramer, 1897)	KR231828-KR231835
<i>Diaphus watasei</i> Jordan & Starks, 1904	KR231847-KR231855
<i>Diaphus garmani</i> Gilbert, 1906	KR231841-KR231846
<i>Diaphus thiollierei</i> Fowler, 1934	KR231836-KR231840

<i>Nemichthys acanthonotus</i> Alcock, 1894	KP244586-KP244587
<i>Laeops macrophthalmus</i> (Alcock, 1889)	KP244572-KP244578
<i>Chascanopsetta lugubris</i> Alcock, 1894	KP244514-KP244518
<i>Samaris</i> sp	KR231811-KR231814
<i>Cynoglossus carpenteri</i> Alcock, 1889	KP244525-KP244532

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Species	Accession number	Species	Accession number
<i>Saurida undosquamis</i>	KR231747-KR231753	<i>Rexea bengalensis</i>	KR231710-KR231712
<i>Saurida</i> cf. <i>micropectoralis</i>	KR231754-KR231758	<i>Neoepinnula orientalis</i>	KR231713-KR231718
<i>Saurida longimanus</i>	KR231759-KR231766	<i>Benthoosema pterotum</i>	KR231719-KR231722
<i>Saurida</i> sp. A	KR231767-KR231771	<i>Benthoosema fibulatum</i>	KR231723-KR231727
<i>Saurida tumbil</i>	KR231772-KR231777	<i>Diaphus thiollierei</i>	KR231728-KR231730
<i>Saurida</i> sp. B	KR231778-KR231781	<i>Diaphus garmani</i>	KR231731-KR231737
<i>Promethichthys prometheus</i>	KR231699-KR231704	<i>Diaphus</i> sp. A	KR231738
<i>Lepidocybium flavobrunneum</i>	KR231705-KR231709	<i>Myctophum</i> sp. A	KR231739-KR231740
		<i>Myctophum spinosum</i>	KR231741-KR231746

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**Appendix IX:** Species with >1% average K2P divergence. Divergent lineages are indicated by region

Species	Family	Overall K2P	Lineages	Lineage by region			Type locality
<i>Acropoma japonica</i>	Acropomatidae	3	3	Arabian sea (1)	Taiwan (1)	South Africa (1)	Japanese Sea
<i>Cyttopsis rosea</i>	Parazenidae	4.4	2	Arabian sea (1)	Portugal		Off Madeira, northeastern Atlantic
<i>Gephyroberyx darwinii</i>	Trachichthyidae	1.49	3	Arabian sea (1)	Tugela deep (1)	Tasman sea (1)	Madeira, North Atlantic
<i>Histiopertus typus</i>	Pentacerotidae	1.62	2	Arabian sea (1)	South China Sea (1)		Nagasaki, Japan
<i>Zenopsis conchifer</i>	Zeidae	2.26	2	Arabian sea (1)	Portugal: Alentejo (1)		Off Madeira
<i>Setarches longimanus</i>	Setarchidae	1.77	2	Bay of Bengal (1)	South China Sea (1)		Andaman Sea
<i>Lepidocybium flavobrunneum</i>	Gempylidae	1.5	2	Arabian sea (1)	Brazil (1)		Cape of Good Hope, South Africa
<i>Promethichthys prometheus</i>	Gempylidae	3.33	3	Bay of Bengal (1)	South Indian Sea (1)	Mid Atlantic bight (1)	Saint Helena Island, South Atlantic
<i>Neopinnula orientalis</i>	Gempylidae	2	2	Arabian sea (1)	South Africa (1)		South Africa
<i>Myctophum spinosum</i>	Myctophidae	2	2	Arabian sea (1)	South Africa (1)		China, Western Pacific
<i>Benthoosema fibulatum</i>	Myctophidae	3.39	2	Bay of Bengal (1)	unknown		Kaiwi Channel, Hawaiian Islands



**Appendix XI.** Details of trawling stations in the southern coast of India by exploratory survey conducted by FORV *Sagar Sampada* and FSI *Matsya Varshini*

<i>Sagar Sampada</i> Cruise 291				<i>Sagar Sampada</i> Cruise 322			
Station No	Latitude	Longitude	Depth (m)	Station No	Latitude	Longitude	Depth (m)
St:1	13 18 .070	80 36.622	300	St:1	10.09.956	75.38.965	200
St:2	18 39.716	85 08.530	543-580	St:2	11.04.195	74.55.430	1000
St:3	18 48.093	85 21.605	655-633	St:4	11.58.355	74.16.791	1000
St:5	18 48.093	85 21.201	614-643	St:5	11.57.317	74.26.081	200
St:6	18 50.45	85 22.021	535-389	St:7	8.59.618	75.55.468	200
St:10	11 53.298	80 08.078	510-757	St:8	8.53.593	75.27.288	1000
St:11	11 52 964	80 07.912	505-785	St:9	8.05.718	76.25.842	1000
St:12	10 54.104	80 22.457	658-681	St:10	8 <sup>0</sup> .21.75	76 <sup>0</sup> .29.800	200
St:13	10 56.376	80 21.055	640-664	St:11	6.58.52	77.26.25	1000
St:14	10 56.376	80 21 055	640-665	St:12	7.13.054	77.29.301	200
St:15	10 35.193	80 33.143	620-676	St:13	8.27.91	78.29.50	1000
St:16	10 52.220	80 23.059	645-654	St:14	8.34.640	78.26.730	200
St:17	11 52.429	80 07.738	528-777				

<i>Sagar Sampada Cruise 281</i>				<i>FSI Matsya Varshini cruise</i>			
<b>Station No</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Depth (m)</b>	<b>Station No</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Depth (m)</b>
St:1	8 24. 351	76 07. 218	900 -1001	St:1	09'9.5	75'50.3	328
St:2	9 53. 681	75 33. 332	500 - 620	St:2	9'02.3	75'50.5	337
St:4	10 05. 580	75 37. 158	450 -475	St:3	9'17.8	75'52.6	141
St:5	11 01. 126	75 01. 995	611 - 650	St:4	10'38.3	75'19.2	435
St:8	11 13. 120	75 53.011	663 - 727	St:5	10'48.6	75'12.4	475
St:9	11 17. 228	74 46. 662	824 - 784	St:6	10'45.4	75'14.4	510
St:13	13 12. 147	73 38. 741	410 - 475	St:7	10'36.9	75'20.5	457
St:17	15 24. 830	72 46. 234	575 - 585	St:8	10'35.2	75'22.4	368
St:22	15 14. 904	72 48. 362	602 - 658	St:9	9'07.4	75'45.3	365
St:23	15 18. 081	72 45. 908	726 - 734				
St:24	15 18. 212	72 49. 761	375 - 407				
St:25	15 24. 470	72 44. 892	676 - 685				
St:30	18 39. 053	70 10 827	879 - 1045				
St:32	19 58. 035	09 27 168	259 - 269				
St:34	20 12. 072	69 19 235	705 - 734				
St:35	20 13. 698	69 21 075	458 - 525				
St:37	20 41. 243	69 13. 679	450 - 513				
St:39	20 39. 889	69 13. 727	568 - 617				