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Molecular and Morphological Investigations of Grouper (Serranidae) Biodiversity in Saudi Arabia

Mohammad Saeed Al Jazza Alqahtani

MOLECULAR AND MORPHOLOGICAL INVESTIGATIONS OF GROUPER (SERRANIDAE) BIODIVERSITY IN SAUDI ARABIA

By

Mohammad Saeed Al Jazza Alqahtani

Bachelor of Science
King Faisal University, Al Hahsa, Saudi Arabia, 2003

Master of Science
King Saud University, Riyadh, Saudia Arabia, 2009

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Accepted by:

Joseph Quattro, Major Professor

Roger Sawyer, Committee, Member

David Reisman, Committee, Member

Jerry Hilbish, Committee Member

Ahmad H. Al harbi, Committee Member

Tracey L. Weldon, Vice Provost and Dean oif the Graduate School

DEDICATION

Most importantly, from the bottom of my heart, I would like to gift my graduation to my father. In the beginning, I would like to gift my work and graduating success to my deceased father, that passed away during the time of my defense, the first week of July. I missed you a lot, my daddy, too much. Also, to my GOD fathers, professor Dr Ahmad Al-harbi and professor Dr. Joseph Quattro.

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ABSTRACT

Chapter 1: The seas surrounding the Arabian Peninsula, which represent the northernmost portion of the Indian Ocean, are considered to have the highest aquatic biodiversity among the worlds marine regions. Seas that surround the Arabian Peninsula include the Red Sea, the Gulf of Aden, the Arabian Sea, the Gulf of Oman, and the Arabian Gulf. In aggregate, this area harbors a large number of endemic and more widespread marine species, including fishes, echinoderms, and corals.

There are unique challenges involved in grouper species identification in the Arabian region including 'familiar' Arabic species designations that are not standardized in the Arabic literature but, rather, based on local variants. This has led to confusion regarding species names and features that are inadequately defined and extremely varied. Previous research lists two pervasive issues with species identification, including differences in localized dialect and an almost complete lack of "informant knowledge" regarding species name variation and uses.

Because of widespread ambiguity in grouper species recognition, many recent systematic studies have instead relied on alternative recognition approaches that utilize molecular techniques, such as DNA sequencing, to identify individual species rather than relying on morphological characters alone.

Chapter 2. The Red Sea is a somewhat peculiar aquatic ecosystem in the world, both from a biological and geological perspective. The basin has seen several episodes of geological and climatic instability that resulted, eventually, in the formation of an incipient

ocean with a noticeable degree of faunal endemism. Chapter 2 develops the case that the Red Sea endemic grouper *Epinephelus summana* is a genetic indicator of Pleistocene events that derived Red Sea fauna endemism. This is substantiated with a pilot investigation of endemism in the Red Sea groupers and Pleistocene-driven speciation of *Epinephelus* species.

Groupers (Serranidae:Perciformes) are reef-associated fishes of great ecological and economic importance. The Summane grouper *Epinephelus summana* is a species native only to the Red Sea and Western the Gulf of Aden. This work aimed to identify the genetic relationship between *E. summana* and the allopatric, but morphologically similar, species *E. ongus*. Also, we were keen to identify the period when species divergence took place. For this, eight grouper species were collected from the coasts of the Kingdom of Saudi Arabia on the Red Sea and the Arabian Gulf. The net results indicated a high degree of endemism in the Red Sea groupers, and a necessity for assessment of possible cryptic speciation within serranids in this area.

Chapter 3: Application of genetic markers for species identification gains crucial importance in the Saudi Arabian national economy because marine products contribute significantly to the Gross Domestic Product (GDP). The current massive increase in the size and outreach of international trade has increased the threats of food misrepresentation and fraud, especially in fish markets. This could be attributable to the insufficiency of classical species identification methodologies that are based only on morphology. The accuracy of these methodologies have been proven to be insufficient to expectations, which may contribute to trading of already endangered or overfished species. This directly leads to fisheries decline due to improper management of fisheries. The issue is becoming more

complicated with the outbreak of unreported fishing, overfishing, and even fraudulence in fisheries markets through representation of low-priced, abundantly-caught fish species as more expensive ones.

In summary, we obtained the first record of *Cephalopholis sonnerati* in the the Red Sea near Jazan which is close to Gulf of Aden. Identified both *Cephalopholis oligosticta* and *Epinephelus summana*. based on morphologically and genetic investigation using 4 different gene markers 16S, 12S, TMO4, and H3. Both are endemic to the Red Sea. First study using morphology and genetics to confirm their related. Finally, the unknown *Epinephelus* species that was found in the Red Sea fresh fish landings showed greater than 98 percent identity with *E. akaara, E. stictus, E. fasciatus,* and *E. anlogus.*

Chapter 4: The identification of species constitutes the first basic step for biodiversity monitoring and conservation. Fish species identification mainly relies on morphometric and meristic characteristics. However, there are pitfalls in relying primarily on morphology when attempting to identify fishes during various stages of their development not considered in original treatments or when examining fragmentary, partial or processed remains.

It has been recently proposed that the use of DNA methods can circumvent such a problem. The reconstruction of phylogenetic relationships based on molecular data in addition to the classical methodologies has helped to resolve taxonomic uncertainties for fishes.

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LIST OF SYMBOLS

SL	Standard length.
TL	Total length.
m	Meters.
cm	Centimeters.
mm	Millimeters.

LIST OF ABBREVIATIONS

ASFIS.....Aquatic Sciences and Fishries Information System

BEAST.....Bayesian Evolutionary Analysis by Sampling Trees, Software Program

BI.....Bayesian Inference

BLAST.....Basic Local Alignment Search Tool

CITES ..... Council on International Trade in Endangred Species

ClustalW .....Multiple Sequence Alignment Program W

COI..... Cytochrome c oxidase I

CPUE ..... Catch Per Unit Effort

DNA..... Deoxyribonucleic acid

EIA.....Environmental Impact Assessment

FAO.....Food and Agriculture Organization of the United Nations

FFC .....Fish Farming Center of the Ministry of Agriculture and Water

GCC .....Gulf Cooperation Council

GDP..... Gross Domestic Product

GenBank .....National Institutes of Health Genetic Sequence Database

H3..... Nuclear Histone

HKY .....Hasegawa-Kishimo-Yano

IMO..... International Maritime Organization

iTOL..... interactive Tree of Life

IUCN.....International Union for Conservation of Nature

KACST .....King Abdulaziz City for Science and Technology

KAUMM..... King Abdulaziz Maritime Museum

KUA..... Kingdom of Saudi Arabia

MCMC..... Markov Chain Monte Carlo

ML..... Maximum Likelihood

MPA..... Marine Protected Area

MYA ..... Million Years Ago

NJ ..... Neighbor-Joining

PAUP ..... Phylogenetic Analysis Using Parsimony

PCR..... Polymerase Chain Reaction

R.....Ratio of Transition/Transversions

RAPD..... Random Amplified Polymorphic DNA

RNA .....Ribonucleic acid

SRI ..... Senckenberg Research Institute, Frankfurt, Germany

TMO-4C4..... a single copy-copy nuclear DNA locus (Streelman and Karl (1997)

UAE ..... United Arab Emirates

UN..... United Nations

CHAPTER 1

A SURVEY OF GROUPER BIODIVERSITY IN THE RED SEA AND ARABIAN GULF

1.1. Introduction

The seas surrounding the Arabian Peninsula, which represent the northernmost portion of the Indian Ocean, are considered to have the highest aquatic biodiversity among the worlds marine regions (Wehe and Fiege, 2002). Seas that surround the Arabian Peninsula include the Red Sea, the Gulf of Aden, the Arabian Sea, the Gulf of Oman, and the Arabian Gulf, that, in aggregate, harbor a large number of endemic and more widespread marine species, including fishes, echinoderms, and corals. For example, 320 species of scleractinian coral and 1078 species of fish have been documented in the Red Sea alone (Veron et al., 2009). This chapter is mainly concerned with those fishes within the subfamily Epinephelinae, or commonly referred to as the groupers. There are at least 110 grouper species inhabiting the marine waters of the Indo-Pacific region (Bariche and Heemstra, 2012). However, a smaller number of species are more commonly found in certain regions surrounding the Arabian Peninsula. For example, common inhabitants of the Red Sea, include at least 16 species of grouper in the genus *Epinephelus* (Golani and Bogorodsky, 2010).

Groupers of the subfamily *Epinephelinae* are members of the speciose family Serranidae and include, at minimum, 475 nominal species within 64 genera. Groupers are

an economically important group of species that are heavily exploited by fisheries and the most important marine fish in many local marine fishing jurisdictions. The grouper and snapper sector in the seafood industry alone accounts for 8.5 percent of all coastal fishes landed and represents 10 percent of the total value of coastal fish landings (FAO, 2016). However, the management of local grouper fishing industries is hampered by confusion and misidentification among grouper species that have led to ambiguous records, taxonomic confusion, and a lack of discriminative morphological characteristics. Indeed, identifications are routinely based on morphological characteristics, color, overall shape, and geographic location of capture (Nurdalila et al., 2015).

There are unique challenges involved in grouper species identification in the Arabian region including 'familiar' Arabic species designations that are not standardized in the Arabic literature but, rather, based on local variants. This has led to confusion regarding species names and features that are inadequately defined and extremely varied. For example, Provencal (2013) lists two pervasive issues with species identification, including differences in localized dialect (e.g., "understanding the informant") and an almost complete lack of "informant knowledge" regarding species name variation and uses. A good example of this confusion involves variations of the Arabic term '*najil*', which is the Arabic name for the roving coral grouper, *Plectropmomus pessuliferus*. However, Sinai Bedouins use '*najil*' as a local name for the lyretail grouper (*V. louti*) despite significant morphological differences between these species. Furthermore, some local names are commonly used to describe a group of fishes rather than a single species. For example, *Kushar* is commonly used to describe a group of five species in some locations, but the name is used for only two species (coral hind and peacock grouper) by fishermen in Sinai.

Because of this widespread ambiguity in grouper species recognition, many recent systematic studies have instead relied on alternative recognition approaches that utilize molecular techniques, such as DNA sequencing, to identify individual species rather than relying on morphological characters alone (Randall, 1998).

1.2. Overview of the Regional Fish Fauna

1.2.1. Epinepheline serranids

Epinepheline serranids, prominent predators in coral reef fish populations, are found globally in tropical and warm temperate environments and are important components of subsistence and commercial fisheries. Given their commercial importance, their biology has received quite a bit of attention (e.g., see reviews in Polovina and Ralston 1987, Sphigel and Fishelson 1989a, 1989b, Gilmore and Jones 1992). Their reproductive ecology has piqued researchers' interest (– for example, Johannes 1981, Thresher 1984, Colin and Clavijo 1988, Colin et al. 1987, Colin 1992, Shapiro et al. 1993), owing to the repercussions for commercial, sport, and sustenance fisheries, as well as a population and community structure preservation (Sphigel and Fishelson, 1991; Gilmore and Jones, 1992; Colin 1992).

1.2.2. Grouper

Fishes and macro-crustaceans are the primary feeding resources for groupers, which are considered a predator, while other groupers, such as the Paranthias and *E. undulosus*, are primarily planktivorous. Furthermore, studies have discovered discordant variation in the adult size between grouper species, where the smaller ones are generally less than 30 cm, such as *Cephalopholis leapardus*, and the larger and giant species reach over 200 cm, such as Lancealatus and E. itajara (Ma, 2014).

Groupers are spawners diffused where spawning occurs among multiple species and move to local spawning sites, such as *Plectropomus leapardus*, and some travel until they reach huge spawning complexes, such as *Plectrompomus areolatus*. These spawning behaviors support species distribution that is as long and wide as the migration journey (Hutchinson, Rhodes, 2010).

According to Koedprang, et al. (2007), worldwide, groupers are divided into 15 genera and have 159 species. All seas' tropical and subtropical waters are home to these creatures (Tupper and Sheriff, 2008). Due to its appealing flavor and strong market need in several regions of the world, including Saudi Arabia, grouper is an economically important marine fish species. Because of their rapid development, tolerance to environmental stress, and quick feed conversion, groupers are the finest fish for intensive aquaculture (Craig and Hastings, 2007). The Red Sea and the Arabian Gulf are home to a variety of grouper species called *Epinephelus* spp (Priest, et al., 2016). For example, there are many important grouper species of *Epinephelus* genera. Orange-spotted grouper *Epinephelus coioides*, greasy grouper *E. tauvina*, king grouper or Malabar grouper *E. malabaricus*, giant grouper *E. lanceolatus*, potato grouper *E. tukula*, and longfin grouper *E. quoyanus* are the most significant grouper species for both capture and aquaculture (Wang, et al., 2011). In the eastern area of the Kingdom of Saudia Arabia, *E. tauvina* is known as an Arabian grouper.

According to Nelson (2006) groupers are classified as 163 species and 16 genera, where 110 species are located primarily in marine waters of the Indo-Pacific, and only 14 species are commonly found in the Eastern Atlantic Ocean and the Mediterranean Sea. Groupers are vital species in the marine fish industry as their length of over 30 cm makes

them easy targets using basic fishing gear, such as spears, nets, and hooks and lines (Barrania and Ibrahem, 2003). In addition, groupers mostly inhabit shallow coral or rocky areas. Furthermore, due to their habitat, they are affected by tectonic changes and climate sea-level variations (Kotb et al., 2004).

1.3. History of Groupers in the Red Sea

In 1775, Swedish naturalist Niebuhr documented 122 marine fish species present in the Red Sea. Of these 122, the Swedish naturalist, Forsskal, had previously documented 58. Both were part of an expedition that went to this area between 1761 and 1763 (Klausewitz and Nielsen 1965; Nielsen 1993; Fricke, 2008; Goren, 2008). Studies of the regions ichthyofauna were subsequently published by the French zoologist Geoffroy Saint-Hilaire (Geoffroy Saint-Hilaire, 1817). Shortly after, the German scientists Ehrenberg and Hemprich led an expedition, funded by the Zoological Museum of Berlin, into Egypt from 1820 until 1826. The marine specimens that they had collected were sent to Cuvier, a French ichthyologist known for writing *Histoire Naturelle des Poisons* (co-authored with Achille Valenciennes) (Cuvier and Valenciennes, 1828, 1849). According to Fricke (2005), Klunzinger, a German ichthyologist, gathered a number of fish specimens for the Stuttgart Natural History museum and documented 501 Red Sea species (Klunzinger 1870, 1871, 1884).

Modifications have been made to the checklist of fish species found in the Red Sea since Niebuhr's initial list of 122 species leading to a final checklist published in 2010. As mentioned, Kunzinger compiled a record of 501 types of fishes, but Klausewitz added 101 species to this initial list in 1964 (Klausewitz, 2002). In 1971, this list was amended by Botros (1971) and brought the total number of Red Sea species to 750. In 1984, Dor

produced a *Checklist of Fishes of the Red Sea* that contained detailed accounts for 1000 fish species. *Checklist of Fishes of the Red Sea II* was issued in 1994 with an additional 250 species (Goren and Dor, 1994). In 2010, the *Checklist of Fishes of the Red Sea* II was revisited, inaccurate species were removed, and the list updated to include newly discovered species. The FAO currently lists 1280 species of fish inhabiting the Red Sea.

1.3.1. Challenges of implementing grouper breeding projects

The challenge of choosing elite species in grouper breeding projects is exacerbated by a dearth of genetic diversity knowledge on grouper species in the Arab Gulf, particularly on the eastern Saudi coast. Furthermore, the grouper is a protogynous hermaphrodite, meaning it starts off as a female and later transforms into a male. The most major barrier to grouper artificial larvae generation is the difficulty in catching mature males due to grouper gender features in nature (Oh et al., 2013; An et al., 2014). Additionally, due to overfishing, marine pollution, and habitat destruction, genetic diversity has reduced among solitary and non-social fish species, particularly groupers (Martinez, et al., 2018)

1.4. The Fish Biodiversity Issues in Saudi Arabia

1.4.1. Environmental stressors

Multiple natural and anthropogenic environmental stresses are plaguing the Arabian Gulf. Extremes in temperature and salinity, combined with anthropogenic influences, create a unique chemical and physical environment that may represent a danger to marine species diversity and ecological stability. Human behavior, ranging from habitat degradation by coastal ecosystems to contamination from a multitude of land-based operations, have a direct or indirect impact on naturally challenged marine ecosystems.

Natural or anthropogenic stresses can have a wide range of environmental effects on marine ecosystems. Because of the intricacy of the ecosystem's responses to a variety of perturbations, distinguishing between natural and manmade stressors may be challenging. Anthropogenic effects on ecosystems, for example, may not be observed until they combine with natural changes in the environment. Furthermore, human activity may have affected some seemingly natural environmental changes in ecosystems (Naser, 2014).

1.4.1.1 Natural stressors

Natural stressors in the maritime environment come in a variety of shapes and sizes, and they can come from a variety of places. Environmental extremes are pressures that wreak havoc on marine ecosystems' basic functioning (Breitburg and Riedel, 2005). The Arabian Gulf's arid physical environment, characterized by high salinity and high temperature, has a significant impact on marine organisms' physiological characteristics, as well as their diversity, abundance, and geographical distribution.

In general, the Arabian Gulf's harsh environmental circumstances are attributed to lower levels of species richness (Price, 2002). The Arabian Gulf, on the other hand, is known for its unique marine assemblages and habitats (Sheppard et al., 1992). As a result, while species richness is relatively modest, variation in species composition along a geographic gradient is rather considerable (Price, 2002).

Biological causes of stress, like invading species and algal blooms, may play a significant influence in ecosystem degradation in the Arabian Gulf. With over 25 000 oil tankers passing thru the Strait of Hormuz yearly (Literathy et al., 2002), aquatic invasive species introduced by coastal waters is one of the most serious dangers to the Arabian Gulf's marine ecology. Some of these foreign species, particularly dinoflagellate

organisms, have been connected to red tide and fish kills in Kuwait, Oman, Saudi Arabia, and the United Arab Emirates (UAE) in recent times (Hamza and Munawar, 2009).

In the Arabian Gulf, large blooms (also known as red tides) have wreaked havoc on the environment and economy. For example, the huge blooms that hit the Arabian Gulf from August 2008 to May 2009 resulted in significant fish fatalities, coral reef damage, fishing restrictions, tourism disruptions, and desalination plant outages. The dinoflagellate species Cochlodinium was discovered for the first time in the Arabian Gulf waters during the toxic algal blooms of 2008-2009 (Richlen et al., 2010).

Despite the fact that ecosystems in the Arabian Gulf are acclimated to extreme environmental circumstances, abnormal sea-surface temperatures caused by climatic warming may have serious consequences for the ecosystems' integrity. Significant bleaching and associated mortality occurred in the Arabian Gulf in 1996 and 1998, when maximum sea-surface temperatures reached 37.3 degrees Celsius and 38.0 degrees Celsius, respectively (Sheppard and Loughland, 2002; Burt et al., 2011).

Increased levels of carbon dioxide (CO2) and other greenhouse gases in the atmosphere have negative consequences for the environment and human health. The Arabian Gulf is a huge CO2 sink, which could cause the marine ecosystem to become acidic. Over a four-year period (2007-2010), assessments of pH concentration in surface waters of the Arabian Gulf revealed that the waters are growing progressively acidic (Uddin et al., 2012). Many creatures, like corals, mollusks, and calcareous phytoplankton, are negatively impacted by increasing acidity in the marine environment.

1.4.1.2. Anthropogenic impacts

Reclamation and dredging

Most of the main residential, cultural, and economic projects in the Arabian Gulf will be concentrated along the coast and in the sea (Naser et al., 2008). In recent years, coastal development around the Arabian Gulf has expanded at an unprecedented rate to handle large-scale projects such as artificial islands, waterfront communities, ports, and marinas (Khan, 2007).

The primary causes of biodiversity loss and environmental degradation in the Arabian Gulf islands are the intense reclamation and dredging projects. Moreover, 40% of the Arabian Gulf's coastline has been improved, according to estimates (Hamza and Munawar, 2009). 'Palm Islands' and 'The World' in Dubai, UAE, 'The Pearl' in Qatar, and 'Al Khaleej' and 'Half Moon Bay' in Saudi Arabia are all examples of large-scale coastal projects in the Arabian Gulf.

Short and long-term ecological, physical, and chemical consequences are connected with dredging and reclamation procedures. These efforts entail removing macrobenthos from the ecosystem and altering it permanently. During the reclamation process, dredging material may be deposited, potentially suffocating coastal and subtidal ecosystems and deoxygenating the subsurface sediments (Allan et al., 2008). Water circulation may be hampered by reclaimed areas, resulting in salinity changes (Al-Jamali et al., 2005). The biodiversity, complexity, abundance, and biomass of marine creatures may be reduced as a result of these chemical and physical changes (Tu Do et al., 2012). Furthermore, dredging actions may lead to the loss of seagrass beds in the Arabian Gulf, either directly or indirectly, by physical removal and burial, as well as a rise in turbidity concentrations (Al-Wedaei et al, 2011).

Industrial effluents

The countries of the Arabian Gulf have experienced remarkable industrial growth, particularly in the oil refining and petrochemical fields. Heavy metals, hydrocarbon hydrocarbons, and nutrients are among the chemicals found in the wastewater discharged by these big enterprises (Sale et al., 2010). Oil and greases, phenols, sulfides, ammonia, suspended particles, and heavy metals such as chromium, iron, nickel, copper, molybdenum, selenium, vanadium, and zinc are among the compounds found in oil processing wastewaters (Wake, 2005). High amounts of hydrocarbons (De Mora et al., 2004; 2010) and heavy metals have been identified in coastal and marine habitats receiving extensive industrial effluents along the Arabian Gulf's coastline (Naser, 2013a; 2013b).

The Arabian Gulf's seawater flushing time varies between 3 and 5 years. As a result, pollutants such as heavy metals and hydrocarbons would most certainly remain in the Arabian Gulf for a long time. Constant industrial wastewater inputs from various anthropogenic sources in the Arabian Gulf could be critical for marine ecosystems as well as people who rely on marine resources for food, leisure, and business.

Desalination effluents

Desalination plant refuse water is dumped to coastal and subtidal regions in the Arabian Gulf on a regular basis in large amounts. As a result, desalination plant emissions of hypersaline water are becoming a severe hazard to the Arabian Gulf's marine ecosystems (Areiqat and Mohamed, 2005).

Chemical and physical changes are common in coastal and marine habitats that receive these discharges. Desalination pollutants are frequently found to contain detrimental chemical properties such as heavy metals, anti-scaling, anti-fouling, anti-foaming, and anti-corrosion compounds (Lattemann and Hopner, 2008). Furthermore,

discharges from desalination procedures may modify the physical and chemical properties of receiving saltwater, such as temperature and concentration. Changes in seawater quality, temperature, dissolved oxygen, and salt content could have a significant impact on a variety of marine creatures and communities.

Sewage discharges

One of the most prominent anthropogenic disruptions of marine ecosystems in the Arabian Gulf is sewage emissions. Despite high sewage treatment standards (for example, secondary or tertiary) (Sheppard et al. 2010), considerable amounts of household wastewater are released to the Arabian Gulf's coastal and marine habitats. High levels of suspended particles and nutrients like ammonia, nitrates, and phosphates describe these wastewaters (Naser, 2011). Biological and chemical contaminants, such as pathogen microorganisms and heavy metals, are frequently present in wastewater discharges (Shatti and Abdullah, 1999). Pathogenic microorganisms and chemical pollutants bioaccumulate and biomagnify as a result of sewage discharges, affect the quality of human food and pose a risk to health.

Oil pollution

The Arabian Gulf is thought to have the world's greatest oil reserves (Literathy et al., 2002). As a result, oil-related pollution poses a constant threat to the Arabian Gulf's coastal and marine habitats. Exploration, production, and transportation of oil have all contributed significantly to pollution in the Arabian Gulf. Offshore oil wells, undersea pipelines, oil tanker collisions, oil terminals, loading and handling operations, weathered oil and tar balls, illegal ballast water disposal, and intelligence deployments are all potential sources of oil spills in the Arabian Gulf (Sale et al., 2010).

1.4.2. Conservation of biodiversity in the Arabian Gulf

In order to conserve and sustain these vulnerable ecosystems, efficient protection and maintenance of marine ecosystems in the Arabian Gulf are becoming increasingly important. Furthermore, well-managed ecosystems provide a variety of critical environmental services that support the Arabian Gulf's economic, social, and cultural goals (Al-Cibahy et al., 2012). As a result, principles of conservation and management practices, such as marine protected areas, environmental impact assessments (EIA), environmental regulations, ecological restoration, and environmental control, may help to protect the Arabian Gulf's fragile marine ecosystems.

1.4.2.1. Marine protected areas (MPAs)

Globally, marine protected areas (MPAs) are regarded as the most essential instrument for in situ conservation (Chape et al., 2005). In coastal and marine areas, MPAs play an important role in the preservation and protection of genetic features, species, habitats, and cultural variety. They may be able to help avoid or slow the current reductions in marine biodiversity, ecosystems, and fisheries productivity. MPAs can also contribute to enhance ecosystem functions and services by preserving ecological procedures and systems that enable commercial and social usage of marine resources (Agardy, 1994). MPAs can also help with adaptation to climate change by bolstering ecological balance and safeguarding critical ecosystem services (McLeod et al., 2009).

Multiple global agreements, such as the Convention on Biological Diversity, the Convention on Wetlands of International Importance (Ramsar Convention), and the World Heritage Convention, work to increase the number and scope of MPAs around the world (Green et al., 2011). In the Arabian Gulf, regional treaties could help to promote the ecosystem services of marine protected zones. The Agreement on the Protection of Species

and Natural Ecosystems in Gulf Cooperation Council Countries (Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and the UAE), for example, lays the groundwork for incorporating conservation areas into national and regional environmental strategies and policies (GCC, 2010). This convention strives to protect ecosystems and wildlife habitats in the most active way possible. It is also concerned with vulnerable species conservation on a regional basis, particularly where the distribution of these species extends beyond the international borders of two or more neighboring nations, or when these species migrate beyond the borders of member countries.

A prospective transboundary marine protected area has been found that extends from the Gulf of Bahrain to the UAE (Knight et al., 2011). These territories, which are inhabited by 4 countries (Saudi Arabia, Bahrain, Qatar, and the UAE), are rich in species and environmental diversity.

MPA classification and execution are undoubtedly crucial for the conservation of the Arabian Gulf's naturally stressed coastal and marine ecosystems. In the Arabian Gulf, approximately 38 officially defined MPAs encompassing around 18,180 km^2 have been constructed to this end (Van Lavieren et al., 2011). Nevertheless, the number and extent of MPAs may not be indicative of their efficacy in fulfilling their conservation objectives (Chape et al., 2005).

1.4.2.2. Environmental Impact Assessment

In most nations around the world, environmental impact assessment (EIA) is regarded as a standard tool for decision-making. It guarantees that authorities have all of the information they need about any potential substantial environmental impact of a proposed project before making a decision. Integrating environmental concerns could lead to a more coherent and organized decision-making process that achieve a balance of

interests between development and environmental protection (Noble and Press, 2011). By addressing potential applications, alternatives, mitigations, potential consequences, and evaluation, EIA reduces or prevents the negative environmental impacts of a proposed development (Cooper and Sheate, 2002).

Recognizing the importance of environmental impact assessments in preventing environmental degradation and pollution as a result of rapid economic expansion, Arabian Gulf countries have incorporated EIA into their environmental laws (El-Fadl and El-Fadel, 2004). In the Arabian Gulf, all coastal development projects, such as reclamation and dredging, must undergo an EIA. Nevertheless, in coastal and marine ecosystems, the efficacy of EIA is limited by a number of characteristics that are also present in many other parts of the world. Absence of suitable legal and regulatory frameworks, restricted public participation, insufficient procedural EIA requirements, and rules pertaining to potential effects and strategic environmental assessment are only a few of them (Van Lavieren et al., 2011; Naser, 2012).

1.4.2.3. National, regional and international environmental regulations

The regions in the Arabian Gulf have enacted a number of laws and regulations relating to environmental and biodiversity protection. Environmental rules, the impoverishment and preservation of living marine resources, the preservation of wildlife and the natural environment, environmental quality standards, environmental assessment, oil pollution control, the prohibition of catching endangered species, and the institution of marine protected areas are all examples of national instruments. Although these national regulations can help to safeguard aquatic habitats in the Arabian Gulf directly or indirectly, their impact may be limited due to lax enforcement (Al-Awadhi, 2002).

Many global accords that can help safeguard coastal and marine environments have been negotiated or signed by nations in the Arabian Gulf. The Ramsar Convention on Wetlands of International Importance (Ramsar Convention), the World Heritage Convention, the United Nations Convention on the Law of the Sea, the United Nations Framework Convention on Climate Change, International Maritime Organization (IMO) conventions, and the Convention on International Trade in Endangered Species (CITES) are just a few examples (the Convention on International Trade in Endangered Species of Wild Fauna and Flora). These international treaties establish methods for dealing with a variety of issues and problems connected to the marine environment, so aiding in the management and preservation of marine ecosystems in the Arabian Gulf.

Ecological restoration

Despite the fact that marine restoration lags behind its terrestrial and freshwater equivalents (Elliott et al., 2007), restoration actions in coastal and marine settings are becoming more common around the world. Similarly, in the Arabian Gulf, various rehabilitation efforts have been carried out (Weishar, 2008). In most of the Arabian Gulf countries, mangrove restoration operations have been carried out. The topographical and hydrological parameters of the chosen site are crucial to the success of mangrove planting, especially low energy shorelines with stable and non-eroding soil, mild slop, adequate depth, amount, and quality of water accessing the building, and the need for low-salinity water (Field, 1999).

1.4.3. Conservation of biodiversity in the Kingdom of Saudi Arabia

In addition to the above, the Kingdom of Saudi Arabia is working to protect the biological diversity of aquatic wealth through the process of fish farming, the most important of which are groupers. The government realized the importance of the

aquaculture industry, thus provided high support to the aquaculture industry by conducting research and providing extension programs, hatchery-reared seeds, commercial and technical information, training, fish feed, and free loans to farmers to afford the purchasing of machinery.

The Gulf of Aqaba, the Red Sea (78 percent of coastline length) to the west, and the Arabian Gulf (or the Persian Gulf) to the east border the Kingdom of Saudi Arabia, which occupies 80 percent of the Arabian Peninsula. The total distance covered is 2640 kilometers. Despite the fact that fish is not a true mainstay of the Saudi diet, demand for seafood is on the rise (Kitto and Regunathan, 2012).

The Saudi Arabian National Centre for Science and Technology (now known as the King Abdulaziz City for Science and Technology) in Riyadh established the Fish Culture Project in 1980, marking the beginning of the country's aquaculture growth (Al-Thobaiti and White, 1989). The Fish Farming Centre (FFC) of the Ministry of Agriculture and Water, created in 1982 in North Obhur near Jeddah with FAO assistance, was a significant contributing factor to mariculture growth. Industrial aquaculture began in the mid-1980s, and productivity has steadily increased since then.

Capture fisheries, which climbed from 49,080 tonnes in 2000 to 68,000 tonnes in 2008, are the main source of seafood. Nevertheless, due to overfishing by traditional fisheries, landings of commercially significant species (groupers, snappers, emperors, Spanish mackerel, and tunas) have decreased or remained stable (Kitto and Regunathan, 2012). There have also been reports of a rise in the number of fish caught per unit effort (CPUE; Amer and Al Gaber, 2006). Based on a UN estimate of 60 million people by 2050, this constrained supply will become increasingly severe.

There was a gradual increase in aquaculture production in KSA from 1980 to 2010. Then, there was a rapid reduction of production in the following years because of the spread of white spot disease. In 2014, the production of aquaculture had recovered, and it has been increased since that time. In addition, the Ministry of Environment Water and Agriculture aims to increase the production of various marine species to reach 600 thousand tons by 2030.

With more additions from planned cage farms, the increase in marine fish output is inclined to maintain. Nevertheless, aquaculture has only been considered for a small number of native species. A decade ago, successful year-round natural spawning and larval rearing of *E. polyphekadion* in captivity and in hypersaline aquatic habitats were described, with a success rate of 42 percent to 43 percent (James et al., 1997). Likewise, the potential of a grouper hybrid (*E. fuscoguttatus* x *E. polyphekadion*) and the production of these two species under develop conditions have been assessed (James et al., 1999; Amenyogbe et al., 2020).

1.5. Fisheries Development in Saudi Arabia

Within the framework of the Ministry's endeavor to develop the fisheries sector and increase its productivity, cooperation has been made with the Agricultural Development Fund, which resulted in the launch of the Fund's seventh initiative to develop fisheries wealth in the Kingdom of Saudi Arabia, which includes a plan for the development of the sector in all its aspects and aims at comprehensive sustainable development in the fields of aquaculture, marine fisheries, and the environment Aquaculture, research, legislation, and localization of marine fish farming techniques in the Kingdom, overcoming the difficulties encountered by this industry, and actively

contributing to the establishment of many aquaculture projects to improve production in quantity and quality, and the establishment of special hatcheries for spawning marine fish species of high economic value (Ministry Of Agriculture, 2018).

Saudi Arabia is the largest nation in the Arabian Peninsula, with accessibility to both the Persian Gulf and the Red Sea, and it encompasses the majority of the Arabian Peninsula's east coast. Despite the fact that Saudi Arabia's Red Sea coastline is three times longer than its Gulf coast, the country's catches are identical on both coastlines. Based on data from a variety of sources, the catches of Saudi Arabian fisheries in the Red Sea are shown starting in 1950. Artisanal, subsistence, industrial, and recreational fisheries were all reconstructed independently. Each sector's overall catch was then broken down into individual species or groups of species. The catch was low at the start of the 1950s, around 7,000 tons in first year, and it climbed slowly. With the widespread motorization of artisanal boats and the emergence of industrial fisheries in the early 1980s, the overall Saudi Arabian catch changed dramatically. Peak catches of around 50,000 t per year1 occurred in the mid-1990s, after which catches dropped to around 40,000 t per year1 by the end of the decade. Artisanal fishing contributed the most to the overall catch (64%), followed by industrial (23%), subsistence (10%), and recreational fishing (3%). While the capture contained a huge number of species, only a few were dominating (Tesfamichael and Pauly, 2016).

The tasks of the Marine Fisheries Department are mainly to set regulations and laws, conduct research and studies, conduct marine surveys and periodic statistics while serving the development of fisheries in the Kingdom and preserving our fish stocks. The goals are to achieve stability and improve the conditions of fishing and fishermen.

The administration supervises the follow-up of the fishermen on the coasts according to the Table 1 (Ministry of Agriculture, 2018). In this regard, in 2011, the King Abdulaziz University in Jeddah, Saudi Arabia (KAU) and the Senckenberg Research Institute in Frankfurt, Germany (SRI) began a scientific research program. The major purpose of the Red Sea Biodiversity Project is to analyze the marine biodiversity along the Saudi Arabian coast and in the deep waters of the Red Sea, as well as to establish a reference collection at the King Abdulaziz Marine Museum (KAUMM) and S19RI.

Since 2011, all marine animal species have been gathered, recognized, preserved, and cataloged for the KAUMM and SRI reference collections. Several species that are novel to the Red Sea or even to science were discovered because of these broad studies.

Table 1.1: The total number of fishermen, workers, and fishing boats in the Kingdom of Saudi Arabia.

	Tabuk	Madina	Mecca	Asir	Jazan	Eastern Province	Total
Number of fishermen	2380	1468	1252	1252	1617	2486	9461
Number of fishing boats	3715	1922	1577	408	1550	2062	11234
Number of Workers	2827	3023	2191	266	5847	9201	23355

Another objective of this project collaboration is to describe and publish the results, with the hope of publishing many of these results in one volume, as well as inviting scientists who are not involved in the described project to contribute investigations on a wide range of topics related to marine biodiversity investigation, includes taxonomy and systematics, ecology, ecosystem health and management, long-term trends, neobiota, and other relevant fields (Sonnewald and El-Sherbiny, 2017).

1.5.1. Related work

Species identification forms the first step in phylogenetic studies, then biodiversity conservation, and monitoring (Moftah et al., 2011). Studies on identifying species have direct management consequences such as recognizing and listing rare and imperiled species under the US Endangered Species Act (Forsman et al. 2010). It is crucial for understanding ecological functions and allows rare views into the processes leading to speciation in marine environments (von der Heyden et al. 2011, Bowen et al. 2013). For example, the Hybridization between two serranids in Bermuda (Bostrom et al., 2002).

In the UAE, Ketchum et al. (2016) identified three genetically distinct species of *Eponephelus*, that are morphologically similar, were managed as a single stock – it is now clear that they need to be managed as multiple stocks. The genetic analysis benefits the management in many ways. It could determine the effectiveness of marine protected areas MPAs (Le Port et al., 2017).

The application of molecular techniques helps fisheries managements to fight illegal, unreported, and unregulated fishing that affect endangered species (Pappalardo et al., 2019). For example, a study conducted in Brazil has used genetic tools to uncover the commercial fraud in the marketing of fillets, which is the substitution of expensive species

with low-value species or species from different fisheries in order to sell it at a high price (Carvalho et al., 2020). In addition, the results of genetic tools inform the management of the proper and effective design of future MPAs (von der Heyden et al., 2014).

The distribution and abundance patterns of rocky intertidal fish assemblages in the Persian Gulf and the Gulf of Oman were studied by Ghanbarifardi and Malek (2009). At low tide, ichthyoid was used to capture specimens from tidal pools. Between May and July 2006, 1497 fish were collected at six different locations, representing 20 different species from eight different families. Permanent tidal pool residents (Gobiidae and Blenniidae) made for 93.5% of the entire fish assemblage, with secondary residents accounting for 6.5%. The most common fish species were Antennablennius variopunctatus (Blenniidae; 23.4%), Istigobius ornatus (Gobiidae; 19.8%), Bathygobius meggitti (Gobiidae; 18.7%) Cryptocentroides arabicus (Gobiidae; 10.5%), Istiblennius pox (Gobiidae; 7.3%), and Omobranchus fasciolatus (Blenniidae; 6.8%). The study found that the Persian Gulf's diversity indices are low when compared to the Gulf of Oman. Despite its location in the Persian Gulf, Qeshm Island has a high variety index, which is most likely due to increased contact with the nearby open ocean, the Gulf of Oman. The Persian Gulf stations are more identical to one another than the stations in the Gulf of Oman, according to hierarchical cluster analysis.

The demersal fisheries of the Arabian Sea, Gulf of Oman, and Arabian Gulf are discussed in Siddeek, Fouda, and Hermosa Jr (1999). The demersal fisheries in the continental shelves of the three regions are supported by over 350 commercial fish species, eight shrimp species, two spiny lobster species, one shovelnose lobster species, one cuttlefish species, one crab species, and one abalone species. Demersal fisheries involved

both artisanal and industrial vessels, totaling about 120 000 fishermen. Fish and shrimp trawlers, huge wooden boats (dhows) with inboard motors, tiny wooden boats (dhows) with outboard engines, and fiberglass boats are all examples of fishing boats. Trawls, bottom gillnets, traps (wire mesh and plastic), barrier traps, hand lines, and bare hands and knives are among the fishing equipment (to dislodge abalone). The two commercially valuable demersal assets were fish and shrimp. Between 1988 and 1993, demersals accounted for roughly 40% of total marine landings, weighing between 198 000 and 214 000 tonnes (t) (475000-552000 t). However, the percentages differed by country: 25% in Oman, 32% in the UAE, 71% in Qatar, 52% in Saudi Arabia, 56% in Bahrain, 55% in Kuwait, nearly 100% in Iraq, and 41% in Iran.

In the middle Red Sea, Kattan, Coker, and Berumen (2017) investigated reef fish biomass in Saudi Arabia and Sudan. They discovered that top predator biomass on offshore Sudanese reefs was nearly three times that of equivalent reefs in Saudi Arabia. Among the most remote reefs observed in Sudan's extreme southern region had biomass values that are comparable to those previously documented in the Northwestern Hawaiian Islands, northern Line Islands, Pitcairn Islands, and other isolated Pacific islands and atolls. The research showed that fishing pressure has had a substantial impact on the fish community structure of Saudi Arabian Red Sea reefs, most notably through the elimination of top predators. The findings highlighted the urgent need for increased control and enforcement of fishing practices in Saudi Arabia, as well as a compelling case for protection in the form of no-take marine protected zones to preserve the comparatively pristine southern Sudanese Red Sea.

The visual census technique was used to analyze the likely impact of industrial operations on species diversity, abundance, and richness of the fish population in Al-Zibdah research (2008). For comparison of fish assemblages at the research location, three zones (ZI, ZII, and ZIII) and two depths (6 and 12 m) were explored. At coral reef habitat, rocky boulders, and the sandy bottom, a total of 36 transect counts were conducted. The abundance, diversity, and spatial arrangement of species were all recorded. The 54 species discovered in this study belonged to 16 different families. Pomacentridae and Serranidae had the highest relative abundance (RA) values at both depths, with 65.9% and 10.6%, respectively. At the three zones at both depths, similar results were reported in terms of species richness and diversity in coral reef habitats. A shallow sand ecosystem, on the other hand, had a limited abundance of fish. In both depths, the Pomacentridae and Labridae families of fish had the highest frequency of appearance (FA). ZII, at a depth of 12 meters, had the highest density (36 species per zone). The most common fish was Neopomacentrus Mirae, and the least common was Lethrinus borbonicus. All fish indices calculated at the research site had comparable results.

1.6. Summary

One of the world's largest fish stocks is found in the Red Sea. Nevertheless, it does have a burgeoning economy and transportation network. The Red Sea faces ongoing degradation of marine habitats. For example, coral reefs, seagrass beds, and mangroves, are degraded by pollution from oil spills, mining operations, and a variety of industries. Results include degraded fisheries, enhanced life conditions for sea urchins that further harm coral, and overexploitation of threatened species. Several occurrences of organisms suffering direct degradation as a result of human actions have been documented, such as

the overfishing of the sea cucumber Holothuria scabra and the overharvesting of giant Tridacna species. In comparison to coral reefs, livestock is reported to be numerous in the Red Sea. Researchers have emphasized that when reefs are properly preserved and fishing is managed, grouper populations remain high.

Serranids are a bony fish family that can be located in both tropical and temperate waters. The family is varied, with over 475 species scattered across 64 genera, all of which have a three-spined operculum and a tip of the maxilla exposed when the mouth is closed. Members of the Epinephelinae subfamily, which includes approximately 160 species and 15 genera, are known as groupers. The majority of groupers are protogynous hermaphrodites who are known to be bottom-dwelling lie-in-wait predators who ambush their prey as it swims by. In tropical and temperate climates, groupers are a high-priced commercial food fish.

CHAPTER 2

INSIGHTS ON THE ROLE OF PLEISTOCENE GLACIATIONS ON THE ENDEMISM OF THE SUMMAN GROUPER *EPINEPHILUS SUMMANA* IN THE RED SEA

2.1. Introduction

The Red Sea is one of the most unique marine ecosystems in the world and has been identified as a 'hot spot' for the generation of marine biodiversity. It exhibits a high level of aquatic species endemism, exceeding, at least in the level of shore fishes, that in other Indian Ocean hotspot areas, such as the Arabian Gulf and the Gulf of Oman (DiBattista et al. 2016). The Red Sea harbors 95 endemic coral reef fish species (11 % of the world's endemic reef fishes), 12.6 % of the world's endemic polychaetes, 8.1 % of the world's echinoderms, 16.5 % of the world's endemic ascidians, and 5.8 % of world's endemic scleractinian corals (Allen et al. 2008; DiBattista et al. 2016). Of the 346 coral species recorded in the Red Sea, 5.5% are endemics as are 33 % of recorded crustaceans (DiBattista et al. 2016; Arrigoni et al. 2016).

At present time, the Red Sea is directly connected to the Gulf of Aden through the narrow (29 km) and shallow (137 m) Bab Al Mandab Strait. The Gulf of Aden is separated from the Arabian Gulf by a cold, nutrient-rich water barrier (Bailey et al. 2007; DiBattista et al. 2016). The endemic fauna of the Red Sea is similar to that of the Western Gulf of Aden, but clearly different from the Eastern Gulf of Aden and the Arabian Gulf. This is likely due to the monsoonal-driven upwelling of cold, nutrient rich water that occurs

seasonally between the coast of Somalia and Oman (Izumo et al. 2008; DiBattista et al. 2016). Moreover, and during the summer, the Monsoon drives a subsurface influx of colder, fresher, and nutrient rich waters from the Gulf of Aden to the Southern Red Sea (Dreano et al. 2016). The intrusion of Gulf of Aden intermediate water is a part of Winter two-layer, Summer three-layer water exchange between the Red Sea and the Gulf of Aden, the system that is suggested to be stable throughout the glaciation periods (Biton et al. 2008; 2010). The exchange of relatively low salinity waters of the Indian Ocean with the high salinity waters of the Red Sea via the Gulf of Aden alleviates hypersalinity in the Red Sea (Mitchell et al. 2015). The intrusion and seasonal upwelling, together with Bab Al Mandab Strait, formed a strong isolating barrier that continued throughout the glaciations isolating the Red Sea as a hot spot for speciation and faunal endemism (DiBattista et al. 2016).

The connection between the Red Sea and the Gulf of Aden was opened and became the only source of water supply to the former since 14-13 MYA (Bailey et al. 2007; DiBattista et al. 2016). Yet, the conditions in that epoch were hypothesized to be of high temperature and excessive evaporation, conditions that are not conducive to the survival of a diverse reef fauna (DiBattista et al. 2016). However, since 5-4 MYA, seafloor spreading in the Red Sea proceeded due to the separation of Arabia, forming this incipient basin in its nearly present-day form. The continental expansion led to the formation of the mid axial trough of the basin, while the uplift of the rift margins formed the Red Sea Mountains (Bailey et al. 2007; Liddy et al. 2016). The Red Sea reef fauna was putatively only recently established, within the last 3-4 MY, simultaneous with the cooling of the northwestern Indian Ocean that enhanced the upwelling and productivity in the region (DiBattista et al.

2016). Since about 2 MYA, at the start of the Quaternary epoch, a period of glacial events led to a drop in water levels, and strong isolation between the Red Sea and the Gulf of Aden. This produced somewhat inhospitable environmental conditions that deeply affected the distribution of fauna in the Red Sea, extirpating some fraction of the marine fauna, but, hypothetically, hastening speciation in others (Bailey 2015; Mitchell et al. 2015). These events, collectively, are believed to have played key roles in triggering speciation and likewise facilitating endemism that define the endemic marine fauna of the Red Sea.

The exact origin of these endemics and their ancestors in the Red Sea has been the focus of recent research. Some suggest that the Red Sea can be attributed directly as a major incubator for endemic animals (Froukh and Kochzius, 2007), while others have suggested that the Red Sea is a peripheral system in the Indian Ocean that produces and subsequently exports new species (Bowen et al., 2013; DiBattista et al. 2013, 2016). Other research has focused primarily on the degree of isolation between the Red Sea fauna and conspecifics in adjacent marine systems (for example, Iacchei et al., 2016).

The Summana grouper, *Epinephelus summana,* species has received little attention in terms of genetic variability and conservation (Galal-Khallaf et al., 2018), despite being one of the endemic coral reef fishes of the Red Sea and the Western Gulf of Aden. Additionally, putative errors in species discrimination often occurs due to the very similar morphology between *E. summana* and *E. ongus*, another tropical and subtropical grouper species that is present in the Indo-Pacific, but that does not occur in the Red Sea and the Arabian Gulf. This has, unfortunately, led to some erroneous reports regarding the presence of *E. summana* outside of its native range, the Red Sea and Western Gulf of Aiden (for example, Kohno et al. 1988; Mamauag et al. 2009). Hence, the aim of this study was

to provide insights on the major genetic relations among *E. summana* and other sympatric and allopatric groupers species that were hypothesized as ¨related species¨, as well as to identify the possible origin of this species endemism in the Red Sea. The hypothesis of this study is that *E. Summana* is most closely related to the *E. ongus*. In particular, a phylogenetic analyses of the genus Epinephelus is conducted to identify the sister taxon to *E. Summana,* and then molecular clock estimates are used to roughly date the origin of the Red Sea endemic *Epinepheles summana*.

2.2. Materials and Methods

2.2.1. Samples collection and DNA processing

Groupers in commercial fish catach were collected from KSA coasts in the Red Sea (Jazan and Jeddah areas) and the Arabian Gulf (Dammam area) From the Red Sea, 23 samples of *E. summana* samples, 23 of *E. chlorostigma*, 6 of *E. stoliczkae* and 2 of *E. awoara* were collected. From both the Red Sea and the Arabian Gulf, 43 samples of *E. areolatus*, 13 samples of *E. bleekeri,* and 4 of *E. coioides* were collected. 4 samples of *E. polylepis* were collected from the Arabian Gulf only (Fig.1). Small fin biopsies were taken from each individual and stored in 95% ethanol. All specimens were stored frozen at the laboratories of King Abdulaziz City for Science and Technology (KACST) laboratories, Riyadh, KSA. Total DNA was extracted using QIAgen DNEasy columns following the manufacturer's instructions. PCR reaction volumes (25µl) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1% Tween 20, 5% DMSO, 200 mM each dNTP, 10 pmol of each primer (universal 16srRNA or 12srRNA) and one unit of Taq DNA polymerase. Cycling conditions were: initial denaturation at 94°C for 4 minutes, followed by 40 cycles of a denaturation at 94°C for 1 minute, an annealing at 48°C for 1 minute, and

an extension at 72°C for 1 minute, followed by a final extension of 72°C for 6 minutes. Amplification success was confirmed by agarose gel electrophoresis. Positive PCRs were sequenced in both directions using the Big Dye Terminator 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosciences). Cycle sequencing products were analyzed on an Applied Bioscience 3130 automated sequencer.

These two markers were chosen because they: (1) are easy to amplify in most fish; (2) are generally variable at the population level; (3) facilitate comparisons with published sequences; and (4) have had molecular clock rates estimatedbased on reef fishes (Bowen et al., 2001; Lessios, 2008; Reece et al., 2010). Also, see DiBattista et al. (2013) for an overview.

2.2.2. Phylogenetic and dating analyses

Sequence trace files were edited 'by eye' using Sequencher (Applied Biosciences) and aligned to other *Epinephelus* sequences from GenBank (Craig and Hastings, 2007). 16srRNA and 12srRNA sequences from each sample, as well as from GenBank sequences for grouper species located in the Indian Ocean, Red Sea, and the Arabian Gulf were joined. These concatenated sequences were aligned using ClustalW (Thompson et al. 1994). The best substitution model, that was identified through Modeltest implemented in Mega (Kumar et al. 2018), was applied for the subsequent analyses. Phylogenetic analyses utilized the Neighbor-Joining (NJ) criteria, as well as pairwise distances, were carried out first using PAUP*4.0 (Swofford 2002). The observed percent pairwise differences was used as a distance under the NJ criterion, after considering the weighing of transitions and transversions equally, a priori weighing of transitions and transversions according to R criteria (=Ti/Tv), and excluding transitions. Bootstrapping (Felsenstein 1985) was used to

estimate the reliability of individual clades in all phylogenetic reconstructions (1,000 replicates). Pairwise estimates of percent sequence divergence were used to estimate divergence times using a clock calibration for marine fish mitochondrial genes of ~1%/MY between pairs of taxa (Tringali et al. 1999; Bowen et al. 2001; Lessios 2008; Reece et al., 2010). Moreover, the NJ tree was validated by carrying out a maximum likelihood phylogenetic analysis using BEAST 2.1.3 (Bouckaert et al., 2014), applying a strict molecular clock based on the same net 1% divergence per million years for marine fishes mtDNA, and a run consisting of 10 million generations, sampling every 1000 generation. A maximum clade credibility tree was generated with median ages and 95% highest posterior density intervals using TreeAnnotator 2.1.2, and viewed using FigTree v 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Bayesian inference (BI) for phylogenetic relations among assessed species was carried out using MrBayes 3.2.1 (Ronquist et al. 2018), after partitioning the sequences as 16srRNA and 12srRNA and indetifying the best selection model using the same software. Four Markov Chains Monte Carlo (MCMC) chains were analyzed for 10 million (ngen=10,000,000) generations, saving a tree each 1,000 generations. The subsequent analysis started when the average standard deviation of split frequencies reached 0.002. Tracer 1.7 (Rambaut et al. 2018) was applied for calculating effective samples size and number of burn-ins. Tracer 1.7 exhibited that 25 % of the saved trees are to be discarded as burn-ins. This information was transferred to MrBayes 3.2.1. for constructing the summarized tree, which was later opened also using FigTree v 1.3.1.

2.3. Results

The 664 bp-long sequences could be resolved for the 16srRNA gene, while this was 450 bp for the 12srRNA. For phylogenetic analysis using all species sampled in the current study and others belonging to the same and close geographical areas, 376 bp and 205 bp were the products of alignment of 16srRNA and 12srRNA genes that were merged for each species and sample. The best fit substitution model was Hasegawa-Kishino-Yano (HKY) model. Tree topologies were almost identical between NJ (Fig 2-2), ML (Fig 2-3), and BI trees (Fig 2-4) ones. Testing different weighing matrices did not result in significant tree topologies differences neither. The trees coincided in exhibiting polyphyletic lineages for the groupers of the Indian Ocean, Red Sea, and Arabian Gulf. More clearly, ML and BI trees (Fig 2-3,2-4) showed that most groupers assessed belong mainly to two monophyletic lineages separated by 8 % divergence. One of these groups included the grouper species that are more related to the Western Indian Ocean, the Red Sea and the Arabian Gulf. The other group encompassed grouper species that are more widely distributed in the Indian Ocean and the Indo-west Pacific. The first group included a single subclade encompassing *E. summana*, the endemic grouper to the Red Sea, in a sister relation to *E. coeruleopunctatus* that exist in both study areas as well as along a wide geographical range in the Indian Ocean and the IndoWest Pacific. Another clade in the same group included two species that are completely absent from the Arabian Gulf, that are *E. polyphekadion* and *E. fuscoguttatus*, despite both being endemic to the Red Sea and the Indian Ocean. *E. ongus*, that exist in neither the Red Sea nor the Arabian Guld, but solely in the Indian Ocean and Indo West-Pacific region, exhibited in all trees (Fig 2-4) a sister relation with *E. fuscoguttatus* and *E. polyphekadion* in the same clade, but in a different subclade. The third

clade of the same group encompassed *E. coioides*, common to the Red Sea and the Arabian Gulf, besides the Indian Ocean, in a sister relation to *E. malabaricus* present in the Red Sea and the Indian Ocean. These two species were directly related in the same subclade with *E. tukula,* of the Red Sea and the Indian Ocean, and *E, bruneus* that is native only to the South West of China. In contrast to *E. summana* and all other species samples in the current study*, E. coioides* samples exhibited strong intraspecific divergence (Fig. 2-4).

Pairwise distances (Fig 5, Table 1) also exhibited an increasing pattern that was closely related with the phylogeny results. Using clock calibration indicated that the major separation events in the clade including *E. summana* and its related species occurred 2-7 MYA. The latest separation of all was that between *E. summana* and *E. coeruleopunctatus*, that dated back to about 2.8 MYA. The departure between *E. summana* and *E. ongus* was calculated as 4.7 MYA. Interestingly, other inhabitants of the Red Sea and the Indian Ocean phylogenetically related to *E. summana,* that are *E. polyphekadion* and *E. fuscogutattus*, exhibited 6.2 and 7.2 MYA period of separation from *E. summana. E. stoliczkae,* that is native to very limited area in the Indian Ocean, and the entire Red Sea, was separated by more than 10.1 MYA.

Curiously, the intraspecific phylogenetic differences among haplotypes of some species that were found in all trees was related to intraspecific differences in pairwise distances could be identified in three of the samples species, that are *E. coioides, E. stoliczkae,* and *E. areolatus* (Tables 2-4). Comparing *E. coioides* haplotypes to the closes phylogenetically-related species, that was *E. malabaricus*, the *d* values were 0.016, 0.024, and 0.026 for the pure Red Sea haplotype (H1), the common Red Sea-Arabian Gulf H2, and the sole Arabian Gulf H3, respectively. *E. stoliczkae* was phylogenetically related to

E. rivulatus, *E. quoyanus,* and *E. macrospilos*, being the first is the only one present in the Gulf of Aden, while the others are more related to the South Eastern and South Western Indian Ocean, as well as the Easter Pacific Ocean. No interspecific differences in *d* value could be identified between different *E. stoliczkae* with all these species, but an intraspecific variability among haplotypes of this species could be detected (*d=0.007).* Finally, the Reds Sea-Arabian Gulf common *E. areolatus* haplotype exhibited the least distance (*d*=0.022) with the *E. undulosus*, while this value was higher (*d=0.024)* upon comparing the Red Sea haplotypes with *E. undulosus*.

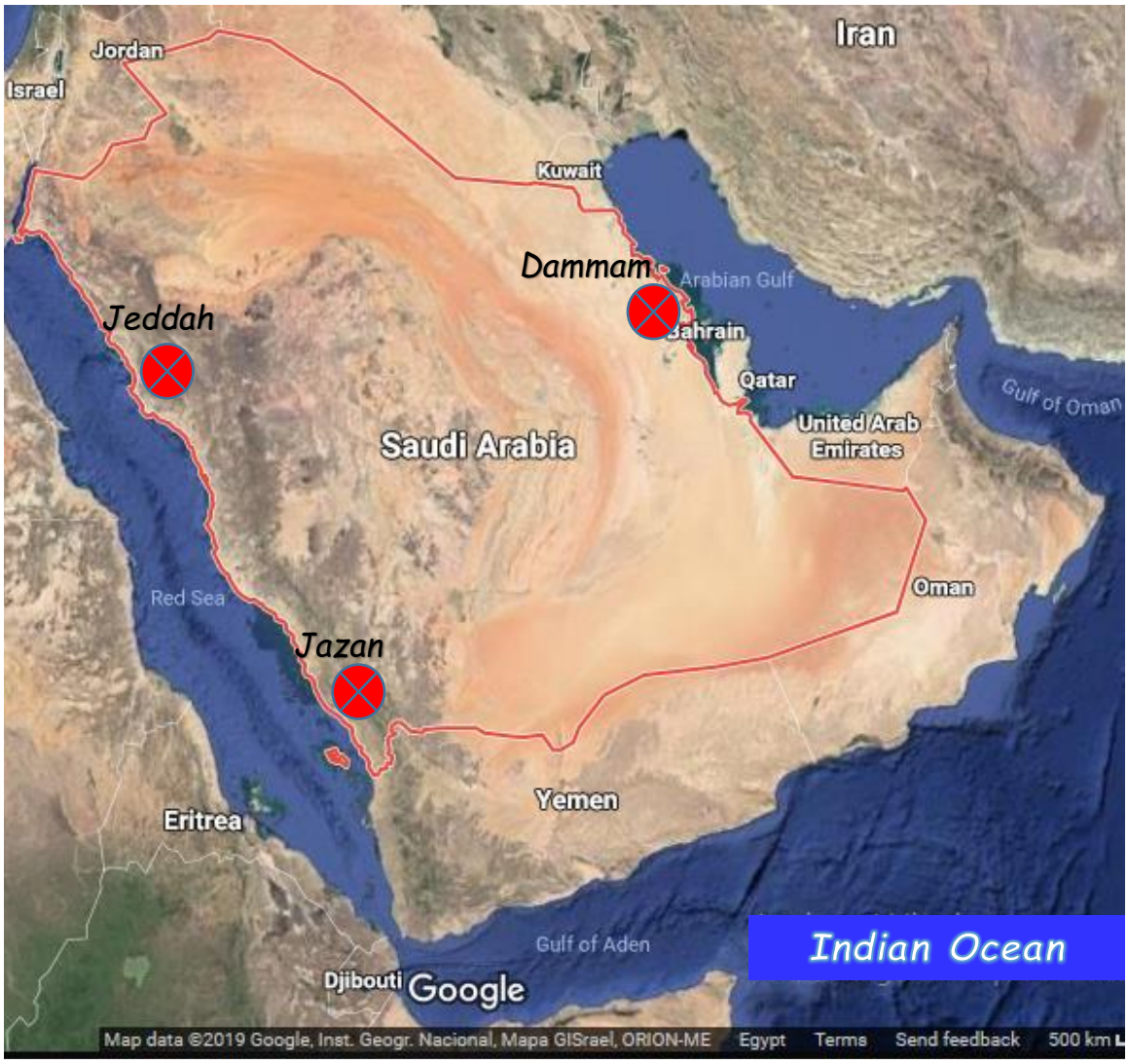

Figure 2.1: Kingdom of Saudi Arabia showing sampling sites for groupers in the Red Sea coasts (Jazan and Jeddah) and the Arabian Gulf (Dammam). Photo credits: Google Maps® (shown below the image).

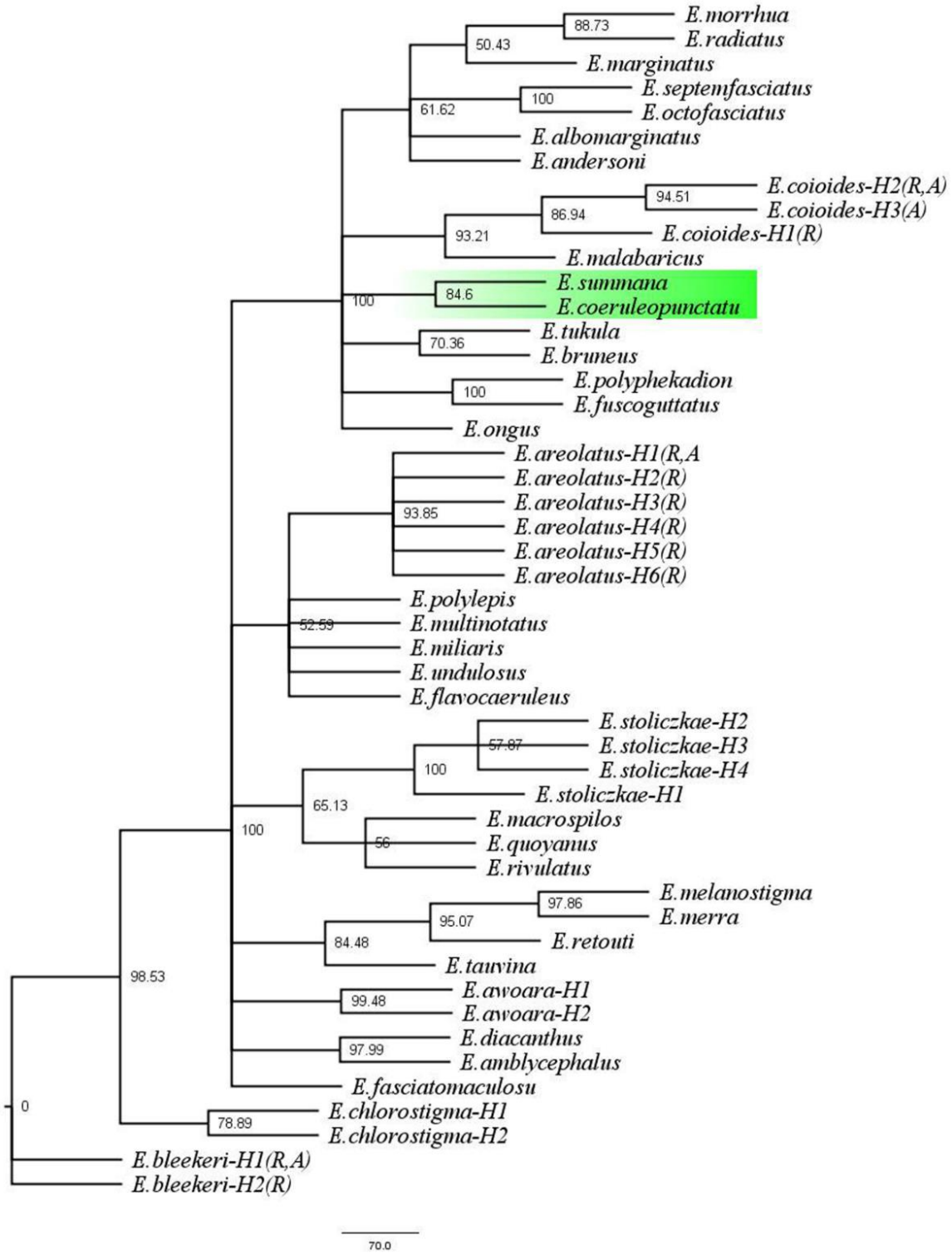

Figure 2.2: NJ bootstrap consensus tree for groupers included in the current study. Bootstrap support is shown in front of nodes. Only bootstraps ≥ 50 % are shown. The clade for *E. summana* is highlighted in green. Abbreviations: H: haplotype, R: Red Sea, A: Arabian Gulf.

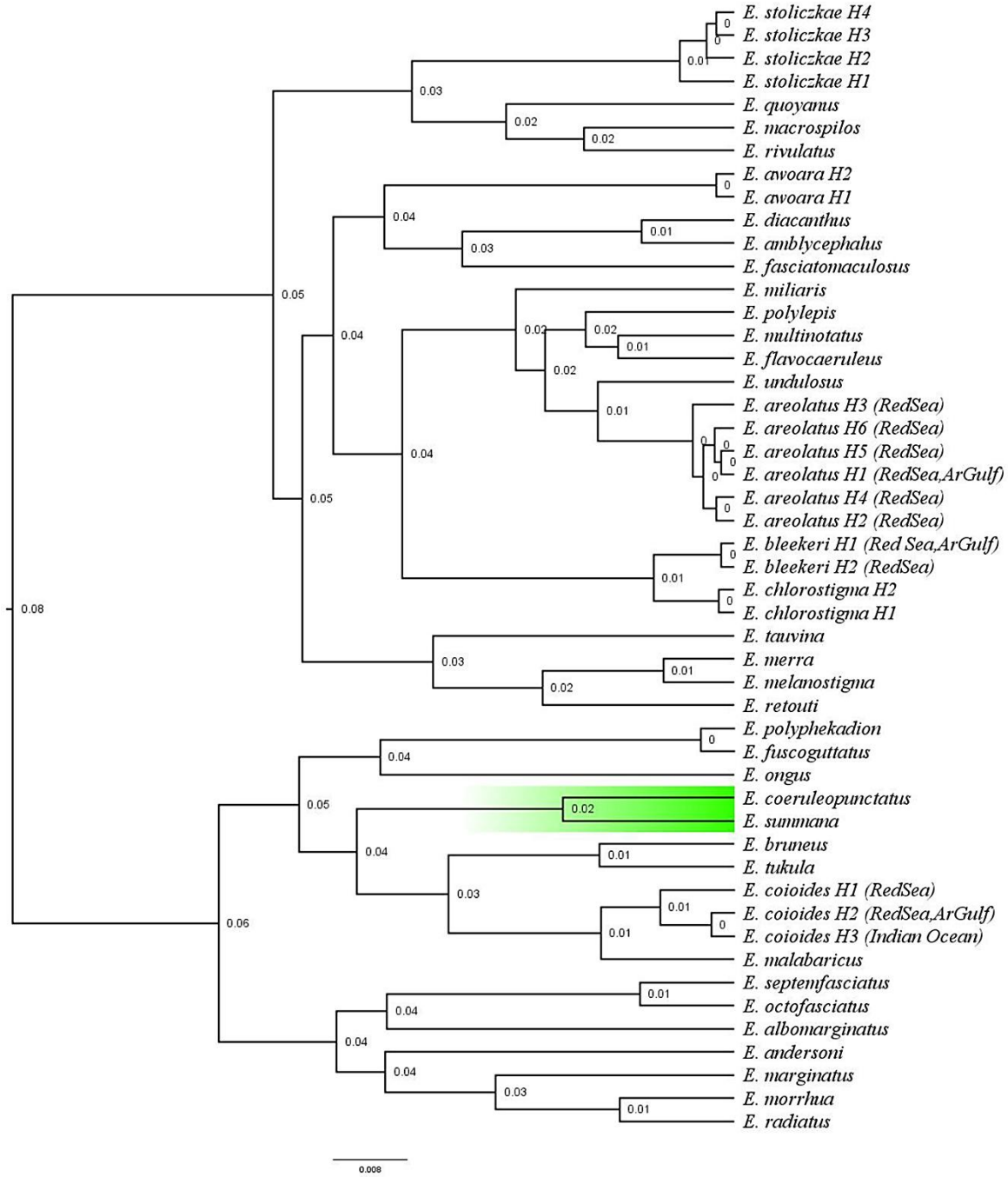

Figure 2.3: ML tree for groupers included in the current study. The tree was generated after analyzing 10,000,000 Markov Chains. Node ages is shown in front of nodes. The clade for *E. summana* is highlighted in green. Abbreviation: H: haplotype.

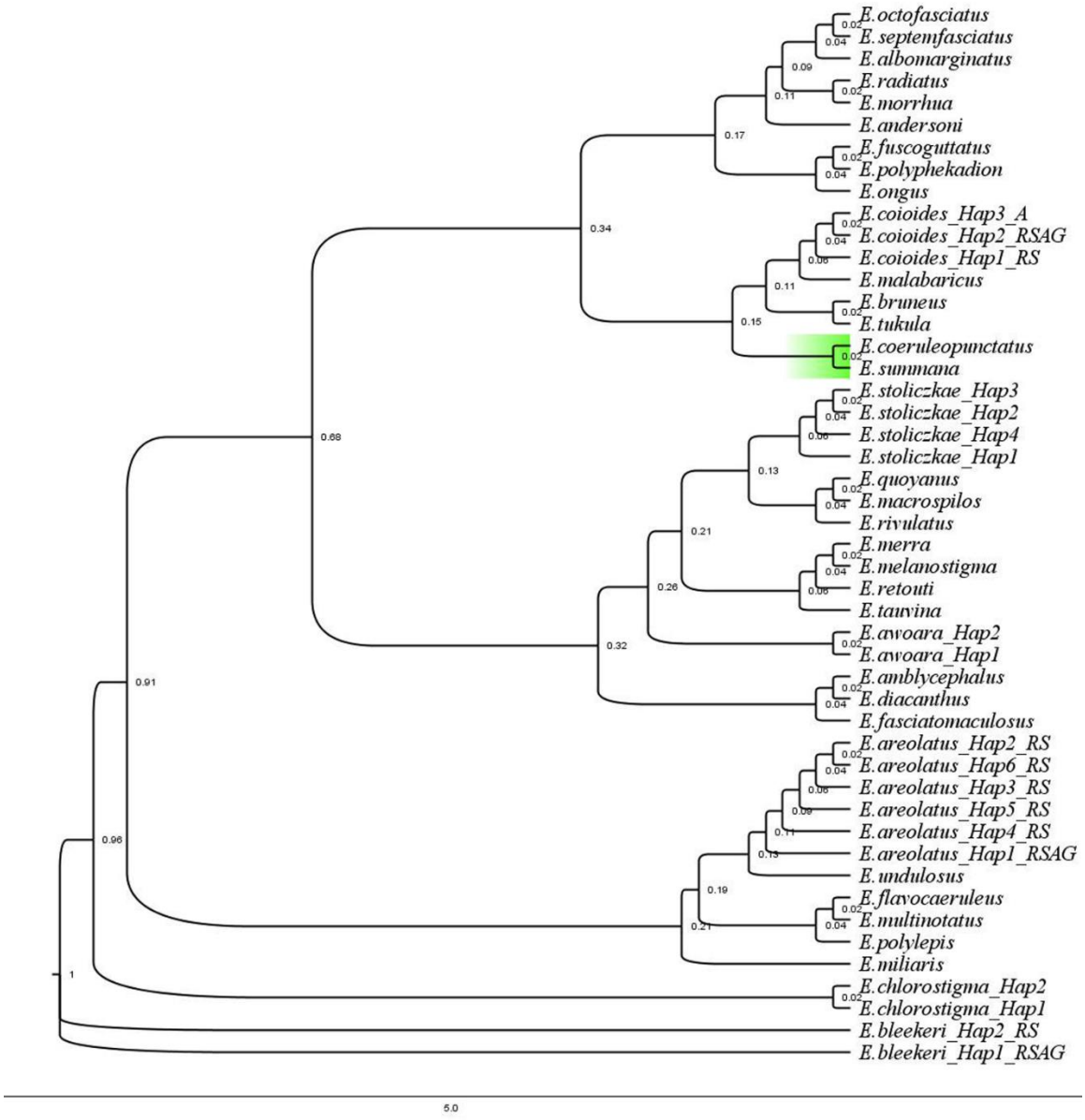

Figure 2.4: BI tree for groupers included in the current study. The tree was generated after analyzing 10,000,000 Markov Chains. The clade for *E. summana* is highlighted in green. Node ages is shown in front of nodes. Abbreviation: Hap: haplotype, RS: Red Sea, AG: Arabian Gulf.

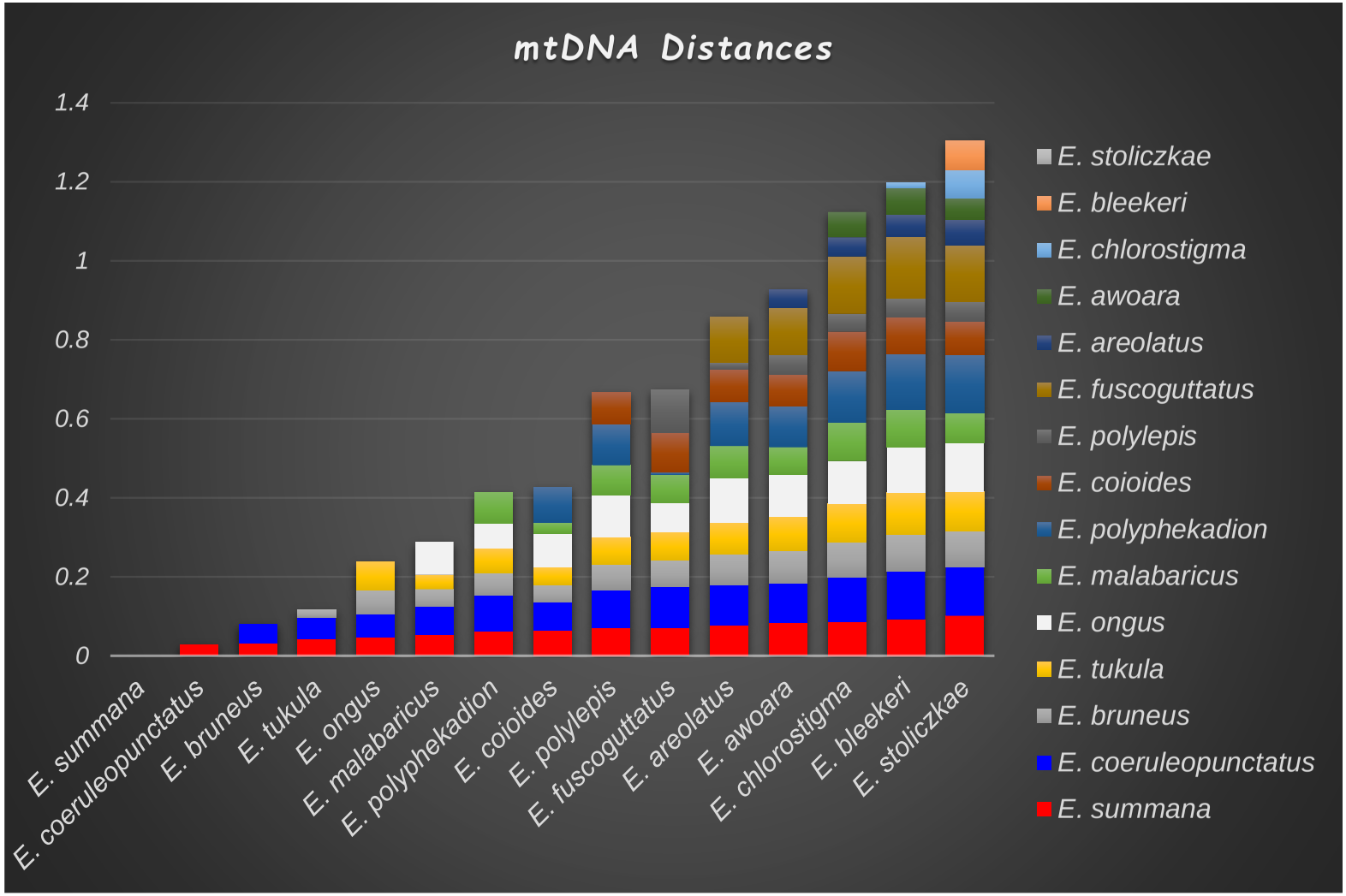

Figure 2.5: Combined 16srRNA-12srRNA genetic pairwise distances among studied species.

Table 2.1: Combined 16srRNA-12srRNA genetic pairwise distances among studied species.

	E. summana	*E. coeruleopunctatus*	*E. bruneus*	*E. tukula*	*E. ongus*	*E. malabaricus*	*E. polyphekadion*	*E. coioides*	*E. polylepis*	*E. fuscoguttatus*	*E. areolatus*	*E. awoara*	*E. chlorostigma*	*E. bleekeri*	*E. stoliczkae*
E. summana															
E. coeruleopunctatus	0.028														
E. bruneus	0.032	0.048													
E. tukula	0.042	0.056	0.019												
E. ongus	0.047	0.059	0.060	0.071											
E. malabaricus	0.054	0.070	0.044	0.037	0.084										
E. polyphekadion	0.062	0.091	0.058	0.062	0.063	0.077									
E. coioides	0.064	0.071	0.045	0.045	0.085	0.028	0.089								
E. polylepis	0.071	0.096	0.066	0.068	0.106	0.074	0.105	0.081							
E. fuscoguttatus	0.072	0.103	0.067	0.072	0.073	0.072	0.005	0.101	0.109						
E. areolatus	0.077	0.103	0.078	0.080	0.113	0.080	0.111	0.083	0.017	0.116					
E. awoara	0.084	0.100	0.082	0.088	0.106	0.068	0.104	0.081	0.050	0.117	0.047				
E. chlorostigma	0.086	0.113	0.091	0.096	0.108	0.097	0.131	0.100	0.045	0.146	0.049	0.063			
E. bleekeri	0.093	0.121	0.094	0.104	0.116	0.097	0.140	0.093	0.047	0.156	0.058	0.067	0.013		
E. stoliczkae	0.101	0.124	0.092	0.098	0.123	0.077	0.147	0.084	0.050	0.144	0.064	0.056	0.070	0.074	

Table 2.2: Intraspecific pairwise distances among different haplotypes of *E. coioides* present only in the Red Sea (H1), common between the Red Sea and the Arabian Gulf (H2), and in the Arabian Gulf only (H3).

E. coioides	H1	H2
H1		
H2	0.0120	
H3	0.0138	0.0052

Table 2.3: Intraspecific pairwise distances among different haplotypes of *E. stoliczkae* sampled from the Red Sea.

E. stoliczkae	H1	H2	H3
H1			
H2	0.0069		
H3	0.0069	0.0034	
H4	0.0069	0.0034	0.0034

Table 2.4: Intraspecific pairwise distances among different haplotypes of *E. areolatus* found in both the Red Sea and the Arabian Gulf (H1) or the Red Sea only (H2-H6).

E. areolatus	H1	H2	H3	H4	H5
H1					
H2	0.0017				
H3	0.0017	0.0034			
H4	0.0017	0.0034	0.0034		
H5	0.0017	0.0034	0.0034	0.0034	
H6	0.0017	0.0034	0.0034	0.0034	0.0034

2.4. Discussion

There is a great debate about the causes of uniqueness of Red Sea fauna, between the possible eradication during the glacial cycles of the Pleistocene, or the presence of Red Sea inside or close outside refuges in response to low sea levels and unfavorable life conditions (DiBattista et al. 2016). *E. summana,* one of the major endemic groupers of the Red Sea, was found to belong phylogenetically to a group of species that are widely spread in the Indian Ocean and the IndoWest Pacific.

Our clock calibration to the speciation in *E. summana* and other sympatric and allopatric groupers of the same genus exhibited that the separation between *E. summana* and its closest relative *E. coeruleopunctatus* occurred 2.8 MYA, that is almost exactly at the onset of the Quaternary period, strictly the early Pliocene-Pleistocene epochs transition, when a major isolation of the Red Sea took place (Bailey 2015; Mitchell et al. 2015). *E. summana* and *E. coeruleopunctatus* splitting was 2 million years after the divergence of their common ancestor and *E. ongus (4.7 MYA).* This latter species has long been identified as the closest relative to *E. summana* (for example, Randall and Ben-Tuvia 1983; Mamauag et al. 2009). The period identified as the dawn for *E. summana* was characterized by versatile geological fluctuations, hypresalinity, and desiccation in the Red Sea. These harsh conditions separated its fauna and their evolutionary history from that of the Indian Ocean and the Gulf of Aden. This period was characterized by a severe drop of sea level to 115-130 m below the current sea level, due to the global climate oscillation, which eventually led to limiting strongly the connection between the Red Sea and the Indian Ocean through shoaling of Bab Al Mandab strait, plus changing the monsoons and the system they trigger of marine currents, besides the reduction of upwelling current productivity (Tribovillard et

al. 1996; Ludt and Rocha 2015)). This reduction in Red Sea connection to the cooling, salinity-reducing Gulf of Aden led to intensifying the glacial-interglacial variations in the Red Sea to 2-3 times those of the global oceans, which dramatically led to hypersalinity (50 ‰, Biton et al., 2008), reduction of plankton availability, and increasing the residence times of water masses in the Red Sea (Biton et al., 2008; DiBattista et al., 2016; Mitchell et al., 2015). All these events were reversed following the melt water pulse events that started 14,300 years before present when water levels started to rise by 30-40 mm annually and the full connection to the Indian Ocean was restored (Hanebuth et al., 2000; Ludt et al., 2015).

Assessment of genetic variability between Red Sea organisms and conspecifics in the Gulf of Aden were extensively carried out. These studies resulted in the presence of such differentiation in several organisms, but not all. The effects of Pleistocene glaciations were, in most of cases, a key player in mediating the connectivity patterns of such cases, more specifically due to the extreme changes in salinity and nutrients distribution in this epoch, as triggers for the endemism, populations' structuring, and speciation in versatile taxa in the Red Sea. Another peculiar finding in this work is some degree of intraspecific phylogenetic separation withing *E. coioides, E. stoliczkae,* and *E. areolatus*. These differences were pronounced in samples from even the same area (i.e. only the Red Sea), and all of them can be provisionally attributed also to the Pleistocene glaciations-interglaciations period (1.3-0.17 MYA). This may indicate a further role of this period in intraspecific variability in the groupers of the Red Sea, Gulf of Aden, and the Arabian Gulf. Moreover, these variations may refer to a possible cryptic speciation within those three groupers. Similar results for cryptic species separation and the role Pleistocene glaciations

was found in cases of the yellowfin hind *Cephaolpholis hemistictos* among population in the Red Sea, Gulf of Aden, and the Arabian Gulf (Priest et al., 2016), the Indo-Pacific goatfish *Mulloidichthys flavolineatus* in the Red Sea (Fernandez-Silva et al., 2015), and other species. Besides, many other reef fish species showed clear genetic separation with their conspecifics out of the Red Sea. For examples, complete genetic fixation in the Red Sea populations of the reef fishes *Neoniphon sammara* and *Pygoplites diacanthus*, modest differentiation in *Acanthurus nigrofuscus*, *C. argus* and *Chaetodon auriga*, but lesser differentiation in *Halichoeres hortulanus* and *L. kasmira*, in comparison to their conspecifics from the Western Indian Ocean, could be clearly identified (DiBattista et al., 2013). Likewise, a prominent genetic separation between two mitochondrial lineages of the Indo-Pacific *M. flavolineatus* could be identified between the Red Sea and the Indo-Pacific, and the separation was dated to the same period when the Red Sea was isolated from the Gulf of Aden (Fernandez-Silva et al., 2015). Pleistocene Red Sea (0.71 MYA) isolation produced significant structuring of populations of the pronghorn spiny lobster, *Panulirus penicillatus* between the Red Sea and the East Pacific (Iacchei et al. 2016). The relatively recent speciation of the scleractinian coral specie *Stylophora mamillata, S. wellsi*, and *S. pistillata* might have been promoted by the strong environmental changes encountered in the Red Sea during Pliocene and Pleistocene through possible favoring of niche partitioning and ecological differentiation (Arrigoni et al., 2016).

2.5. Conclusion

In conclusions, genetic phylogeography could elucidate the role of Pleistocene glaciations in the divergence between the Summan grouper *Epinephelus summana* and its closest relatives that assume wider geographical distribution in the Red Sea and the Indian

Ocean. Moreover, the degree of genetic separation within some Red Sea groupers may indicate a necessity for more work on the level of characterization of cryptic speciation and its impacts on ecological conservation and management of Red Sea fisheries. Further assessments of grouper species structuring within the Red Sea and in comparison to the Indian Ocean can provide more data about the effects of hydrological and geological conditions that these regions suffered during the Qauternary period in the evolution and diversity of this animal group.

CHAPTER 3

DNA BARCODING OF COMMERCIALLY IMPORTANT GROUPER (HAMMOUR) SPECIES (PERCIFORMES, SERRANIDAE) IN SAUDI ARABIA

3.1. Introduction

Application of genetic markers for species identification gains crucial importance in economies where marine products contribute significantly to the Gross Domestic Product (GDP) of the Saudi Arabian national economy. The current massive increase in the size and outreach of international trade has increased the threats of food misrepresentation and fraud, especially in fish markets. This could be attributable to the insufficiency of classical species identification methodologies that are based only on morphology. The accuracy of these methodologies have been proven to be insufficient to expectations, which may contribute to trading of already endangered or overfished species. This directly leads to fisheries decline due to improper management of fisheries (da Silva Ferrette et al., 2019; Behrens-Chapuis et al., 2021). The issue is becoming more complicated with the outbreak of unreported fishing, overfishing, and even fraudulence in fisheries markets through representation of low-priced, abundantly-caught fish species as more expensive ones (Galal-Khallaf et al., 2014). These threats caused by improper identification can be more prominent in fish families characterized by sexual polymorphism, age polymorphism, or external similarities as a result of surviving in complicated environments where different camouflage strategies are assumed, such as

groupers and other coral reef fishes (McKeown et al., 2020; Bhaskar et al., 2021; Fadli et al., 2021).

Owing to the vast coverage of aquatic areas to our planet´s surface (i.e. more than 70 % of total earth´s area), it can be expected that methodologies for authentication of current biodiversity can provide valuable tools for making decisions about environmental protection and sustainable economies. Polymerase Chain Reaction (PCR)-based amplification and sequencing of short, standardized DNA fragments has been proven over more than two decades as a very efficient tool for fish and other marine species´ discrimination. Upon comparing the resulting sequences to specific databases, including for example GenBank (https://www.ncbi.nlm.nih.gov/nuccore/) and Barcode of Life Database (BOLDSYSTEMS: http://www.boldsystems.org/), the task of species identification becomes more and more accessible and reachable to taxonomists, ecologists, and other specialists in different disciplines related to marine life. Since the introduction of DNA barcoding concept by Hebert et al. (2003), more than 10,378 works were deposited in and related to the database of the U.S. National Library of Medicine (https://www.ncbi.nlm.nih.gov). Of these, 937 studies are related to DNA barcoding of fishes. DNA barcoding is actively playing key roles in many fields related to the marine environments, such as recording and authentication of native fish fauna in given regions, identification of different-shaped stages of fish species, early-alert against invasive species, identification of new species, characterization of sibling species, marine conservation, fisheries management, and even detection of presence of food misrepresentation, such as illegal market substitution and use of endangered or threatened species in an undeclared manner (Galal-Khallaf et al. 2017; 2019).

The seas surrounding the Arabian Peninsula, which represent the northernmost portion of the Indian Ocean, are considered to have the highest biodiversity among worldwide marine regions (Wehe and Fiege, 2002). The Arabian Peninsula seas include the Red Sea, the Gulf of Aden, the Arabian Sea, the Gulf of Oman, and the Arabian Gulf, which harbor a wide biodiversity of endemic species, including fishes, echinoderms, and corals. Researchers over many years have documented around 320 scleractinian coral and 1078 fish species in the Red Sea alone (Veron et al., 2009). There are at least 110 serranid species inhabiting the marine waters of the Indo-Pacific region (Bariche and Heemstra, 2011). However, some species are more commonly found in certain regions surrounding the Arabian Peninsula; for example, common inhabitants of the Red Sea, including *Epinephelus areolatus, E. chlorostigma, E. coioides, E. stoliczkae, E. summana, E. tauvina*, *Cephalopholis sonnerati, Cephalopholis miniata, C. oligosticta, C. sexmaculata*, *c. hemistiktos, Variola louti*, and *Aethaloperca rogaa* (Randall and Ben-Tuvia, 1983, Randall, 1986; Golani and Bogorodsky, 2010; Priest et al., 2016).

Despite being ranked as the tenth most important group of fish species caught from the Saudi fisheries in 2019, according to Aquatic Sciences and Fisheries Information System (ASFIS, FAO, 2019), most grouper species in the Kingdom are still understudied, and their conservation status insufficiently known. For example, of the above mentioned species, only few species were evaluated by the IUCN. Of these, *Epinephelus areolatus* was considered as "Near Threatened" in the Arabian Gulf (Choat et al., 2015b). *E. coioides* was considered as "Vulnerable" (Choat et al., 2015a). Most other *Epinephelus* species are categorized as either *Least Concern* or *Data Deficient.* Similarly, all the above mentioned

Cephalopholis species, together with *V. louti* and *A. rogaa*, are all categorized as ¨List Concern¨.

In some of these species, and in many other serranid species, extensive morphological similarities were reported. For this, nomenclature differences were found between fishermen, even in proximate geographical areas, which led to confusion in their proper identification and nomenclature (Provençal, 2013). Furthermore, several studies that applied either or both of morphological and genetic identifications of serranids belonging to various species or genera, based on DNA barcoding, revealed interspecific/intraspecific discrepancies, as a clear result of improper previous identification methodologies (for examples, see Aziz et al., 2016). More commonly, the external morphological similarities among some of these species lead to many cases of species inaccurate identification by both fishermen and related authorities. This can produce inconsistencies in statistics of catch and conservation statuses of some serranid species. Furthermore, another aspect in serranids that has not been sufficiently studied (yet it led to apparent morphogenetic discrepancies in identification of serranid species), is the presence of intraspecific hybridizations (Herwerden et al., 2002; Qu et al., 2018).

Therefore, the current work was designed to carry out accurate tools for identification of different grouper, hind, and lyretail species that inhabit the Red Sea and the Arabian Gulf, the main marine regions around the Arabian Peninsula. This identification was based on DNA barcoding through PCR amplification and sequencing of the mitochondrial gene which is represented in versatile taxonomic levels, i.e. the 16srDNA. For cases of inefficiency of this gene as a barcode in terms of barcoding database inconsistencies, other species marker genes were also sequenced. These genes were the

mitochondrial 12srDNA, and the nuclear Histone H3 and TMO gene. A corner stone of this work was the previously provided morphological key for these species in the current Ph.D. Thesis.

3.2. Materials and Methods

3.2.1. Collection of samples and DNA extraction

Samples of different serranid species (n=8-10 each) were randomly collected from different fish markets that receive landings from Kingdom Saudi Arabia coasts in the Arabian Gulf (Dammam area) and the Red Sea (Jazan and Jeddah areas) (**Figure 3-1**). These samples were initially morphologically identified as groupers (Genera: *Epinephelus* Bloch, 1793 and *Aethaloperca* Forsskål, 1775) belonging to the species *A. rogaa*, *E. stoliczkae, E. coioides, E. chlorostigma, E. bleekeri, E. areolatus,* and an unknown *Epinephelus* species. *A*lso, four hind species (Genus: *Cephalopholis* Bloch and Schneider, 1801) were collected, belonging to the species *C. hemistictos*, *C. sonnerati,* and *C. miniata*, and *C. oligosticta*. A common lyretail (Genus: Swainson, 1839) species in the Saudi fisheries was also collected and identified as *Variola louti* (**Figure 3-2**). Later on, the morphological key was applied to check the identity of each species, as mentioned in previous sections in this Ph.D. thesis, and appended to the barcoding trial that was carried out in the current section. Fin clips from each sample were stored in 95% ethyl alcohol, then stored frozen at the laboratories of King Abdulaziz City for Science and Technology (KACST), Riyadh, KSA. The genomic DNA from fin biopsies (~30 mg) was extracted from each sample using QIAgen DNEasy spin columns kit (Qiagen, Valenica, CA, USA) according to the manufacturer's instructions.

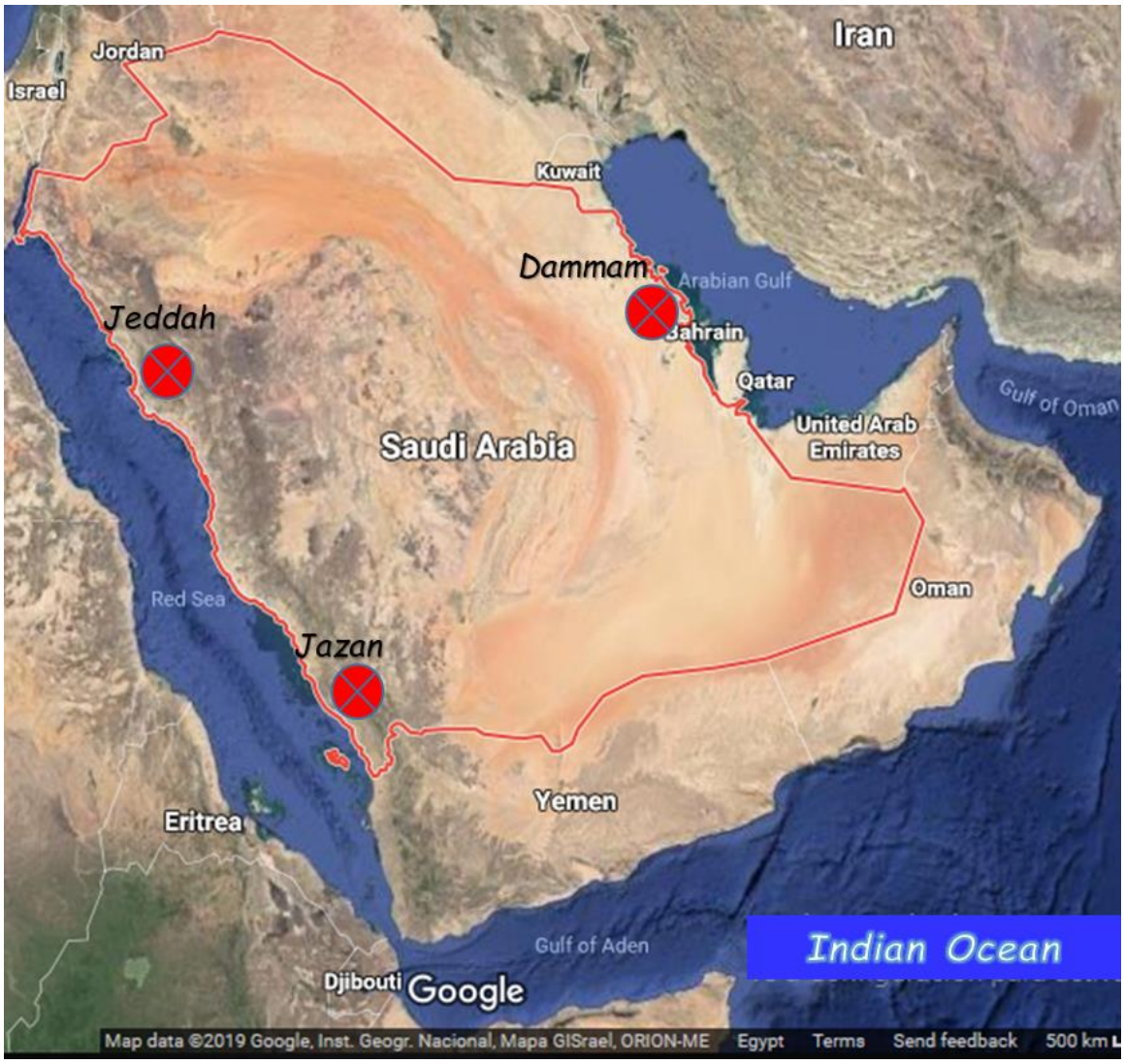

Figure. 3-1. Kingdom of Saudi Arabia showing sampling sites for groupers in the Red Sea coasts (Jazan and Jeddah) and the Arabian Gulf (Dammam). Photo credits: Google Maps® (shown immediately below the image).

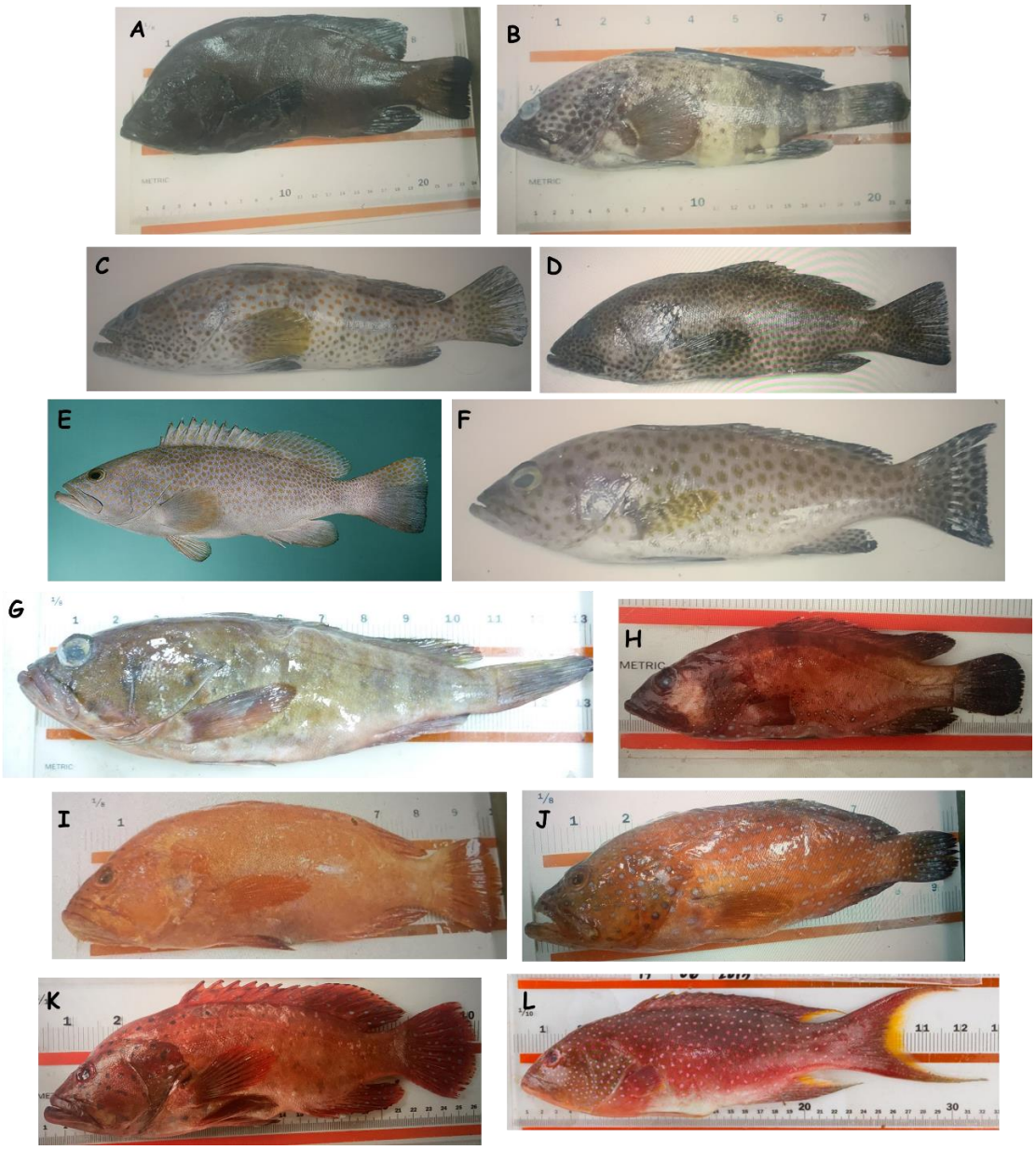

Figure 3-2. Serranid species collected in the current study from Saudi Arabian markets, coming from the Red Sea and the Arabian Gulf coasts of KSA. A: *A. rogaa*, B: *E. stoliczkae*, C: *E. coioides*, D: *E. chlorostigma*, E: *E. bleekeri*, F: *E. areolatus*, G: an unknown *Epinephelus* species. H: *C. hemistictos*, I: *C. sonnerati*, J: *C. miniata*, K: *C. oligosticta*, L: *Variola louti*.

3.2.2. DNA barcoding

For DNA barcoding, the primers used for amplification of the mitochondrial 16S rDNA gene (16SA: 5'-ATGTTTTTGATAAACAGGCG-3' and 16SBr: 5'-CCGGTCTGAACTCAGATCACGT) (Palumbi 1996) were used. The expected amplicon size was 600 bp. Furthermore, three primers´ sets were also applied in case of barcoding results´ inconsistencies. Included are:

i) The mitochondrial 12S rDNA gene (12SA: 5'-AAACTGGGATTAGATACCCCACTAT -3' and 12SF (5'-GAGGGTGACGGGCGGGCGGTGTGT-3'), amplicon size: 400 bp (Palumbi 1996)];

ii) The nuclear histone H3 gene (H3A-L: 5′-ATGGCTCGTACCAAGCAGACVGC-3′:, H3B: 5′-ATATCCTTRGGCATRATRGTGAC-3′), amplicon size: 325 bp (Colgan et al. 1998); and

iii) The nuclear TMO-4C4 gene (TMO-F1: 5′-CCTCCGGCCTTCCTAAAACCTCTC-3′:, TMO-R1: 5′-CATCGTGCTCCTGGGTGACAAAGT-3′), amplicon size: 418 bp (Streelman and Karl 1997).

The amplification reactions were carried out individually for each gene in the samples whose barcodes to be amplified. The following reaction components and volumes were used in a PCR reaction volume of 25 µl: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1% Tween 20, 5% DMSO, 200 mM each dNTP, 10 pmol of each primer (universal 16SrDNA or 12SrDNA) and one unit of Taq DNA polymerase. Cycling

conditions for the 16SrDNA included an initial denaturation at 94°C for 4 minutes, followed by 40 cycles of a denaturation at 94°C for 1 minute, an annealing at 48°C for 1 minute, and an extension at 72°C for 1 minute, followed by a final extension of 72°C for 6 minutes. For H3, the following cycling conditions were applied: one cycle at 94 °C for 3 min; 34 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; and one cycle at 72 °C for 10 min. For TMO-4C4 primers, the cycling parameters were 1 cycle of 2 min at 95 °C and 30 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1 min at 72 °C, The PCR products were electrophoresed in a 1 % agarose gel, together with a 1000 bp DNA ladder (Thermo Scientific Cat No. SM0314); purified; then processed through BigDye™ Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific) for two direction-conventional Sanger sequencing, following the manufacturer's protocol. The DNA strands (forward and reverse amplicons) were sequenced using an Applied Biosystems 3130 Automated Sequencer (Applied Biosystems, USA).

3.2.3. Analyses of sequences

3.2.3.1. Sequences' identities

The gene sequences obtained for serranid species were checked and trimmed to remove the non-informative nucleotide peaks whenever required. The edition of sequences was carried out using Sequencher version 5.4.6 (Gene Codes Corporation, Ann Arbor, MI USA) and Chromas Lite software v. 2.6.5 (Technelysium-Pty Ltd, available from the URL, *http://technelysium.com.au/*). The results were compared to the GenBank database for confirmation of species assignment, using Basic Local Alignment Search Tool (BLAST) from Altschul et al. (1990). Comparisons were restricted to highly similar sequences (megablast) only. An identity level between 98 %-100 % was considered acceptable for species identity level.

In case of having DNA barcoding low efficiency owing to the presence of mixed barcode databases identities (i.e., a barcode identity that is equal to or exceed 98 % with more than single species), confirmation steps using nuclear DNA marker genes was appended to the species in question. This was carried out by PCR amplification, sequencing of partial fragments, and sequence analyses for the three genes mentioned previously (section 2.2.1).

3.2.3.2. Phylogenetic analyses

To confirm the efficiency from using 16SrDNA as a DNA barcodes for targeted serranid species, the mitochondrial DNA sequences for the 16SrDNA for the analyzed serranid specimens were aligned using CLUSTALW algorithm integrated to Mega11 software (Tamura et al., 2021). Sequences from the same, closely related, or barcode-similar barcoded species that were available in the GenBank database were appended to this alignment. This alignment was used to construct Bayesian Inference phylogenetic trees using MrBayes 3.2.1 software (Ronquist et al. 2012). For doing this, the 16SrDNA alignment was exported as nexus files to MrBayes 3.2.1. Four Markov Chains Monte Carlo (MCMC) samples were analyzed for 10 million (10,000,000) generations, saving a tree every 1,000 generations. The analysis was stopped, for each gene, upon achieving standard error of calculations of below 0.001. The number of burn-ins was calculated using Tracer 1.7 software (Rambaut et al. 2018). Tracer 1.7 exhibited that 25 % of the saved trees are to be discarded as burn-in. These burn-ins were removed from the final tree, then the tree was visualized using the interactive tree of life platform (iTOL) (Letunic and Bork, 2019).

3.3 Results

PCR amplifications resulted in the expected amplicon sizes as mentioned in the *Materials and Methods* section. Most of them produced perfect quality chromatograms.

Trimming and alignment of the resulting chromatograms resulted in 550 bp common fragment for the 16srDNA, and 387 bp for the 12SrDNA. Both were used for subsequent barcode analyses.

3.3.1. Sequences' identities

3.3.1.1. 16srDNA Sequences' identities

Epinephelus areolatus 16SrDNA sequences shared 99.35% -99.78% identities with other *E. areolatus* samples deposited in the GenBank under the accession numbers (acc. no.), for examples, of LC127001.1 and KC593374.1. Yet, the same *E. areolatus* samples showed strikingly high identities (ID) with other *Epinephelus* species, including *E. chlorostigma* (98.7 %-98. % ID, acc. no. LC126986.1- LC126988.1) and *E. polylepis* (acc. no. KM656830.1). Below the 98 % barcoding ID, i.e., 97.13 %-97.83 % ID range, barcode similarities appeared with many other *Epinephelus* species, including *E. miliaris, E. flavocaeruleus, E. undulosus, E. fuscoguttatus,* and others. *Epinephelus chlorostigma* showed >98 % 16SrDNA sequence identity with a reference from the same species in the Genbank (acc. no. LC126988.1), but also with *E. bleekeri* (acc. no. KT835671.1). *Epinephelus coioides* showed 99.5 %-99.8% sequence identities with 16srDNA sequences with the same species, yet they also showed high identity with *E. rivulatus* and *E. malabarcius* (98-99.6 % ID, acc. no. AY947586.1, acc. no.; respectively). For *E. stoliczkae,* GenBank database lacked any 16SrDNA sequences for this species, which produced an unexpectedly high level of sequence identity, i.e. 98.03 %, with the Indo-West Pacific grouper *E. bontoides* (acc. no. KT619054). Lower *E. stoliczkae* 16SrDNA sequence identity, i.e. 97 % was found with *E. akaara, E. rivulatus,* and *E. howlandi* (acc. no. KM458971.1, KM077985.1, and KM077977.1; respectively). *Epinephelus bleekeri* 16SrDNA barcodes shared 98%-100 % identities with their counterparts of the same

species available in the GenBank database (e.g. acc. no. AY947626.1- KT835671.1). Lower level of identity (i.e. 97 %) was achieved upon comparison to *E. chlorostigma* (acc. no. LC126987.1). *Aethaloperca rogaa*, however, showed clear, unambiguous 16SrDNA identity with the same species' barcodes in the GenBank database, e.g. the ones with accession numbers KC593376.1. Similarly, the yellow-edged lyretail *V. louti* shared 100 % sequence identity with its GenBank references, such as KC593369.1 and KC593369. Finally, the unknown *Epinephelus* species that was found in the Red Sea fresh fish landings showed >98 % identity with *E. akaara, E. stictus, E. fasciatus*, and *E. anlogus.*

The hinds (genus *Cepalopholis*) also showed several inconsistencies in some species. *Cephalopholis oligosticta* showed 99 %-100 % 16SrDNA sequence identity with its GenBank references (for example, acc. no. KX298691.1), but also with *C. sonnerati* (acc. no. KX298695.1- KX298697.1). *Cephalopholis sonnerati* showed high 16SrDNA identity with its references (first report in the Red Sea), e.g. 100 % with acc. no. KX298697.1, but also high identity (> 99 %) with *C. oligosticta* (acc. no. KX298696.1). *Cephalopholis miniata* showed 100 % 16SrDNA sequence identity with its GenBank reference (KM261612.1). Yet, it also showed 98.63% identity with *C. sexmaculata* (KJ469385.1). In contrast to the previous three hind species, *C. hemistiktos* showed no 16SrDNA barcode inconsistencies, showing very specific barcode identity, being its 16SrDNA sequence exhibiting 100 % 16SrDNA sequence identity only with its GenBank reference KM656816.1.

3.3.1.2. Barcode authentication using other gene markers

Roles of 12SrDNA, TMO, and H3 in confirming species identity through barcoding results' authentication also faced the problem of inappropriate species naming in the GenBank database. Sequencing of 12SrDNA restricted *E. areolatus* barcode identity (>99

%) to either the same species (acc. no. LC650573.1) or *E. chlorostigma* (acc. no. KR872887.1). Same results could be obtained for *E. chlorostigma.* For *E. coioides,* the three applied gene markers could not provide clear barcode identity with certain species, being more than 5 different species with >98 % sequences identity as the ones applied for that species in the current study. Similar to its 16SrDNA, sequences for *E. stoliczkae* 12SrDNA, TMO and H3 were not available in GenBank, so no comparison could be carried out at the level of these genes.

For the hinds whose 16SrDNA barcodes could not provide definitive species level identity, 12SrDNA, TMO and H3 provided better sequences' identities than those found in case of groupers. For *C. sonnerati*, the three genes provided >98 % sequences' identities with GenBank references of this species (e.g. 12SrDNA: KU681001.1, TMO: EF517742.1, H3:AY949534.1). Identities and coverages for similar hinds were lower than those for *C. sonnerati.* Similarly, 12SrDNA exhibited higher sequences identities for *C. miniata* (i.e. GenBank reference acc. no. AY949400.1) over other *Cephalopholis* species. Yet, TMO and H3 genes' sequences were similar among different *Cephalopholis* species.

3.3.3. Phylogenetic analyses

The constructed BI tree (Figure 3) agreed with DNA barcoding results in most cases. Some species showed perfect clading with their GenBank references. These included *V. louti, A. rogaa, C. miniata, C. hemistictos,* and *E. bleekeri.* Other species showed good clading with their references, but presence of awkward records in the GenBank led to appearance of some references in inaccurate clades, such as the cases of *C. sonnerati, C. oligosticta, E. chlorostigma* and *E. coioides.* Absence of GenBank references led to the lading of *E. stoliczkae* with *E. bontoides.* Meanwhile, the unindtified grouper species that

was found during samples' collection showed claded with a morphologically distant species, that was *E. analogus.*

In Figure 3.3 four Markov Chains Monte Carlo (MCMC) chains were analyzed for 10 million (ngen = 10,000,000) generations. Posterior probability values are shown above the branches. Colored names refer to sampled specimens. blue labels: groupers (Genera: *Epinephelus* and *Aethaloperca*), red labels: hinds (Genus: *Cephalopholis*), and brown labels: lyretail (Genus: *Variola*), and Black labels: GenBank references.

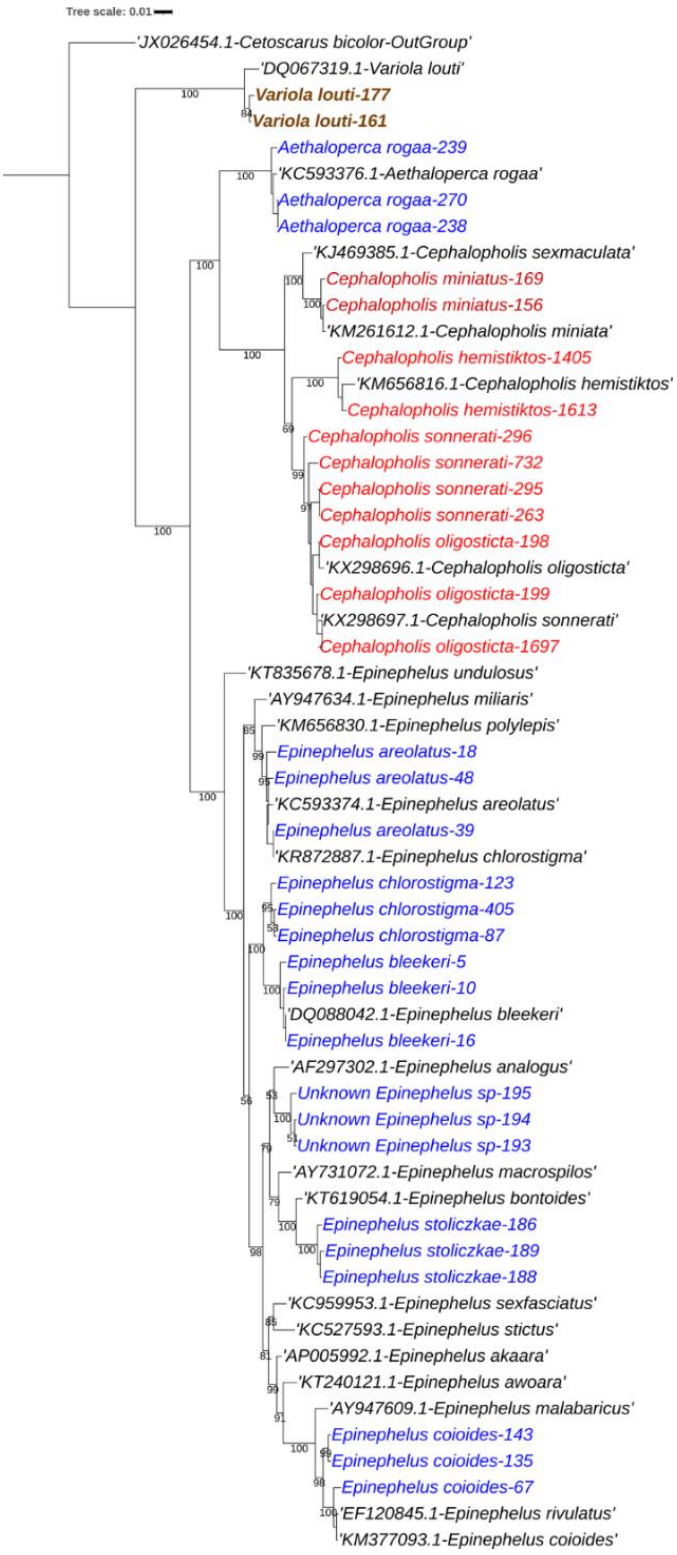

Figure 3-3. BI phylogenetic analysis for the serranid species barcoded in the current study using 16SrDNA in relation to different GenBank references.

3.4. Discussion

The roles of DNA barcoding in marine biodiversity research have been well established since Hebert et al. (2003) suggested that methodology as a taxonomic tool. It exhibited great potentials to detect species diversity, identify new and / or exoticspecies, and also reveal the presence of undeclared or unintentional species substitutions (for examples, Galal-Khallaf et al., 2019; Velkeneers et al., 2022). These issues seem to be crucial for exploration in members of the family Serranidae, owing to their great diversity, interest for fisheries, and vulnerable status of many of them. However, low interspecific variability among members of some taxonomic groups produced several problems with their barcoding using the conventional COI protocols. For this issue specifically, 16S rDNA, as well as 12S rDNA, genes were suggested as suitable alternatives within the mitochondrial genome to identify a wide range of fish and shellfish species (Fernandes et al., 2021). They were considered suitable for universal primer design because they include highly conserved regions across taxa, which are interspersed with species-specific short variable regions (Staats et al., 2016). Therefore, the current study applied 16SrDNA, aided in some cases by 12SrDNA, H3 nad TMO-4C4 as markers for serranid species discrimination.

In the current study, application of 16SrDNA sequencing exhibited several advantages. Of these, no specific genetic differences could be identified between the Arabian Gulf and the Red Sea, since all haplotypes for common species are shared between the two geographical areas. Moreover, it was shown to be an effective way to produce unambiguous species identification for *E. coioides, E. bleekeri, A. rogaa,* and *C.*

hemistiktosi, owing to the high specificity of barcoding databases' comparison results for these species.

Some confusion appeared in comparing our morphogenetically-authenticated species with their GenBank references. Some confusions were also found in other studies that used different barcodes for serranids. Of these, phylogenetic analysis using 16SrDNA and 12SrDNA detected also the close proximity between *E. areloatus* and *E. chlorostigma;* as well as between *E. coioides* and *E. malabaricus* (Craig and Hastings, 2007). Similarly, *E. areolatus* and *E. chlorosotigma* exhibited this phylogenetic proximity at the levels of COI and 12SrDNA sequences (Galal-Khallaf et al., 2019). A simple explanation for that is the presence of morphological similarities among these species (Craig et al., 2011). Some cases were reported for such confusion secondary to morphological similarities, such as in the Indian waters, and it was attributed to their morphological similarities and overlapping distributions (Darwin et al., 2020). The similarity and geographical proximity were expected since early to produce false reports about abundance of each species whereas the exact species abundance could not be accurately reported (Heemstra and Randall, 1993). Almost all GenBank 16srDNA sequences for *E. chlorostigma* that produced 98-100% identity with *E. areolatus* collected in the current study came from unpublished studies or works that were not strictly necessitating morphogenetic species identification, and mostly from the Western Pacific (for examples, the ones with the accession numbers KR872887, LC126988, KM077973, etc). Another possibility for such inconsistent identities is the existences of hybrids. Hybridization is common in serranid species, at both natural and aquaculture levels (Kiriyakit et al., 2011; Huang et al., 2016). To the best of author´s knowledge, no known hybrids between *E. areolatus* and *E. chlorostigma* have been

described. Therefore, this may support the role of misidentification of *E. areolatus* as *E. chlorostigma* as a result of their morphological similarity. Likewise, the morphological similarity in external coloration and banding patterns is a common cause for confusion between *E. coioides* and *E. malabaricus* (Rimmer and Glamuzina, 2019; Hassanien and Al-Rashada, 2021).

In spite of the application of other gene markers to aid the specific molecular identification of the target species, the applied markers did not show definitive capability to annotate the barcodes to the morphologically identified species. This, again, came from the improper depositing of references in the GenBank database. Morphological misidentification of some of the target species is possible owing to the presence of various morphological similarities. As mentioned in the third chapter of the current work, *C. miniatus* and *C. sexmaculata*, are covered with blue spots over the entire body with no darkness observed at the dorsal and caudal fins. Besides, they also have one additional dorsal fin ray; thus, it has 15 rays in total. Also, both *E. chlorostigma* and *E. areolatus* are commonly confused, owing to the brown spots covering the body and the truncate or emarginate caudal fins with the white posterior margin Heemstra and Randall, 1993; Craig et al., 2011). Similarly, confusion between *E. coioides* and *E. malabaricus* is frequent (Samoilys et al., 2018), especially due to the similar body coloration patterns (Heemstra and Randall, 1993). These confusions can cause serious losses in fisheries of those species due to the possibility of underestimating overfishing of certain species, which eventually result in fisheries collapse, especially in case of species that are considered as "Vulnerable", such as *E. coiodes* (Calosso et al., 2020).

Moreover, the current work is the first to add DNA barcodes for *E. stoliczkae* to the GenBank database. This species is common in the Saudi Arabian waters, yet it received lesser attention for DNA barcoding than other serranid species. This study is the first to provide 16SrDNA, 12SrDNA, TMO4C4 and H3 sequences for it in the GenBank database. This is of high importance for fisheries management, for one hand to provide an accurate base for proper management of species fisheries. In the other hand, to cover different morphs and stages of this species. For example, a very recent report detected the presence of new color variant of *E. stoliczkae* in the Gulf of Oman (Jawad and Al-Kharusi, 2013). Furthermore, some recent studies point to high sensitivity of *E. stoliczkae* to pollution (Jawad et al. 2018).

Interestingly, the current study identified the presence of an unusual grouper species in the Saudi waters. Works are still ongoing for this species. Data about molecular markers for this species is completely unavailable in GenBank. It exhibited direct phylogenetic proximity to a species which are not common in the area, including *E. analogus*. Yet, it could not be directly assigned to certain known species.

3.5. Conclusions

In conclusion, DNA barcoding of groupers in Saudi Arabian waters resulted in detection of various species confusion cases in the major DNA barcoding database, i.e. GenBank. These identification faults and confused reports apparently resulted from inappropriate morphological identification for species prior to their depositing in the barcoding database. Another possible region is the presence of different morphs for a single species. These misunderstood barcodes, either for inaccurate morphological identification or for presence of different morphs, can directly impact the future efforts for serranid

fisheries management. It is strongly recommended to provide more wide spectrum revision for DNA barcoding system related to serranid species, adding specifically more thorough morphogenetic analyses for this group of species.

With this research we obtained the first record of *Cephalopholis sonnerati* in the the Red Sea in Jazan which is close to Gulf of Aden. We identified both *Cephalopholis oligosticta* and *Epinephelu summana* based on morphologically and genetic investigation using 4 different gene markers 16S, 12S, TMO4, and H3. Both are endemic to the Red Sea. First study using morphologi anf genetics to confirm related. Finally, the unknown *Epinephelus* species that was found in the Red Sea fresh fish landings showed greater than 98 percent identity with *E. akaara, E. stictus, E. fasciatus,* and *E. anlogus.*

CHAPTER 4

TAXONOMIC KEY OF COMMERCIALLY IMPORTANT GROUPER SPECIES (PERCIFORMES, SERRANIDAE) IN SAUDI ARABIA

4.1. Introduction

The identification of species constitutes the first basic step for biodiversity monitoring and conservation (Dayrat, 2005). Fish species identification mainly relies on morphometric and meristic characteristics (Strauss and Bond, 1990). However, there are pitfalls in relying primarily on morphology when attempting to identify fishes during various stages of their development not considered in original treatments or when examining fragmentary, partial or processed remains. Even when intact adult specimens are available, the morphological characteristics used to discern species can be so subtle that identification is difficult even for trained taxonomists (Ward, Hanner, and Hebert, 2009).

It has been recently proposed that the use of DNA methods can circumvent such a problem (Hebert and Gregory, 2005). The reconstruction of phylogenetic relationships based on molecular data in addition to the classical methodologies has helped to resolve taxonomic uncertainties for fishes (Hanel and Sturmbauer, 2000). The rise in molecular biological techniques in marine forensic science has facilitated the development of accurate taxonomic identification of shark species by sampling biological tissue (Holmes et al., 2009; Moftah et al., 2011).

4.2. Biological Taxonomy of Marine Life in the Red Sea

A taxonomic bias has dominated much of the Red Sea's marine biological research—collecting, recording, preserving, characterizing, and naming novel species of fish and invertebrates. The Red Sea's high degree of endemicity has made it ripe for enthusiastic amateurs and devoted experts alike. However, the collaboration between amateur collectors and professional taxonomists has repeatedly blurred the line between hobbyists and specialists (Vine, 2019).

The problem is further exacerbated by the relatively recent finding of cryptic speciation (Bickford et al., 2006), phenomena in which individuals formerly ascribed to a single species have enough divergent genetic make-up to be considered separate species. The Grouper Cephalopholis hemisktos, which can be found in the Red Sea/Gulf of Aden as well as the Gulf of Oman/Arabian Gulf, is an instance of this. Because the species have been separated for over 800,000 years, disparities in pectoral fin size, pectoral fin ray count, oblique scale rows, and asymptotic size have emerged (Randall and Ben-Tuvia, 1983; Priest et al., 2016).

4.3. Types of Grouper and Their Characteristics

Aethaloperca rogaa

Aethaloperca rogaa (Red Mouth Grouper). ***(***Forsskål, 1775**,** distributed along Red Sea to southern Africa to Gilbert Island east. *Aethaloperca rogaa,* body features: Craig et al., 2011, Randell, 1983, Heemstra and Randell, 1993). Has compressed body, body depth greatly 2.1-2.4 SL, Head Length 2.5-2.7 SL, with concave at interorbital area. Max length 60cm TL Has total 9 dorsal spins and total 17-18 dorsal soft rays, 3 anal spins, 8-9 anal soft rays, 17-19 pectoral fin rays, with truncate caudal fin. Has 8-10 gill rakers on upper

limb, and 15-17 on lower limb. Colour: Dark brown body to black with whit vertical bar at middle abdomen with orange – red large mouth and reddish upper jaw part. Spawning in the any time in year and matures at 35cm SL. Habit: reef associated in costal and lagoons, in 1-10 m depth. Feeds on small fishes, stomatopods and crustaceans.

Anyperodon leucogrammicus

Anyperodon leucogrammicus (Heemstra and Randell, 1993) **(**Slender Grouper) is widely distributed grouper at Red Sea, has a body features. Has a remark elongate body and head shapes, with great depth 3.1-3.7 SL, head length 2.3-2.5 SL Max length 65 TL. Rounded caudal fin. Maxilla extend and past eye, without pone at rear end of maxilla, non-teeth palatines with canines' absence at front jaw. Has total 11 dorsal spines, total 14-16 dorsal soft rays, 3 anal spines, and 8-9 anal soft rays. Has 7-9 gill rakers on upper limb, and 14-17 on lower limp. Spawning: this species spawning as female for years then change its functionality as male in posterior spawning process, with open substrate spawners. Color: greenish to brownish grey adult with orange red spots. Spot distribution: Orange – red spots scattered at head, body, dorsal fin with basis dense on caudal fin, with clear appearance of whitish long bars or series strikes on post head and body, the juveniles have dark edge and pale grey stripes and blue edge black spot some cases two spots on the caudal fin. Habit: reef associated found in coral rich, clear water on lagoon and sea reefs. Piscivorous, feeds on fishes mainly, and crustaceans.

Cephalopholis argus

Cephalopholis argus (Peacock Hind) has one of the most diverse natural ranges of any Grouper, stretching from the Red Sea to the middle Pacific Ocean. The fish was imported in 1956 to develop a new fishery in Hawaii, which was naturally devoid of

Groupers. By spearfishing on SCUBA in Hawaii in July 2003, a total of 285 *C. argus* specimens were collected from 17 sites. In Dierking and Meyer (2009) speared fish were quickly sealed in plastic bags upon the catch, following the method of DeMartini et al. (1996) for estimating regurgitation (i.e. underwater). Regurgitated objects were gathered from the surrounding water and placed in the specimen bag in rare occasions where regurgitation occurred before closing. When the specimens were returned to the lab, they were examined for signs of regurgitation (i.e. prey found in the mouth cavity or between the gill rakers, or completely expelled from the mouth cavity and found in the bags). On a scale of external characteristics, the M of prey items was recorded to the nearest milligram, and the degree of digestion was classed as "Little," "Medium," or "Strong" (not shown). In moreover, the standard length (SL) and mass (M) of *C. argus* specimens were determined to the nearest mm and 5 g, respectively.

C. argus body is clearly very deep, and it has a common body length of 40 cm TL. The body depth ranges from 2.7–3.3 times the SL, and the head length is 2.4–2.7 times the SL (Heemstra and Randall, 1993). The maximum length is 60 cm (“Coastal fishes” 57), and it has small eyes. It has a concavity in the interorbital area, 9–11 upper limb gill rakers, and 17–19 lower limb gill rakers. It has a total of 9 dorsal spines, 15–17 dorsal soft rays, 3 anal spines, 9 anal soft rays, 16–18 pectoral fin rays, and a rounded caudal fin. It has daily courtship behavior from afternoon to sunset and repeated single male to multiple female mating groups, and the mating is paired and pelagic (Donaldson 364). It is distinguished by a black-edged blue covering the dark brown body, a large pale bar on the chest compared with a small pale bar on the posterior part with a narrow white edge on the rear of the median fins with orange-gold on the rectangular dorsal fin ends. This varies, but it is

commonly associated with coral reef habitats of 40 m in depth. It Feeds on fishes and crustaceans mainly in the dark (night); thus, it is called a crepuscular feeder. It has also been observed feeding in the early morning or evening.

C. argus is sometimes misidentified as *C. cyanostigma* as they share similar color characteristics. The latter is differentiated from *C. argus* by 8 anal-fin rays in addition to the reduction of gill rakers in the upper and lower limbs (7–9 vs. 14–18, respectively).

Cephalopholis hemistiktos

Cephalopholis. hemistiktos (Yellowfin Hind) is endemic to the sea border of the Arabian Peninsula (Randall and Ben-tuvia, 1983: 380). However, it is categorized as a near-threatened species due to the lack of management of fishery behaviors. *C. Hemistiktos* body features. include a body depth equal to 2.7–3.0 times standard depth, with 2.4–2.6 times the standard head length (Craig et al., 2011: 383; Heemstra and Randall, 1993; Randall, 1983). It has a flat interorbital area and rounded preopercle. The maxilla reaches past the vertical line of the rear edge of the eye in some cases. It also has 6–8 gill rakers on the upper limb and 13–15 on the lower limb. It has a total of 9 dorsal spines, 8–10 dorsal soft rays, 3 anal spines, 8–10 anal soft rays, and 16–18 pectoral fin rays. The base body color is brownish to brownish-red to reddish as the depth increases. There are ocelli on the head, a dark blue edge with a darker color than the body on the caudal fin, a rear dorsal fin, anal fins in addition to blue ocelli and a line, and an orange dorsal spine fin, while the pectoral fins are brownish to reddish with small blue ground ocelli bordered with yellow. It lives at a depth of 4-55 m. It is a monogamous species, where each pair occupies 62 m2 of territory. It feeds on fishes, mostly pomacentrids and crustaceans (ambush predator), eating throughout the day. It is closely related to *C. miniata* and *C. sexmaculata*, which are

covered with blue spots over the entire body with no darkness observed at the dorsal and caudal fins. They also have one additional dorsal fin ray; thus, it has 15 rays in total.

C. hemistiktos (Serranindae; Ruppell, 1830), an economically important fishing species limited to the Arabian Peninsula and Pakistan's coast (Hashim, 1993; Gladstone, 2002). *C. hemistiktos* has a limited range and is not found in the western Arabian Sea (southern Oman) (Craig et al., 2011). Furthermore, previous researchers have mentioned that these two populations differ morphologically in terms of pectoral fin size, pectoral fin ray count, oblique scale rows, and asymptotic size (Randall and Ben-Tuvia, 1983; Appendix S1 in Supporting Information); these discrepancies suggest isolation on evolutionary time scales. They conducted genetic and age-based demographic evaluations of *C. hemistiktos* over most of the species' range due to the species' spatial spread and physical variances across places. To assess gene flow, dispersal barriers, and connection among populations, they employed one mitochondrial and one nuclear genetic marker, and also otolith-based age estimations to look for differences in life-history characteristics (Priest et al., 2016).

Priest et al. (2016) collected *Cephalopholis hemistiktos* from 10 different locations, covering the majority of the species distribution. Individuals were collected utilizing hand spears while snorkeling or SCUBA diving in the Gulf of Aqaba, Red Sea, Gulf of Aden, and the Arabian Sea from 2005 to 2014, or from local fish markets (the Gulf of Oman and Arabian Gulf). Individuals from the entire size range available at each location were sampled. To the nearest millimeter, total length (TL) measurements were taken. Extracting sagittal otoliths, cleaning them in ethanol, and storing them dry till sectioning was done. Tissue samples for genetic analysis were kept at room temperature and preserved in 70%

ethanol or a saturated salt-dimethyl sulfoxide buffer (Seutin et al., 1991). They did not gather both DNA and otolith samples from every site since samples were collected across many years for several research initiatives. As a result, some sites only provide genetic and otolith information.

Cephalopholis miniata

Cephalopholis miniata (Coral Hind) as previously mentioned, the *C. miniata* is closely related to *C. hemistikos*. Based on this, some could argue about *C. miniata* existence in the Arabian Gulf, but it does not (Heemstra and Randall, 1993; Craig et al., 2011; Randall, 1983). It has a body depth equivalent to 2.6-3 time of the standard Length, with head length 2.4-2.6 times of head standard length, the maximum total length is 50cm. Has rounded caudal fin. It has a slight concave appearrance in the interorbital area, the maxilla exceeds rear of the orbit. It has 9 dorsal spins, with 14-15 dorsal soft rays, 3 anal spines, 8-9 Anal soft rays and 17-18 pectoral fin rays. It has a 7-9 gill rakers on the upper limb, while the lower has 14-16 rakers. its color is orange -red range from dark to light degree with darkish in some parts, with pale blue –grey spots, while the juveniles' color is yellow with faint pale blue spots. the posterior parts are darker than the rest of body, the spot were narrow and smaller than the pupil, but the spot appears in scatter pattern in the juveniles, with distinguished blue margin occurred on the soft dorsal, caudal and anal fins parts. *C. miniata* female matures on 25 cm of total length, it occurs in haremic groups with prevalent males patrol certain territories occupied with 2-12 females with sub internal territories defined for each single female. it dwells in the reef associated with high dominance in the exposed reefs areas such as shallow knolls. Having two activity period during the morning

and afternoon (Shpigel and Fishelson, 1989). Fishes and crustaceans considered main food for the *C. miniata,* but it shows inclination for *Anthias squamipinnis* and *Pseudanthias*.

Cephalopholis oligosticta

Cephalopholis oligosticta (Vermilion Hind) as previously mentioned, this species is only found and distributed in the Red Sea. It is an endemic species to the Red Sea and near-threatened by fisheries, as even though it is too small to be of major interest, it is caught accidentally by fisheries (Choat et al., 2008). However, the literature on *C. oligosticta* research is poor compared with that of other species. *C. oligosticta* body length is equivalent to 2.6–3.0 times the SL (16–22 cm) and 2.4–2.6 times the standard head length, the depth of body is 2.6–3.0 times the SL, and the width depth is equal to 19 times the SL (equal orbit diameter). Females are 17–19 cm long, and the mature male length is 22 cm, with a maximum length of 30 cm. It has a slight concaveness in the interorbital area. It also has a total of 9 dorsal spines, 14–15 dorsal soft rays, 3 anal spines, 9 anal soft rays, and 16–18 pectoral fin rays. It The maxilla extends past the eye. Contains 7–8 gill rakers on the upper limb and 14–15 on the lower limb. It has a Orange-red Color. It has a pale blue spots widely distributed on the whole body, fins, and head, while they become closer and narrower pale spots on the soft dorsal and caudal fins. it has a reef-associated habitat commonly found at a nearly shallow depth between 20–50 m from surface, where juveniles are commonly found in coral rubble areas. Closely related to *C. miniata*, which has more-dense blue spots than *C. oligosticta*, with shorter pelvic fins and different habitats (Heemstra and Randall 1993).

Cephalopholis sexmaculata

Cephalopholis sexmaculata (Six-Spot Grouper or Sixblotch Hind) one of the sympatric grouper in the Red Sea, which named *Serranus zanana* but the International Commission on Zoological Nomenclature refused to use this name as a specie name, favoring the widely used *C. sexmaculata*. *C. sexmaculata* has the following body features: (Heemstra and Randall 1993, Craig et al., 2011; Randall 1983). It has a 2.5 -3 of standard length body depth, with 2.3-2.5 standard head length, maximum total length is 50cm. it has a slight concave on the flat interorbital area, with distinguished concave above the eye. It has 9 dorsal spines, 14-16 dorsal soft rays, 3 ananl spines, 9 anal soft rays, and 16-18 pectoral fin rays, with rounded caudal fin. It has 7-9 gill rakers in the upper limb, and 14-16 rakers on the lower. It has a very brown color degree range in brownish, brownish red, and reddish body base associated the deep in the water, and has asmall blue ocelli. It has six quadrangular blotches, four observed in the dorsal fin base and other extended to fin, the spaces between these six blotches filled with very pale bars, also, there are pale blue lines radiating from the eye, with more-dense small elongated blue ocelli in the head compared with the lower part of body. matures on the 24.91 cm length, Habitat *C. sexmaculata* dwells on the deep reef wall or caves, at depth exceeding 30m, it has two activity cycles, nocturnal activities exposed in shallow water, and diurnally active in deep water (Shipgel and Fishelson, 1989). *C. sexmaculata* feeds dominantly in fishes. It has confused with both *C. miniata* and *C. hemistiktos*, but the *C. sexmaculata* distinguished from *C. hemistiktos* in colour, where the last does not have small blue spots on the dorsal part of body, further, the *C. hemistiktos* has darker part occurred in the anal fins and the

dorsal, where *C. sexmaculata* isn't darker on these parts. However, *C. sexmaculata* differ with their black blotches on the dorsal fin base which not exists in the *C. miniata*.

Cephalopholis sonnerati

Cephalopholis sonnerati (Tomato Hind) has a distinguished body depth equal to the head length and the concave of dorsal head in the adults as described in the body features below. (Heemstra and Randall, 1993; Craig et al., 2011). It has body depth of 2.3-2.8 times of standard length, with head length equivalent to 2.5-2.7 of standard length, slight straight to concave dorsal head in the adults, maximum length is 75 cm total length. slight to concave interorbital area with rounded preopercle, also pelvic fins reaching further the anus, and the caudal fin is rounded. It has 9 dorsal fins, 14-16 dorsal soft rays, 3 anal spins, 9 anal soft rays and 18-20 pectoral fin rays. *C. sonnerati* has 7-9 gill rakers on the upper limb, and 14-16 rakers in the lower front is orange red to reddish brown body base with whitish blotches, where head color is purplish to reddish with orange- red spots. The second pattern recognized with light reddish to yellowish brown body base, with brownish red spots on the head, body and fins, first pattern mentioned has a dense network of purple on head, maxilla and lips, in addition to whitish or purple spots scattered on the body, and orange distally in pectoral fins, with blackish tips occurred in the tips of dorsal, anal, pelvic and caudal fins. In the second pattern mentioned the spots were small brownish red to dark brown, with whitish projection in the rear part of caudal fins and pectoral fins. spawns in particular prolong seasons in the open water where the substratum egg scatters, fertilization occurs external, has a protogyny mode where female matures at 28 cm of standard length, while male matures at 34cm standard length. dwells on the lagoon reef and steep outer reef,

juvenile dominant sponges and coral heads, while adults dwell in moderate depth in range of 30-100 m, and feeds on the crustaceans and small fishes

Dermatolepis striolata

Dermatolepis striolata (Smooth Grouper). Considered rare, this species has body depth equal to 2.4-2.6 of the standard length, head length 7.2-7.8 of standard length, the eye diameter less than snout. It has 11 dorsal spins, 18- 19 dorsal soft rays, 3 anal spins and 9 -10 anal soft rays and 17-19 pectoral fins, and 5-7 gill rakers on the upper limb, and 13-16 rakers on the lower limb.

Yellowish to reddish brown base body with small round dark spots and pale blotches, with a small elongated dark brown spot distributed over the whole body and head, in horizontal elongation thus its poses short lines, the blotches were irregular pale black distinct in the head. Fertilization occurs external, has a protogyny mode. It found in the shallow water, turbid coastal rocky and coral reef, and feeds on fishes predominantly (Heemstra and Randall, 1993; Craig et al., 2011).

Epinephelus areolatus

Epinephelus areolatus (Areolae Grouper) has a very wide distribution in the Red Sea and Arabian Gulf, but is threatened by overfishing. It has a body depth equivalent to 2.8–3.3 times the SL with a head length equal to 2.4–2.8 times the SL with concaveness at the interorbital area. With a total of 11 dorsal spines, 15–17 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 17–19 pectoral fin rays, 8–10 gill rakers on the upper limb and 14–16 on the lower limb. The maxilla reaches past the vertical line of the center of the eye.

It has pale head, body, and fins with close, dense, and large brown or brownish-yellow or greenish-yellow spots with pale pectoral fins and a white margin at the caudal

fins. It has a one-male-to-multiple-female spawning manner in a respective ratio of 1:6 in a restricted period and builds aggregation with pelagic eggs. Maturity occurs at female length equal to 19.5 *cm* TL and at 29 *cm* TL for males. It is reef-associated and frequently found in seagrass beds or in the upper part of fine sediment and around rocky reefs at a depth of 2–200m, and feeds on fishes, prawns, and crabs as primary benthic invertebrates. Often confused with *E. chlorostigma*, as mentioned previously (Craig et al., 2011).

Epinephelus bleekeri

Epinephelus bleekeri (Dusky Tail Grouper) recognized with the blackish to bluish lower half part of the caudal fin, also it has replacement name, *Serranus Coromandelicus*. It has elongate body with depth equal to 3 -3.5 times of standard length, and 2.4-2.7 times of standard head length, maximum length 76 cm, 11 dorsal spines, 16-18 dorsal soft rays, 3 anal spines, 8-9 anal soft rays, 17-19 pectoral fins rays, rounded truncate caudal fin, also the pelvic fins were shorts.

It has a slight concave in the interorbital area, and has a greyish brown body base with dark reddish brown to black spots, where the fins are darker than body. In addition to narrow pale yellow or white margins occurred in the anal fins. the dark reddish brown spots are well –distributed over the body, the spots are smaller than the pupil and elongate horizontally, also the small dark spots projected on the median fins. It matures at 36 cm total length, and found at 30-104 m water depths, in the shallow rocky banks (Heemstra and Randall, 1993; Craig et al., 2011).

Epinephelus chlorostigma

Epinephelus chlorostigma (Brown Spotted Grouper) has a body depth equal to 2.8-3.3 of standard length, and 2.4-2.7 head length on standard, with a slight concave on the

interorbital area. It has 11 dorsal spines, 16-18 dorsal soft rays, 3 anal spines, 8 anal soft rays, 17-19 pectoral fin rays. Whitish body base colour with dark brown spots. The spots are small and scattered over all body and head in irregular close-set network form. White line is projected on the posterior margin of caudal fin. Spawning period varies and prolonged among this species, show a protogynous mode, where female matures at 25 cm total length, at 34 cm to 56 cm the sexual changes occurs, but not all female experienced sex changes. Fertilization are external in the aggregation form of matures. It dwells in the large different areas at depth 280m, such as sea grasses beds, outer reef slopes. It main food is fishes and invertebrates (Heemstra and Randall, 1993; Craig et al., 2011; Randall, 1983).

Epinephelus coeruleopunctatus

Epinephelus coeruleopunctatus (White Spotted Grouper) characterized by their color and has a body depth contained 2.9-3.4 times of standard length, head length equal 2.3-2.5 times of standard length. Maximum length 76 *cm* total length, 11 dorsal spines, 15-17 dorsal soft rays, 3 anal spines, 8 anal soft rays and 17-19 pectoral fin rays.

The adults body base is brownish grey with pale spots and blotches, while juveniles has a dark grey to black body base with white spots and dots. the adults' body has distributed small white pale spots and large white pale blotches, also there are five black blotches in the base of dorsal fins, while the juveniles covered with small white spots and dots.

It is mature at 42 cm of total length, has a protogyny mode and fertilization occurs externally. Dwells near to the coral reefs caves, rocky coral rich areas and outer reef slopes, juveniles found in tidepools, and feeds on fish and crustaceans (Heemstra and Randall, 1993).

E. coeruleopunctatus misdefined with the other three white spotted grouper *E. ongus, E. summana, and E. corallicola* where these has similar pattern of colour and spots, but it can be defined based on the dorsal fins and pectoral fins of each of them, where *E. summana* fewer dorsal fins and pectoral fins, also *E. ongus* has fewer pectoral fins in addition to the distinguished convex of operculum and a blackish brown margin in the white edge of anal fins. The most identical of morphometric features is between both *E. coeruleopunctatus* and *E. corallicola*, but its differant in the color pattern where the last does not have white spots.

Epinephelus coioides

Epinephelus coioides (Orange-Spotted Grouper) is considered a main and common species target in aquaculture and has high value in fish markets. It is widely distributed in marine areas (McIlwain et al. 2016). *E. coioides* has an elongated body with a length equivalent to 2.9–3.7 times the SL, which is equal to 10–78 *cm*, a head length equal to 2.3–2.6 times the SL, and a maximum length of 120 *cm*. The mature female TL is 25–30 *cm*, with a flat interorbital area with a slightly convex shape.

The maxilla passes or approaches the vertical line at the eye rear edge, occupying 4.2–5.5% of the SL, with a lower eye parameter with respect to the head length, which is 7 times the SL. The lower midlateral jaw contains 2–3 subequal teeth rows. It has a total of 11 dorsal spines, 14–16 dorsal soft rays, 3 anal spines, 8 anal soft rays, 18–20 pectoral fin rays, and a rounded caudal fin. It spawns in aggregation regions in a specific period (probably from March to June). The successful and surviving larvae need 30°C water temperature conditions.

It has a tan color on the dorsal part of the head and body, with a white shade in the ventral region and small scattered orange or reddish-brown spots distributed on the body, head, and fins in the middle, with two dark spots on the interopercle in addition to two junctions in the sub- and interopercles. It also has five unique, random, ventrally fork slanting and pale dark rods; the first rod is located in the lower region of the dorsal fin spines, and the far rod is located on the caudal peduncle. Meanwhile, note that the orange spots convert to brown in air-exposed conditions. It is reef-associated and found in shore and coastal regions at a depth of 100m. Juveniles are commonly found in shallow water areas. It feeds on small fishes, shrimps, and crabs Heemstra and Randall, 1993; McIlwain et al., 2016).

Epinephelus epistictus

Epinephelus epistictus (Dotted Grouper) has body depth 3.0-3.3 times standard length, and the head length is 2.2-2.25 cm standard length, maximum total length is 80 *cm*, and had a slight concave on the interorbital area and head part, with dorsal spines, 14-15 dorsal soft rays, 3anal spines, 8 anal soft rays, 17-19 pectoral fin rays, 7-10 gill rakers in the upper limb, and 15-19 on the lower limb.

It has a pale brownish to greenish body base, with brownish black spots, also has a second pattern where the colour body base is brown to olive with brownish black spots. The spots were small, scattered in the dorsolateral part, and disappeared in the posterior part of head and median fins, also, has brownish pectoral fin rays, has three faint dark brown band radiant from the eye and extend to the operculum end, where the juveniles have dark spots on the head and body perform three longitudinal rows.

It spawning has protogyny, the fertilization occurrs external, and dwells in the deep water range from 71 *m* to 200 *m*, in the continental shelf and associated with coral reefs and rocky bottoms (Heemstra and Randall, 1993; Craig et al., 2011).

Epinephelus fasciatus

Epinephelus fasciatus (Blacktip Grouper), *Epinephelus fasciatus* (Forsskal, 1775) is one of the most abundant species in the Indo-Pacific and one of the two most extensively dispersed grouper species on the planet (Heemstra, 1993). In the Red Sea, it is fairly prevalent (Randall, 1983; Randall and Ben-Tuvia, 1983). *Epinephelus fasciatus* is a coral reef and rocky bottom species that can be found at depths of up to 160 meters but is most commonly found between 20 and 45 meters (Heemstra, 1993). It feeds on fish, decapods, stomatopods, and cephalopods and is active both during the day and at night (Harmelin-Vivien and Bouchon, 1976; Randall and Ben-Tuvia, 1983). It can grow up to 40 cm long and is sometimes seen in tiny groups on the bottom (Heemstra, 1993; Taquet and Diringer, 2007). On the 1st of February 2011, a single blacktip grouper was taken off the coast of Lebanon, north of Tripoli (34828′ N 35852′ E). The specimen was caught in a trammel net deployed at a depth of 20–25 meters over a soft, rocky bottom. The fisherman snapped the fish just after it was caught, and it was later sold mixed with other species. The identification of the common Indo Pacific E. fasciatus was made possible by the characteristic traits evident on the images and unique to the species. The species' distinctive traits include the pale yellowish-red body with orange-red bars, a series of prominent black triangles behind the tip of the dorsal fin spines, and a dark reddish-brown dorsal region of the head and neck. The previous studies contain a detailed description of the species

(Randall and Ben-Tuvia, 1983; Heemstra, 1993). Based on the photos, the specimen's total length is estimated to be around 22 cm (Bariche and Heemstra, 2012).

Epinephelus fasciatus

Epinephelus fasciatus (Black Trip Grouper) has a distinguished incised interspinous of dorsal fins as described and has body depth equivalent to 2.8-3.3 times of standard length, with 2.3-2.6 times head length, maximum total length 40 cm. it has a flat interorbital area, with convex in the head. It Caudal fin is moderately rounded to truncate shape. It has a body color base ranging from greenish grey, to pale reddish yellow to scarlet, with varied dense dark bars, the median part of body is pale where the rear part is darker, the dorsal area if head and upper jaw has a darker reddish or reddish brown color, while the other parts are pale orange. There is no spots appears in such species, but have a distinguish black triangle projected on the incised interspinous of dorsal fin, with 5-6 conspicuous dark bar.

It has also 11 dorsal fin spines, 15-17 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 18-20 pectoral fin rays. It is performed hermaphroditism at juvenile phase, and in the older stage it deprived of female functions, and performed male function only, matures at 24 cm of total length, and Fertilization occurred externally.

It’s a reef associated fish, commonly found in the outer reef slopes in 15-160 *m* water depth. While, in the protected bays and lagoons it found at 4 m depth, and feeds on brachyuran, crabs, stomatopods, fishes, ophiuroids, and octopus, feeds predominantly on fishes and some crustaceans (mainly crabs) (Randall, 1983; Heemstra and Randall, 1993; Craig et al., 2011).

Epinephelus fuscoguttatus

Epinephelus fuscoguttatus (Brown Marbled Grouper) has a body depth equvilant to 2.6-2.9 times of standard length with head length equal 2.3-2.5 times of standard length, maximum total length is 120 *cm*. it has slight convex on the interorbital area; also has a convex point on the dorsal part of head extended to the posterior part of dorsal fins. It has 11 dorsal spines, 14-15 dorsal soft rays, 3 anal spines, 8 anal soft rays and 18-20 pectoral fin rays. The 3rd and 4th dorsal spines are the longest compared with dorsal spines and shorter than the longest dorsal fin rays. However, it has a incised interspinouse membranes. With 10-12 gill rakers on the upper limb, and 17-21 on the lower limb.

It has a pale yellowish brown body base, with dark brown blotches, brown spots, and darks bar at side of the jaw, with the small dark brown spots distributed in close set irregular form over the 5 irregular bars performed by dark brown blotches, and 2-3 faint bar on the jaw. spawning season starts from November to January; form large spawning aggregation, exhibits protogyny hermaphrodite, where female changes sex at 68 cm total length, with external fertilization. Dwell in the shallow coral reefs and rocky bottom at 60m depth, some juveniles found in seagrasses, and Feeds on fishes, crabs, and cephalopods (Randall 1983, Heemstra and Randall, 1993; Craig et al., 2011).

E. fuscoguttatus confused with related *E. polyphekadion* species, the difference between both species based on the pectoral fin rays and gill rakers, where the last has fewer pectoral fins and gill rakers, also, the last has less incised of interspinous dorsal fin.

Epinephelus geoffroyi

Epinephelus geoffroyi (Red Sea Spotted Grouper): Endemic to the Red Sea and Gulf of Aden, recently resurrected. *E. geoffroyi* has an elongated body 2.9 times the SL

depth, head length 2.7 times the SL, and small eyes. The lower jaw is projected, and the maxilla extends to the central vertical eye line. It has various anal fin shapes, which can be pointed or round, with 8 gill rakers on the upper limb and 17 on the lower limb, and a total of 11 dorsal fins, 17 dorsal rays, 3 anal spines, 8 anal soft rays, and 17 pectoral fin rays.

It is color beige over the whole body with large, dense, dark brown spots. The spots are scattered over the whole body in a close-set manner, with pale spots in the lower part that are nearly orange in color and one single dark spotted bar at the caudal fins. Substrates in rocky and coral habitats at a depth of 12 *m* (Randall et al. 1971; Golani et al., 2010: 143)

Misidentified as *E. chlorostigma* or *E. areolatus*. Regarding the similarity with *E. areolatus*, the latter has larger spots than *E. geoffroyi*. However, the confusion with *E. chlorostigma* occurs based on the larger gill raker on the *E. geoffroyi*, wide spot on the belly, unique vertical bar of dark spots on the caudal fin, and absence of a clear white margin on the caudal fin.

Epinephelus lanceolatus

Epinephelus Lanceolatus (Giant Grouper) has body depth contained 2.4-3.4 times in standard length, and head length 2.20-2.70 times of standard length, slight convex on the flat interorbital area, head is convex at the dorsal. Maximum total length is 270 *cm*. it has 11 dorsal spines, 14-16 dorsal soft rays, 3 anal spines, 8 anal soft rays, 18-20 pectoral fin rays, and 8-10 giller rakers in the upper limb and 14-17 on the lower limb for juveniles. Its color changes based on the age, the base body color of juveniles is yellow with black bar, while the body color of adults is yellow to greenish to dark brownish with yellow, white, black spots.

The juveniles are characterized by three irregular wide bars; the first bar extends from spinous dorsal fin to the belly, until reach head, the second bar extends from base of soft dorsal rays to the anal fin, and the third bar is projected on the base of the caudal fin. However, the spots on an adult body are distributed irregularly, the yellow and white spots distributed over the darker part of body, while the black spots occur on the fins. The species matures at 129 cm, exhibits protogyny mode, not known if it from spawning aggregation, but potentially. It inhabits solitary, juveniles were secretive, but the adults commonly found in the coral reefs area, shallow water, estuaries and in the caves. It has various foods such as spiny lobsters, fishes, small sharks, batoids, and juvenile turtles and crustaceans (Heemstra and Randall, 1993; Craig et al., 2011).

Epinephelus latifasciatus

Epinephelus latifasciatus (Striped Grouper) has body depth 2.9-3.4 times of standard length, and head length of 2.3-2.6 times of standard length, maximum total length 157 *cm*. it has convex interorbital area, and dorsal head. It has 11 dorsal spines, 14-16 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 17-19 pectoral fin rays. However, the interspinous dorsal incised sharply. It has 8-11 gill rakers on the upper limb, and 15-18 rakers on the lower. lavender –grey or pale brownish, where juveniles has whitish shades at median, with 2 black longitude edge bar, white bars and black spots, adults don't have white bars, just dark edges. The black bars on juveniles start from the eye and extend edgy to upper dorsal fin rays, and lower to the caudal fins, also, black spots and streaks distributed on caudal and dorsal fin. As adults, the dark edges were breaking into dashes and spots. It matures at 86 *cm* total length, exhibit protogyny mode, also fertilization occurs externally. Is found at depths of 20 to 230 *m* in water, prefers continental areas, and bottoms

of low relief. Large found on coarse sand or rocky locations, but the juvenile individuals found on the silty sand or muddy bottom (Heemstra and Randall, 1993; Craig et al., 2011).

Epinephelus malabaricus

Epinephelus malabaricus (Malabar Grouper): Known to exist in the Red Sea, but not in the Arabian Gulf. It is threatened by adult and juvenile fishing behaviors (Choat et al., 2008). *E. malabaricus* has an elongated body, with a body depth equal to 3.0–3.7 times the SL and 2.3–2.6 times standard head length. It Slightly concave at the flat interorbital area and has a maximum length of 234 cm TL. It has a total of 11 dorsal spines, 14–16 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 18–20 pectoral fin rays. It has 8–11 gill rakers on the upper limb and 14–18 rakers on the lower limb.

It has a brownish body and head with blackish-brown spots, irregular white spots and blotches, and dark brown bars. With the Dark brown oblique bars, with small, well-separated blackish-brown spots scattered on the body (even the lower part and mouth roof) and small black spots on the fins, with white spots and blotches on the head and body. It matures at 64 *cm* SL. Sex reversal is likely to occur after 10 years of age or between 97 and 113 cm TL. The spawning period is from September to February (Gaspare and Bryceson 2013). It Enjoys a variety of habitats, such as coral or rocky reefs, tide pools, estuaries, mangrove swamps, and sand or mud, from the shore to 150 *m* in depth. Feeds equally on fishes and crustaceans and rarely on octopuses (Heemstra and Randall, 1993; Craig et al., 2011).

Epinephelus marginatus

Epinephelus marginatus (Dusky Grouper) has a body depth of 2.6-3.1times standard length, and head length is 2.3- 2.5 standard length. Maximum total length 143 *cm*.

It has convex interorbital area. It has 11 dorsal spines, 14-16 dorsal fin rays, 3 anal spines, 8-9 anal soft rays, and 17-19 pectoral fin rays. It has 7-10 gill rakers on the upper limb, and 14-16 rakers on the lower. It has dark reddish brown body base, with yellowish projection ventrally and greyish dorsally, distributed white, pale greenish yellow or silvery grey blotches. It has a blotches perform vertical series. exhibits protogynous hermaphrodite forms spawning aggregation, spawning occurs on December, where females mature at 45 *cm*, sexual changes occur after lengths of maturity exceed ten years. It juveniles prefer shore in tidal rocky pool in brackish environments, adults prefer rocky bottom, lives solitary and territorial. It Feeds on fishes and invertebrates (Craig et al., 2011).

Epinephelus microdon

Epinephelus microdon **(**Small-Mouth Grouper); this name of grouper is the oldest name for the *E. polyphekadion* or the synonym name, even the name of *E. micodon* was rejected and replaced with *E. polyphekadion* but a lot of previous studies and research occasionally utilized the name of *microdon* for this type of Grouper, especially in those studies which concern the sexuality cultures of species. Furthermore, *E. microdon* is the replacement name of the oldest name *Epinephelus dispar* (Playfair), that was used by Morgans (Randall, 1964).

E. microdon considers a threatened species. It has a body depth of 2.7-3.2 of standard length and head length of 2.4-2.5 of standard length. It has a flat interorbital area, with slight convex in the head dorsal. Maximum total length 490 mm and has rounded caudal fin, and 11 dorsal spines, 14-15 dorsal soft rays, 3 anal spines, 7-9 anal soft rays (commonly 8 rays) and 16-17 pectoral rays. It has 14 -18 gill rakers in the lower limp and 8-10 on the upper limp. It has a brownish body bases, with dark brown spots, and dark

blotches, with small dark spots but much larger than pupil eyes, covers the whole body, with dark blotches such as the one at the caudal peduncle. its protogynous hermaphrodite species, the spawn season represents in two to three month per year, the sex inversion occurs at the resting period after spawning (Brusle'-Sicard et al., 1992; Rhodes et al., 2011) and feeds on fishes and crustaceans.

E. microdon is related to *the E. fuscoguttatus*, the differences between both can be observed based colour key, where the spots on the *E. fuscoguttatus* is less regular in outline compared with those *in the E. microdon*, also the last mentioned has only one dark blotch projected on the base of fifth dorsal spines, while the *E. fuscoguttatus* has two closet blotches or two dark lobes.

Moreover, the difference between these two species could be observed based on several distinguished keys such as: body depth key where *microdon* is more slender compared to the *fuscoguttatus*, Dorsal rays key where the dorsal rays in *microdon* usually 15 rays while it is 14 rays in *fuscoguttatus*, and gill rakers key where *fuscoguttatus* has more gill rakers than *microdon* (Randall, 1964; Morgans, 1982; Heemestra and Randall, 1984).

Epinephelus morrhua

Epinephelus Morrhua (Comet Grouper) has body depth 2.8-3.1 times in standard length, and head length 2.3-2.5 times in standard length. Maximum total length is 100 *cm*. it has moderate convex interorbital area, with slight convex on head dorsal. It has 11 dorsal spines, 14-15 dorsal soft rays, 3 anal spins, 7 -8 anal soft rays, and 17-18 pectoral fin rays. It has 8-10 gill rakers on the upper limb, 15-18 rakers on the lower limb. It has a tan body base with brawn bands and blotches. It has a brown bars radiant bifurcately from the edge

of eye, the upper one extend to the brown blotches on the posterior part of dorsal spins, the lower band forked in other sub-bars curving to the upper 3rd to 7th, and last 4 dorsal fin rays, also to 5th to 9th dorsal spines, last band extend breaking in blotches curving to caudal fin. It exhibits protogyny. It found deeply at 80-370 *m* in water, on the sea mounts, and continental shelves, and Feeds on benthic fishes and large invertebrates (Craig et al., 2011; Heemstra and Randall, 1993).

Epinephelus multinotatus

Epinephelus multinotatus (Whiteblotches Grouper) has body depth equal to 2.6-2.9 of standard length, and the head length reaches 2.4-2.7 of standard length, maximum total length is 100 *cm* and has a convex interorbital area with 11 dorsal spines, 15-17 dorsal soft rays, 3 anal spines, 8 anal soft rays and 18-20 pectoral fin rays, and 9-11 gill rakers on the upper limb, and 15-17 on the lower limb. It has an olive to dark purplish gray body base of adults, while the juveniles have a dark greyish blue body base with yellow part cover the rear edge of caudal fin, peduncle, soft dorsal fin, and anal fin, with pale whit spot and blotches. The spots distributed irregularly on the body and head, these spots and blotches were better developed as gets larger, corresponding with loss of yellow coloration. It matures at 41 – 50 cm of total length, and is protogynous hermaphrodite. However, it is spawning aggregately over the entire year but the high activity season represent from August to October. Commonly found in rocky reef region at depth of 110 m, while juveniles found in inshore coral reef. Feeds on the small fishes and crabs the juveniles imitate the herbivorous damsel fish approaching to their unsuspecting prey (Craig et al., 2011; Heemstra and Randall, 1993).

Epinephelus poecilonotus

Epinephelus poecilonotus (Dot – Dash Grouper) has body depth equivalent to 2.6-3.1 times of the standard length, and head length of 2.3-2.5 of standard length, maximum total length is 65 *cm*. it has a slight concave on the interorbital area and 11 dorsal spines, 14-15 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 17-19 pectoral fin rays. With 8-10 gill rakers on the upper limb, and 15-18 rakers on the lower. the juveniles have faint yellowish grey with oval black blotches, and pale white, brown and brown black semicircular bands, the rear phase of juveniles has series black spots breaking from the blotches and dark brown bands, while the black spots in the adults disappear completely, and the bands' color becomes more faint.

The juveniles have three bands described as: first bands its dark brown starts from the nape and divided into upper brand curving dorsally and extended broadly over the basal half of the dorsal fin between the 9^{th} spine and 4^{th} dorsal soft rays, while the lower extend to last 4 dorsal fin rays, the second band is brown band corresponding to the first band, start from the interorbital area extending dorsally to black saddle spot on the caudal peduncle. The third band is dark brown start from the lower edge of the eye expanding as a series of dark dots reaching the base of caudal fin. On the adults the bands were pale and fins become yellowish brown, and dorsal fin margin will be orange –yellow, while the other fins part shading to blackish ending with bluish white edge.

E. poecilonotus female matures at 41 *cm* of standard length, exhibits protogyny mode. It dwells in 45-375 *m* depth, in the reef margins and feeds on fishes and crustaceans (Craig et al., 2011; Heemstra and Randall, 1993). *E. poecilonotus* is related to *E. morrhua*, the distinguished between adults of these two species where difficult, but at the juveniles

it is obvious and easily to distinguished based on the morphology feature, the last has a dark blotch at 5th to 9th dorsal spines while at the *E. poecilonotus* projected between 9th spines to the 4th dorsal soft rays.

Epinephelus polylepis

Epinephelus Polylepis (Small Scaled Grouper) has body depth 2.6-3.3 of standard length, and head length of 1.8-2.4 of standard length, maximum total length is 75 *cm*. it has slight convex interorbital area. It has 11 dorsal spines, 16-17 dorsal soft rays, 3 anal spines, 8 anal soft rays and 18 -19 pectoral fin rays with 9-10 gill rakers on the upper limb, and 17-18 on the lower limb. It has a pale grey body base with dark spots, spots intensely distributed on the head and dorsal part of body and appeared in smaller close –set scattered pattern compered to those distributed on the ventral, with white margin projected at the edge of caudal fins. exhibits diandric protogynous hermaphrodite. It is found adjacent to the rocky area at 10-155 m (Craig et al., 2011; Heemstra and Randall, 1993). *E. Polylepis* is related to *E. Chlorostigma*, where the last has fewer scales and more pointed anal fin at adults.

Epinephelus polyphekadion

Epinephelus polyphekadion (Camouflage Grouper): A grouper species that is well known to exist in the Red Sea. It is a threatened species due to overfishing ("International Union" 36). *E. polyphekadion* has a body depth equal to 2.7–3.1 times the standard depth with a head length equal to 2.3–2.5 times the head length, flat interorbital area, and rounded caudal fin. Its maximum length is 90 cm SL. It has 8–10 gill rakers on the upper limb and 15–17 rakers on the lower limb. The maxilla extends past the rear edge of the eye, with a total of 11 dorsal spines with 14–15 dorsal soft rays, 3 anal spines, 8 anal soft rays, and

16–18 pectoral fin rays. It has pale brown basis for the body with dark brown and white spots and dark blotches, with Small dark brown spots cover the whole body, even the inner part of the mouth, with irregular dark blotches over small spots and a large black distinguished saddle blotch on the caudal fin in addition to small white spots scattered on the head, body, and fins.

It matures at 27–30 *cm* SL. Spawning occurs on full-moon nights between February and April and sometimes between January and February. There are separate colonies for each sex, where the female releases hundreds to thousands of eggs, and then the male spreads smoky sperm for fertilization. Throughout spawning activity, the background body color of the fish becomes lighter. It is reef-associated, found in coral-rich areas of lagoons at a 2–64-*m* depth. Primarily feeds on fishes and crustaceans, in addition to cephalopods and gastropods. It can be misidentified as *E. fuscoguttatus*, which has more gill rakers and pectoral fin rays.

Epinephelus radiatus

Epinephelus radiatus (Oblique – Banded Grouper), considered a rare species, has body depth 2.6-3.0 of standard length, and 2.1-2.3 of standard length is head length. maximum total length is 70 *cm*, and a flat interorbital area with slight convex at the dorsal part of head. It has 11 dorsal spines, 13-15 dorsal soft rays, 3 anal spines, 8 anal soft rays and 17-18 pectoral fin rays, with 8-9 gill rakers on the upper limb and 16-18 on the lower limb. The color varies based on size and age. For juveniles it is tan body bases with dark brown and black edged pale bands and black spots, for small adults, it is tan body base with dark edge pale bands and dark brown spots, while the large adults it is tan body bases with only dark spots.

Small adults have five curved and bifurcated oblique edged pale bands with small black spots scattering in addition to pale blotches on the dorsal. The large adults have series of dark spots disappeared on the third ventral part, also small dark spots covered the dorsal fin and caudal fin. While juveniles have dark brown with black, edged pale brown bands confined black spots. Adults commonly found at depth of 80-383 m, while juveniles at depth of 18-20m over hard substrates (Heemstra and Randall, 1993). *E. radiatus* has a similarity and relation to the *E. morrhua*, where the first mentioned has wider and steeper angle of the oblique bands (Craig et al., 2011; Heemstra and Randall, 1993).

Epinephelus stoliczkae

Epinephelus Stoliczkae (Epaulette Grouper) has a body depth of 2.8-3.3 of standard length, and head length equals 2.3-2.6 time of standard length, with maximum total length is 38 *cm*. it has moderate convex at the interorbital area. It has 11 dorsal fin spines, and 16-18 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 17-19 pectoral fin rays, with a yellowish grey bases with dark orange spots, dark grey bars and dark oval semicircular blotches. The orange spots were scattered intensely on the posterior part of head and body until ventral, the bars project under the posterior of dorsal fin spines, and two under soft dorsal fin and on the caudal peduncle. The blotches presented on the pectoral fin. The spinous of the dorsal fin is yellowish, with dark red spots at bases, while the median fins have yellowish in the posterior area. In spawning it exhibits protogynous mode. It dwells in coral reef at 5-50 *m* depth (Craig et al., 2011; Heemstra and Randall, 1993).

Epinephelus summana

Epinephelus summana (Juvenile or Speckled-Fin Grouper), as previously mentioned, the species has only been found and distributed in the Red Sea and Gulf of

Aden (Heemstra and Randall, 1993). *E. summana* has a body length is equivalent to 2.7–3.1 times the standard length (SL) (15–43 *cm*), 1.8–2.3 times the standard deep width, and 2.2–2.6 times the standard head length. The lengths of juveniles are smaller than 4 *cm*. It has some concaveness in the interorbital area and has a bottom nostril diameter length equivalent to 2–4 times the interior length. The vertical expandable maxilla to the orbit rear edge and lower midlateral jaw contain 2–4 subequal teeth rows. It has a total of 11 dorsal spines, 14–16 dorsal soft rays, three anal spines, 8–9 anal soft rays, and 16–18 pectoral fin rays.

The color varies between dark olive brown to dark brownish grey, and it has blotches larger than the eye size. The juveniles are dark grey with random black-tipped pectoral fins. The head is restricted with pale white blotches/spots, small white spots cover the fins, where the pectoral fin spots are enclosed in the base color, and the white spot size is variable in juveniles. It has a reef-associated habitat and is commonly found at a shallow depth. It feeds on small fish species and crustaceans.

Spawning is serial and occurs at midnight after six days of the new moon phase. Number of spawnings: 3. Total length (TL) at first spawning: 25–28 *cm*. 71% fertilization success rate. 65–750*10^3 eggs released per day. Fertilized egg diameter range: 0.75–0.80 *mm* (Alava et al., 1996; Heemstra and Randall, 1993).

Epinephelus tauvina

Epinephelus tauvina (Greasy Grouper) has body depth of 3 -3.6 times of standard length, and head length 2.1-2.4 times of standard length. Maximum total length is 75 *cm*. it has flat interorbital area with slight convex, 11 dorsal fin spines, 13-16 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 18-19 pectoral soft rays, and 8-10 gill rakers on the

upper limb, and 17-20 rakers on the lower limb. It has a faint greenish grey or brown body base shading to whitish ventrally, with orange –red to dark brown spots and slight dusky bars. Spots were darker on the center and smaller on the head, with black blotch visible on the four dorsal fin spines, and white edge projected on the caudal, anal and pectoral fins. Spawning; it exhibits protogynous that matures at 61 *cm* total length. Spawning occurs in the period extending from April to July each year. It dwells in coral reefs at depths of 50 *m* and feeds on fishes and crustaceans (Craig et al., 2011; Heemstra and Randall, 1993; Randall, 1983).

Epinephelus tukula

Epinephelus tukula (Potato Grouper) has a body depth of 2.9-3.5 times of standard length, and 2.3-2.6 of standard head length. Maximum total length 200 *cm*, it has a slight convex on the interorbital area, with 11 dorsal fin spines, 14-15 dorsal fin rays, 3 anal spins, 8 anal soft rays, and 18-20 pectoral fin rays, and 8-10 gill rakers on the upper limb and 15-18 on the lower limb. It has a pale brownish grey body, with dark brown to black blotches. The blotches were widelyspaced blotches distributed irregularly with small brown spots on head, and dark spots on the fins. exhibits protogynous and aggregation spawning, which matures at 90 *cm* of standard length. It dwells in reef associated areas, channels, seamounts, and prone area at depths 10-400 *m*, and feeds on fishes and invertebrates.

Epinephelus undulosus

Epinephelus undulosus (Wavy-Lined Grouper) has a body depth of 2.7-3.1 of standard length, and head length of 2.5-2.7 in standard length. Maximum total length is 120 *cm* with convex interorbital area. It has 11 dorsal spines, 17-19 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 18-19 pectoral fin rays, and 12-16 gill rakers on the upper limb,

and 20-23 gill rakers on the lower limb. It has a purplish grey to brownish grey body bases, with brown to golden brown dots. The dots irregularly distributed and radiates wavy longitude golden brown lines. It exhibits protogynous which matures at 41 *cm* of total length. It dwells in various areas such as: offshore banks, silty sand and mud substrates in depth of 25-90 *m*. Juveniles commonly inhabit shallow depths of 5m in coral colonies, and feeds on the small fishes, and crustaceans (Craig et al., 2011; Heemstra and Randall, 1993).

Grammistes sexlineatus

Grammistes sexlineatus (Spotted Coral Grouper or Goldenstriped Soapfish) has a body depth of 2.3-2.8 of standard length, with head length equals to 1.8-2.5 of standard length. Characterized by elongate body. Maximum total size is 28 *cm*. It has 7 dorsal spines, 13-14 dorsal soft rays, 2 anal spines, 9 anal soft rays, and 16-18 pectoral fin rays, with a rounded caudal fin, and 1-3 gill rakers on the upper limb, and 7-9 rakers on the lower limb. It has a dark brown body base with yellow stripes that increase accordingly with size. Juveniles have small spots. The stirpes are divided into dashes and spots as adult individuals. It dwells in rocky bottom and coral reefs, at shallow water, and is commonly hidden beneath ledges and small caves at day time hours, and feeds on the fishes (Al-Jufaili, 2010; Randall, 1983; Smith, 2003; Fischer and Bianchi, 1984).

Hyporthodus octofasciantus

Hyporthodus octofasciantus (Eight-Bar Grouper) considered rare. This could be based on its habitat behaviors. Has a body depth of 2.2-2.7 of standard length, and head length of 2.4-2.5 standard length, Maximum total length 130 *cm*. It has convex interorbital area, with 11 dorsal spines, 14-15 dorsal soft rays, 3 anal spines, 9 anal soft rays, and 18-19 pectoral soft rays, 7-9 gill rakers on the upper limb, and 15-17 on the lower limb. It has

a buff body shading, blackish with brown bars, with 8 brown bars projected onthe nape and dorsal fin posterior. Also, the 7th bar is wider than other bars, 8th bar covered cadual peduncle. It dwells on rocky reefs at depths of 80-200 *m* (Craig et al., 2011, Heemstra and Randall, 1993).

Plectropomus areolatus

Plectropomus areolatus (Squaretail Coral Grouper) has confined body depth of 2.9-3.9 times of standard length, and head length 2.7-3.1 times of standard length. Maximum total length is 73 *cm*. it has flat interorbital area and has 7-8 dorsal spines, 10-12 dorsal soft rays, 3 anal spines, 8 anal soft rays and 15-16 pectoral fin rays, and 2-7 gill rakers on the lower limb and 0-2 rakers on the upper limb. It has a greenish grey to brownish red body base, with oval dark edge blue spots, blackish margin bands. The spots are distributed irregularly on the body, with dorsal blackish marginal bands and white edged margin on the caudal fin. Exhibits protogynous with females mature at 41 *cm* of total length. Spawning period varies, associated to the area. It spawning in aggregation behaviors, and dwells in the outer reef channel and slopes at depths of 30m. Depends on fishes completely (Craig et al., 2011; Heemstra and Randall, 1993).

Plectropomus areolatus was recognized and morphologically defined in fish samples obtained from the Red Sea's Yanbu coast in Saudi Arabia. The 194-bp fragment of the TMO-4C4 gene was discovered during PCR amplification of the sample DNA. The TMO-4C4 gene and the reported accession sequences had a maximum homology of 97 to 98 percent, according to sequence alignment. The *P. areolatus* sample matched 97 percent of the two *P. areolatus* accessions in GenBank, whereas the other four *Plectropomus* species, *P. oligacanthus*, *P. leopardus*, *P. maculates*, and *P. laevis*, matched 98%of the

Yanbu sample. When the TMO-4C4 gene's sequence was aligned against the *Plectropomus* GenBank species, a total of 14 nucleotide positional changes with base-pair substitutions were discovered. The nucleotide sequences of TMO-4C4 indicated seven transversion interchanges, one transition from A to G, and four transitions from TDC. The phylogenetic tree depicting the link among Yanbu samples and *Plectropomus* species in NCBI GenBank revealed an interesting result, with Yanbu sample serving as the real root source for all separated *Plectropomus* species groupings. The TMO-4C4 gene was translated in a *P. areolatus* sample, resulting in a 46-amino-acid polypeptide with a molecular mass of 5421.02 kDa and an isoelectric point (pI) of 4.19. A study of amino acid sequences demonstrated 100 percent genetic similarity with six GenBank *Plectropomus* species. *P. areolatus* with accession AAY68548 and *P. areolatus* Yanbu sample occupied the first distinct cluster isolated from all and derived from *P. areolatus*. Phylogenetic relationships based on amino acid sequences split all species into one primary discrete cluster (AAY68548). The studies emphasized variations in the TMO-4C4 Yanbu sample gene and translated protein (Gharbawi, 2015).

Plectropomus pessuliferus

Plectropomus pessuliferus* (Roving Coral Grouper), is one of the endemic species in the Red Sea; also found in Zanzibar, the Maldives, St. Brandon's Shoals, Sri Lanka, Chagos, Nazareth Bank, Sumatra, and Fiji. It has an elongated body with a length equivalent to 2.9–3.9 times the SL (63 cm) and 2.7–3.1 times the standard head length. The maximum length is 120 *cm* in the Red Sea region. It has a flat interorbital area, which becomes concave at the edge of the orbits. It has a total of 7–8 dorsal spines, 10–12 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 15–16 pectoral fin rays. Juveniles have a pair

of serrated caudal fins. Color ranges from brown to orange-red with small dark-edged blue spots. Small dark blue spots cover the head and some aspects of the body, while they seem to be elongated on adults. Additionally, the ventral part is spot-poor and has darker spots compared with the head and upper posterior part of the body. It has a reef-associated habitat in a common base found at a depth of 250–147 *m* near coral reefs (Heemstra and Randall, 1993).

Plectropomus pessuliferus, also known as Najil, is a critically endangered Red Sea fish that can be discovered in Egypt, Saudi Arabia, Jordan, and Sudan, as well as scarce species in the Indo-Pacific area (Ashworth et al., 2006). Fishes of the genus *Plectropomus pessuliferus* have been discovered in coral reefs and seaward reefs at depths of 25 to 147 *meters*. In the Red Sea, the *Plectropomus pessuliferus* fish can grow to 120 *cm* in length, while in the Indo-Pacific, it can only grow to 63 *cm* (Heemstra et al., 1993; Morris et al., 2000). The body of these huge fishes is covered in blue spots and their hues range from white to beige to crimson (Durville et al., 2003; Randall et al., 2004; Sattar and Adam, 2005). This species looks a lot like *Plectropomus maculatus* and is frequently mistaken for it.

Plectropomus possesses the same basic body plan as other groupers (strong body and huge head), but its skin is more ornately patterned with brilliant spots, bars, and/or stripes that differ subtly among species. Skin brightness (redness) generally rises with water depth, appears to be rigid over short time scales (days to weeks), and may have a hereditary component (Cai et al. 2013). Skin color patterns, on the other hand, may be modified in seconds to aid in camouflage and courtship (Samoilys and Squire, 1994).

Additionally, some *P. laevis* individuals undergo a permanent drastic color transition (from a light body with black saddles and yellowfins to a dark body with numerous blue spots), although the timing of this occurrence varies according to size, age, and maturity (Heupel et al., 2010).

The most recent studies of morphological characters (Randall and Hoese, 1986) concluded that seven species warrant recognition: *P. leopardus*, *P. laevis*, *P. areolatus*, *P. maculatus* (Bar-Cheek Coral Grouper), *P. oligacanthus* (Highfin Coral Grouper), *P. punctatus* (Marbled Coral Grouper) and, lastly, *P. pessuliferus* (Roving Coral Grouper) which is comprised of two subspecies (*P. pessuliferus marisrubri* and *P. pessaliferous pessuliferus*). Recent genomic analyses, on the other hand, suggest that the two *P. pessuliferus* subspecies should be reclassified as separate species (Ma, 2014). Inter-specific analyses of a variety of nuclear and mitochondrial DNA sequences reveal a wide range of phylogenetic connections, implying inadequate lineage sorting, gene evolution independent of each other, and/or introgressive hybridization. As a result, the phylogeny of *Plectropomus* remains uncertain, necessitating additional research (Frisch et al., 2016).

During the Pleistocene, vicariant processes associated with varying sea levels frequently separated and re-joined reef habitats, leading to the diversification of *Plectropomus*, according to genetic and biogeographic evidence (van Herwerden et al., 2006, 2009). Five of the seven species currently have extensive secondary interaction spanning much of the Indo-Australian archipelago (*P. leopardus*, *P. maculatus*, *P. laevis*, *P. areolatus*, *P. oligacanthus*). This overlap has resulted in the possibility of hybridization (interbreeding), which has been observed in closely related species. Some *Plectropomus* species (1 percent) on the GBR, for instance, exhibit exterior coloration that is intermediate

between *P. leopardus* and *P. maculatus*, and these putative hybrids have DNA sequences that are similar to both parent species (van Herwerden et al., 2002; Frisch and van Herwerden, 2006).

Variola louti

Variola louti (Yellow-Edged Lyretail) is a widespread grouper that is categorized as being of the least concern by the IUCN Red List ("International Union" 21). It can be confused with its congener *V. albimarginata*, where the difference is a concrete color of either yellow or white. It Has an oblong body 2.8–3.3 times the SL depth and 2.5–2.8 times the standard head length with a long pelvic fin. The maximum length reaches 83 *cm* for the male and 81 *cm* for the female. It has a total of 9 dorsal spines, 13–14 dorsal soft rays, 3 anal spines, 8 anal soft rays, 16–19 pectoral fin rays, and a lunate caudal fin. Yellowish-brown to orange-red at the head, body, and median fins, with blue to lavender or pink spots. Small elongated blue to lavender or pink spots, yellow rear margin on median fins, and red to brown pectoral fin rays, with abrupt yellow on the distal third. Juveniles have 3 irregular black spots, with a wide pale yellow to white bar on the mid-dorsal extending from the lower jaw tip to the dorsal fin origin, where the smaller one does not have a black band and spot on the dorsal part. Spawning occurs in December and February; in the Indian Ocean, it occurs in March, April, and October, and coral reef-associated at a 3–240-*m* depth. In addition, feeds on reef fishes, crustaceans, shrimps, and stomatopods (Craig et al., 219; Heemstra and Randall, 1993; Randall, 1983).

4.4. Discussion

4.4.1. Commercially Important Grouper Species in the Kingdom of Saudi Arabia

There is a large market potential for Groupers (family Serranidae) especially in the Middle East and Southeast Asia. Local names for species in two families in the Saudi Arabian region include Taradi, Kusher and Najil. They bring high prices at market and are therefore important marine fish species for some local economies. Groupers are farmed in Southeast Asia using earthen ponds or floating net cages (Kohno et al., 1988, Manzano, 1990, Hanafi et al., 1991).

Worldwide, the Serranid subgroup *Epinephelinae* contains 159 marine fish species with common names sea bass, hind and grouper. All are considered to have significant economic value, particularly for the fisheries located along the coasts of subtropical and tropical areas. Research suggests that the majority of the world's marine food harvest comes from these Groupers and associated artisanal fisheries. In addition, Serranids or Groupers are unfortunately summarized in a few categories under landings; that is, statistics for individual species is often not considered. Misidentification of individual species is common and leads to a lack of species-specific information available to fisheries and conservation managers. For example, *Epinephelus tauvina* (Indo-Pacific) and *E. guaza* (Atlantic and Mediterranean) are often incorrectly identified. Clearly, comprehensive fisheries and conservation management plans must include the proper identification of species.

Twenty-five species of Grouper species are found in the Red Sea (Heemstra and Randall, 1993) and 22 species in the Arabian Gulf (Randall and Ben-Tuvia, 1983). Some species, for example, *E. polyphekadion* (synonymous with *E. microdon*) are known from

the southern portions of the Red Sea in association with coral reefs and are commercially significant for Saudi Arabia. Similarly, *E. tauvina* is extensively present in the Arabian Gulf and there is commercially valuable. However, some of the gGroupers have somewhat restricted distributions. For example, *Cephalopholis oligosticta*, is described as restricted, and therefore endemic, to the Red Sea. Other species are listed as present in the Red Sea, but their existence is questionable. For example, *C. boenack* is apparently an invalid species name (Weber and de Beaufort 1931). Similarly, the existence of *E. tauvina* in the Red Sea is likely attributable to misidentification and/or taxonomic confusion with *E. merra* (Steinitz and Ben-Tuvia, 1955; Roux-Esteve and Fourmanoir, 1955; Roux-Esteve, 1956). Clearly, taxonomic confusion and misidentification are an issue with Groupers in the Red Sea and Arabian Gulf.

4.4.2. Taxonomic Issues

Groupers are taxonomically distinguished through various traditional morphological characters, color patterns, and body configuration (e.g., size and length), but a caution is needed for variation in color, a characteristic that can vary for the same species between adults and juveniles (Nelson, 2006). Thus, Grouper identification is based on its spawning behaviors, variation of common characteristics in the same species, unreliable morphologic aspects, confusion between and taxonomy in relatives, and similarities between different species. This issue can be solved through biochemical methods and properties to determine the species and population (Sujatha et al, 2011).

Scrranid fishes, often recognized as Groupers, are among the most lucrative tropical and subtropical seafood species. Despite their significance, there is a lot of disagreement about how to classify them, especially the huge genera *Epinephelus Bloch* and

Cephalopholis Schneider. These two families' Indo-Pacific species are in desperate need of systematic review. Because the Red Sea has only 22 recognized species of Groupers, which is a tiny amount when comparing to most other main Indo-Pacific regions (Randall and Ben-Tuvia, 1983), it has been possible to provide the Taxonomic issues in this chapter.

Randall (1983) classifies Groupers in the *Epinephelinae* subfamily, adopting Katayama et al. (1960). Serraninae (one species of Serranus-Klunzinger, 1870), Liopropominae (two species of Liopropoma-Lubbock and Randall, 1978), and Anthiinae are other serranid subfamilies found in the Red Sea (one species of Plectranthias- Randall, 1980, and four species of Anthias, Heemstra, and Randall, MS). Randall et al. (1971) separated the two soapfishes located in the Red Sea into a new family called *Grammistidae* (though several authors have regarded this group as a subfamily of the Serranidae).

Despite the fact that there has been a significant amount of classification research on the fishes of the Arabian Peninsula, the classification of several species remains a mystery (Harrison et al, 2015). The Grouper faces issues including defining the exact taxonomy of the species of Grouper where the morphological aspects of the species overlap, making taxonomy complex. *E. coioides,* for instance, is frequently confused with *E. tauvina* and *E. malabaricus* (Rimmer and Glamuzina, 2019).

For preservation, fishery administration, and the creation of fish breeding programs, awareness of population and subpopulation (stock) structure is critical (Grandcourt, et al., 2005). For the identification of fish stocks, molecular marker technologies are routinely used (Cuéllar-Pinzón, et al., 2016). Several studies have argued on the classification, genetic diversity, and estimation of genetic variation among Grouper species using PCR-based approaches, including microsatellite analysis (Antoro et al., 2006,

Koedprang et al., 2007, Wang et al., 2011, Yang et al., 2011, An et al., 2014; Vaini et al., 2019), mitochondrial DNA (Maggio et al., 2005; Jackson et al., 2014; Jefri et al., 2015; Ketchum et al., 2016; Galal-Khallaf et al., 2019), random amplified polymorphic DNA (RAPD) (Govindaraju and Jayasankar, 2004; Noikotr et al., 2013; Roy et al., 2014) and inter simple sequence repeat (ISSR) (Chiu et al., 2012).

Some species are only known from the Red Sea and Gulf of Aden, such as *E. summana* and *C. oligosticta*; these are also endemic to the Red Sea (Randall and Ben-tuvia, 1983). However, some species have not been found in the Red Sea and Arabian Gulf, such as *E. diacanthus* and *E. flavocaeruleus*, despite being found in the Gulf of Aden, and *E. multinotatus* has been found in the Arabian Gulf, but it has not been found in the Red Sea or Gulf of Aden. Furthermore, *E. undulosus* has not been found either in the Red Sea or Arabian Gulf, but is frequently found in the Gulf of Oman.

Based on a review of the current literature and other treatments of grouper biodiversity, I list in Table 4.11, Table 4.2 and Table 4.3, a consensus of opinion on those species inhabiting the Arabian Sea (23 species), the Arabian Gulf (11 species) and the Red Sea (30 species), respectively.

Table 4.1 (1 of 3): Grouper species known or suspected to occur in the Arabian Sea.

No.	Species	Common Name	Habitat	Source
1	*Anyperodon leucogrammicus*	Slender grouper	Reef-associated	Randall & Ben-tuvia (1983)
2	*Cephalopholis argus*	Peacock hind	Reef-associated	Randall & Ben-tuvia (1983)
3	*C. hemistiktos*[1]	Yellowfin hind	Reef-associated	Randall & Ben-tuvia (1983)
4	*C. miniata*	Coral hind	Reef-associated	Randall & Ben-tuvia (1983)
5	*C. sexmaculata*	Sixblotch hind	Reef-associated	Randall & Ben-tuvia (1983)
6	*C. sonnerati*	Tomato hind	Reef-associated	Craig et al. (2011)
7	*Dermatolepis striolata*	Smooth grouper	-	Craig et al. (2011)
8	*Epinephelis areolatus*[2]	Areolae grouper	Reef-associated	Randall & Ben-tuvia (1983)
9	*E. chlorostigma*	Brown-spotted grouper	Reef-associated	Randall & Ben-tuvia (1983)
10	*E. epistictus*	Dotted grouper	Demersal	Randall & Ben-tuvia (1983)
11	*E. fasciatus*	Blacktip grouper	Reef-associated	Randall & Ben-tuvia (1983)
12	*E. fuscoguttatus*	Brown-marbled grouper	Reef-associated	Randall & Ben-tuvia (1983)

Table 4.1 Continued (2 of 3): Grouper species known or suspected to occur in the Arabian Sea.

No.	Species	Common Name	Habitat	Source
13	*E. malabaricus*	Malabar grouper	Reef-associated	Randall & Ben-tuvia (1983)
14	*E. latifasciatus*	Striped grouper	Demersal	Randall & Ben-tuvia (1983)
15	*E. marginatus*	Dusky grouper	Reef-associated	Randall & Ben-tuvia (1983)
16	*E. morrhua*	Comet grouper	Reef-associated	Randall & Ben-tuvia (1983)
17	*E. stoliczkae*	Epaulet grouper	Reef-associated	Randall & Ben-tuvia (1983)
18	*E. tauvina*	Greasy grouper	Reef-associated	Randall & Ben-tuvia (1983)
19	*E. tukula*	Potato grouper	Reef-associated	Craig et al. (2011)
20	*E. undulosus*[3]	Wavy-lined grouper	Reef-associated	Craig et al. (2011)
21	*E. polylepis*[4]	Smallscaled grouper	Demersal	Craig et al. (2011)
22	*E. poecilonotus*	Dot-dash grouper	Reef-associated	Craig et al. (2011)
23	*Grammistes sexlineatus*[5]	Goldenstriped soapfish	Reef-associated	Al-Jufaili et al. (2010)

Table 4.1 Continued (3 of 3) - Notes: Grouper species known or suspected to occur in the Arabian Sea.

[1]As recorded by Randall (1983), *C. hemistiktos* has the common name "half-spotted grouper"

[2] Often confused with *E. chlorostigma*

[3] *E. undulosus* is distributed in the Oman Gulf and Yemen Coast (Craig et al., 2011)

[4] *E. polylepis* is found in the Arabian Gulf and Yemen Coast (Craig et al., 2011)

[5] *Grammistes sexlineatus* is found in the Oman Gulf (Al-Jufaili et al., 2010)

Table 4.2 (1 of 2): Grouper species known or suspected to occur in the Arabian Gulf.

No.	Species	Common Name	Habitat	Source
1	*Aethaloperca rogaa*	Redmouth grouper	Reef-associated	Craig et al. (2011)
2	*Cephalopholis hemistiktos*[1]	Yellowfin hind	Reef-associated	Craig et al. (2011)
3	*Epinephelis areolatus*[2]	Areolae grouper	Reef-associated	Craig et al. (2011)
4	*E. bleekeri*	Duskytail grouper	Demersal	Craig et al. (2011)
5	*E. coeruleopunctatus*[3]	White-spotted grouper	Reef-associated	Craig et al. (2011)
6	*E. coioides*[4]	Orange-spotted grouper	Reef-associated	Craig et al. (2011)
7	*E. epistictus*	Dotted grouper	Demersal	Craig et al. (2011)
8	*E. latifasciatus*	Striped grouper	Demersal	Craig et al. (2011)
9	*E. multinotatus*	Whiteblotched grouper	Reef-associated	Craig et al. (2011)
10	*E. polylepis*[5]	Smallscaled grouper	Demersal	Craig et al. (2011)
11	*Hyporthodus octofasciatus*	Eight-bar grouper	Rocky reefs	Craig et al. (2011)

Table 4.2 Continued (2 of 2) - Notes: Grouper species known or suspected to occur in the Arabian Gulf.

[1]As recorded by Randall (1983), *C. hemistiktos* has the common name "half-spotted grouper"

[2] *E. areolatus* is often confused with *E. chlorostigma*

[3] *E. coeruleopunctatus* is found in the Arabian Gulf and Oman Gulf (Craig et al. 2011)

[4] *E. coioides* is frequently misidentified as *E. tauvina* or *E. malabaricus*

[5] *E. polylepis* is found in the Arabian Gulf and Yemen Coast (Craig et al. 2011)

Table 4.3 (1 of 4): Grouper species known or suspected to occur in the Red Sea.

No.	Species	Common Name	Habitat	Source
1	*Aethaloperca rogaa*	Redmouth grouper	Reef-associated	Red Sea (Forsskål in Niebuhr, 1775)
2	*Anyperodon leucogrammicus*	Slender grouper	Reef-associated	Randall & Ben-tuvia (1983)
3.	*Cephalopholis hemistiktos*[1]	Yellowfin hind	Reef-associated	Randall & Ben-tuvia (1983)
4	*C. miniata*	Coral hind	Reef-associated	Randall & Ben-tuvia (1983)
5	*C. oligosticta*	Vermilion hind	Reef-associated	Randall & Ben-tuvia (1983)
6	*C. sexmaculata*	Sixblotch hind	Reef-associated	Randall & Ben-tuvia (1983)
7	*C. argus*	Peacock hind	Reef-associated	Randall & Ben-tuvia (1983)
8	*Dermatolepis striolata*	Smooth grouper	Reef-associated	Craig et al. (2011)
9	*Epinephelis areolatus*	Areolae grouper	Reef-associated	Randall & Ben-tuvia (1983)
10	*E. chlorostigma*	Brown-spotted grouper	Reef-associated	Randall & Ben-tuvia (1983)
11	*E. coioides*[2]	Orange-spotted grouper	Reef-associated	Golani et al. (2010)
12	*E. epistictus*	Dotted grouper	Demersal	Randall & Ben-tuvia (1983)

Table 4.3 Continued (2 of 4): Grouper species known or suspected to occur in the Red Sea.

No.	Species	Common Name	Habitat	Source
13	*E. fasciatus*	Blacktip grouper	Reef-associated	Randall & Ben-tuvia (1983)
14	*E. fuscoguttatus*[3]	Brown-marbled grouper	Reef-associated	Randall & Ben-tuvia (1983)
15	*E. geoffroyi*	Red Sea spotted grouper	Rocky habitat	Randall et al. (2013)
16	*E. lanceolatus*[4,5]	Giant grouper	Reef-associated	Rouphael et al. (2011)
17	*E. latifasciatus*	Striped grouper	Demersal	Randall & Ben-tuvia (1983)
18	*E. marginatus*	Dusky Grouper	Reef-associated	Randall & Ben-tuvia (1983)
19	*E. malabaricus*[6]	Malabar grouper	Reef-associated	Randall & Ben-tuvia (1983)
20	*E. microdon*	Small-mouth grouper	Water surface	Red Sea (need reference)
21	*E. morrhua*[7]	Comet grouper	Reef-associated	Randall & Ben-tuvia (1983)
22	*E. polyphekadion*	Camouflage grouper	Reef-associated	Craig et al. (2011)
23	*E. radiatus*	Oblique-banded grouper	Demersal	Craig et al. (2011)
24	*E. stoliczkae*[8]	Epaulet grouper	Reef-associated	Randall & Ben-tuvia (1983)

Table 4.3 Continued (3 of 4): Grouper species known or suspected to occur in the Red Sea.

No.	Species	Common Name	Habitat	Source
25	*E. summana*	Summan grouper	Reef-associated	Randall & Ben-tuvia (1983)
26	*E. tauvina*	Greasy grouper	Reef-associated	Randall & Ben-tuvia (1983)
27	*E. tukula*	Potato grouper	Reef-associated	Craig et al. (2011)
28	*Plectropomus areolatus*	Roving coral grouper	Reef-associated	Craig et al. (2011)
29	*P. pessuliferus*	Squaretail coral grouper	Reef-associated	International Union (2001)
30	*Variola louti*	Lunartail grouper	Reef-associated	International Union (2001)

Table 4.3 Continued (4 of 4) - Notes: Grouper species known or suspected to occur in the Red Sea.

[1] As recorded by Randall (1983), *C. hemistiktos* has the common name "half-spotted grouper"

[2] *E. coioides* is frequently misidentified as *E. tauvina* or *E. malabaricus*

[3] *E. fuscoguttatus* is often confused with *E. polyphekadion* (*E. microdon*)

[4] The giant grouper is endemic to the Red Sea and Gulf of Aden and local to the Red Sea. It is frequently misidentified as *E. chlorostigma* (Randall et al., 2013)

[5] The giant grouper can be observed in the Yemen Red Sea (Rouphael et al., 2011)

[6] *E. malabaricus* is closely related to *E. coioides*

[7] *E. morrhua* is sometimes misidentified as *E. poecilonotus*, *E. radiatus*, or *E. tuamotoensis*

[8] The *Epaulet* grouper has the common name sand grouper, as recorded by Randall (1983)

Table 4.4 (1 of 4): A checklist of grouper species inhabiting the marine waters surrounding the Arabian Peninsula (observed species numbers in parentheses).

No.	Species	Red Sea (30)	Arabian Sea (21)	Arabian Gulf (11)
1	*Aethaloperca rogaa*	✓		✓
2	*Anyperodon leucogrammicus*	✓		
3	*C. argus*	✓		
4	*C. hemistiktos*	✓	✓	✓
5	*C. miniata*	✓	✓	
6	*C. oligosticta*	✓		
7	*C. sexmaculata*	✓	✓	
8	*C. sonnerati*		✓	
9	*Dermatolepis Striolata*	✓	✓	
10	*E. areolatus*	✓	✓	✓
11	*E. bleekeri*			✓
12	*E. chlorostigma*	✓	✓	

Table 4.4 Continued (2 of 4): A checklist of grouper species inhabiting the marine waters surrounding the Arabian Peninsula (observed species numbers in parentheses).

No.	Species	Red Sea (30)	Arabian Sea (21)	Arabian Gulf (11)
13	*E. coeruleopunctatus*			✓
14	*E. coioides*	✓		✓
15	*E. epistictus*	✓	✓	✓
16	*E. fasciatus*	✓	✓	
17	*E. fuscoguttatus*	✓	✓	
18	*E. geoffroyi*	✓		
19	*E. lanceolatus*	✓		
20	*E. latifasciatus*	✓	✓	✓
21	*E. malabaricus*	✓	✓	
22	*E. marginatus*	✓	✓	
23	*E. microdon*	✓		
24	*E. morrhua*	✓	✓	

Table 4.4 Continued (3 of 4): A checklist of grouper species inhabiting the marine waters surrounding the Arabian Peninsula (observed species numbers in parentheses).

No.	Species	Red Sea (30)	Arabian Sea (21)	Arabian Gulf (11)
25	*E. multinotatus*			✓
26	*E. poecilonotus*		✓	
27	*E. polylepis*		✓	✓
28	*E. polyphekadion*	✓		
29	*E. radiatus*	✓		
30	*E. stoliczkae*	✓	✓	
31	*E. summana*	✓		
32	*E. tauvina*	✓	✓	
33	*E. tukula*	✓	✓	
34	*E. undulosus*		✓	
35	*Grammistes sexlineatus*		✓	
36	*Hyporthodus octofasciatus*			✓

Table 4.4 Continued (4 of 4): A checklist of grouper species inhabiting the marine waters surrounding the Arabian Peninsula (observed species numbers in parentheses).

No.	Species	Red Sea (30)	Arabian Sea (21)	Arabian Gulf (11)
37	*Plectropomus areolatus*	✓		
38	*Plectropomus pessuliferus*	✓		
39	*Variola louti*	✓		

Table 4.5: Morphological characteristics, No. 1 of 30.

Species: *Aethaloperca rogaa:*

Body shape: Compressed body, with a great body depth equal to 2.1–2.4 times the SL, and the head length is equal to 2.5–2.7 times the SL, with concaveness at the interorbital area. The maximum length is 60 *cm* TL.

Fin: Truncated.

Spines: Has 9 dorsal spines, 17–18 dorsal soft rays, 3 anal spines, 8–9 anal soft rays, and 17–19 pectoral fin rays, with a truncated caudal fin. Has 8–10 gill rakers on the upper limb and 15–17 on the lower limb.

Spawning: Spawns at any time in the year and matures at 35 *cm* SL.

Color: Dark brown to black body with a vertical bar on the middle of the abdomen with a large orange-red mouth and reddish upper jaw part

Feed: Feeds on small fishes, stomatopods, and crustaceans.

Source: Craig et al., 2011; Randall 1983; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No. 2 of 30.

Species: *Anyperodon leucogrammicus.*

Body Shape: A remarkably elongated body and head shape, with a great depth of 3.1–3.7 times the SL, head length 2.3–2.5 times the SL, and maximum length of 65 *cm* TL.

Fin: Rounded.

Spines: Dark brown to black body with a vertical bar on the middle of the abdomen with a large orange-red mouth and reddish upper jaw part.

Spawning: Spawns at any time in the year and matures at 35 *cm* SL.

Color: Greenish to brownish-grey adults with orange-red spots. Orange-red spots are scattered on the head, body, and dorsal fin with a dense basis on the caudal fin and the clear appearance of whitish long bars or a series of stripes on the post location of the head and body. Juveniles have a dark edge, pale grey stripes, and a blue-edged black spot (in some cases two spots) on the caudal fin and the clear appearance of whitish long bars or a series of stripes on the post location of the head and body. Juveniles have a dark edge, pale grey stripes, and a blue-edged black spot (in some cases two spots) on the caudal fin.

Feed: Feeds on small fishes, stomatopods, and crustaceans.

Source: Craig et al., 2011; Randall 1983; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No. 3 of 30.

Species: *C. argus.*

Body Shape: Very deep, and it has a common body length of 40 cm TL. The body depth ranges from 2.7–3.3 times the SL, and the head length is 2.4–2.7 times the SL. The maximum length is 60 *cm* ("Coastal fishes" 57), and it has small eyes.

Fin: Rounded.

Spines: Has 9 dorsal spines, 15–17 dorsal soft rays, 3 anal spines, 9 anal soft rays, and 16–18 pectoral fin rays.

Spawning: Has daily courtship behavior from afternoon to sunset and repeated single male to multiple female mating groups, and the mating is paired and pelagic (Donaldson 364).

Color: Black-edged blue covering the dark brown body, a large pale bar on the chest compared with a small pale bar on the posterior part with a narrow white edge on the rear of the median fins with orange-gold on the rectangular dorsal fin ends.

Feed: Feeds on fishes and crustaceans mainly in the dark (night); thus, it is called a crepuscular feeder. It has also been observed feeding in the early morning or evening.

Source: Craig et al. 2011; Randall, 1983; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No. 4 of 30.

Species: *C. hemistiktos.*

Body Shape: a body depth equal to 2.7–3.0 times standard depth, with 2.4–2.6 times the standard head length.

Fin:

Spines: Has 9 dorsal spines, 8–10 dorsal soft rays, 3 anal spines, 8–10 anal soft rays, and 16–18 pectoral fin rays.

Spawning: A monogamous species, where each pair occupies 62 m^2 of territory.

Color: The base body color is brownish to brownish-red to reddish as the depth increases. There are ocelli on the head, a dark blue edge with a darker color than the body on the caudal fin, a rear dorsal fin, anal fins in addition to blue ocelli and a line, and an orange dorsal spine fin, while the pectoral fins are brownish to reddish with small blue ground ocelli bordered with yellow.

Feed: Feeds on fishes, mostly pomacentrids and crustaceans (ambush predator), eating throughout the day.

Source: Craig et al., 2011; Heemstra and Randall, 1993; Randall, 1983.

Table 4.5 Continued: Morphological characterisitics, No. 5 of 30.

Species: *C. miniata.*

Body Shape: a body depth equivalent to 2.6-3 time of the standard Length, with head length 2.4-2.6 times of head standard length, the maximum total length is 50 *cm*.

Fin: Rounded.

Spines: Has 9 dorsal spins, with 14-15 dorsal soft rays, 3 anal spines, 8-9 Anal soft rays and 17-18 pectoral fin rays.

7-9 gill rakers on the upper limb, while the lower has 14-16 rakers.

Spawning: Female matures on 25 *cm* of total length, it occurs in haremic groups with prevalent males patrol certain territories occupied with 2-12 females with sub internal territories defined for each single female.

Color: Orange -red range from dark to light degree with darkish in some parts, with pale blue –grey spots, while the juveniles' color is yellow with faint pale blue spots.the posterior parts is darker than the rest of body, the spots were narrow and smaller than the pupil, but the spot appears in scatter pattern in the juveniles, with distinguished blue margin occurred on the soft dorsal, caudal and anal fins parts.

Feed: Fishes and crustaceans consider main food for the C. miniata, but it shows inclination for Anthias squamipinnis and Pseudanthias.

Source: Craig et al., 2011; Heemstra and Randall, 1993; Randall, 1983.

Table 4.5 Continued: Morphological characterisitics, No. 6 of 30.

Species: *C. oligosticta.*

Body Shape: The body length is equivalent to 2.6–3.0 times the SL (16–22 cm) and 2.4–2.6 times the standard head length, the depth of body is 2.6–3.0 times the SL, and the width depth is equal to 19 times the SL (equal orbit diameter). Females are 17–19 *cm* long, and the mature male length is 22 *cm*, with a maximum length of 30 *cm*.

Fin: A slight concaveness in the interorbital area.

Spines: Has 9 dorsal spines, 14–15 dorsal soft rays, 3 anal spines, 9 anal soft rays, and 16–18 pectoral fin rays, with 7–8 gill rakers on the upper limb and 14–15 on the lower limb.

Spawning:

Color: Orange-red, pale blue spots widely distributed on the whole body, fins, and head, while they become closer and narrower pale spots on the soft dorsal and caudal fins.

Feed:

Source: Choat et al., 2008; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No. 7 of 30.

Species: *C. sexmaculata*.

Body Shape: 2.5 -3 of standard length body depth, with 2.3-2.5 standard head length, maximum total length is 50 *cm*.

a slight concave on the flat interorbital area, with distinguished concave above the eye.

Fin: Rounded.

Spines: 9 dorsal spines, 14-16 dorsal soft rays, 3 ananl spines, 9 anal soft rays, and 16-18 pectoral fin rays. Also, 7-9 gill rakers in the upper limb, and 14-16 rakers on the lower.

Spawning: Matures on the 24.91 *cm* length.

Color: Varied brown color degree range in brownish, brownish red, and reddish body base associated the deep in the water. Has a small, blue ocelli, six quadrangular blotches; four observed in the dorsal fin base and other extended to fin, the spaces between these six blotches filled with very pale bars, also, there are pale blue lines radiating from the eye, with more dense of small elongated blue ocelli in the head compared with the lower part of body.

Feed: Feeds dominantly in fishes.

Source: Heemstra and Randall, 1993; Craig et al., 2011; Randall, 1983.

Table 4.5 Continued: Morphological characterisitics, No. 8 of 30.

Species: *C. sonnerati.*

Body Shape: Body depth of 2.3-2.8 times of standard length, with head length equivalent to 2.5-2.7 of standard length, slight straight to concave dorsal head in the adults, maximum length is 75 *cm* total length. slight to concave interorbital area with rounded preopercle, also pelvic fins reaching further the anus. Slight to concave interorbital area with rounded preopercle, also pelvic fins reaching further the anus.

Fin: Rounded.

Spines: Has 9 dorsal fins, 14-16 dorsal soft rays, 3 anal spins, 9 anal soft rays and 18-20 pectoral fin rays. Has 7-9 gill rakers on the upper limb, and 14-16 rakers in the lower.

Spawning: Spawns in particular prolong seasons in the open water where the substratum egg scatters, fertilization occurs external, has a protogyny mode where female matures at 28 *cm* of standard length, while male matures at 34 *cm* standard length.

Color: Varies in two patterns: first is orange red to reddish brown body base with whitish blotches, where head color is purplish to reddish with orange- red spots. The second pattern recognized with light reddish to yellowish brown body base, with brownish red spots on the head, body and fins, first pattern mentioned has a dense network of purple on head, maxilla and lips, in addition to whitish or purple spots scattered on the body, and orange distally in pectoral fins, with blackish tips occurred in the tips of dorsal, anal, pelvic and caudal fins. In the second pattern mentioned the spots were small brownish red to dark brown, with whitish projection in the rear part of caudal fins and pectoral fins.

Feed: Feeds on the crustaceans and small fishes.

Source: Heemstra and Randall, 1993; Craig et al., 2011; Randall, 1983.

Table 4.5 Continued: Morphological characterisitics, No. 9 of 30.

Species: *Dermatolepis Striolata.*

Body Shape: Body depth equal to 2.4-2.6 of the Standard length, head length 7.2-7.8 of standard length, the eye diameter less than snout

Fin:

Spines: Has 11 dorsal spins, 18- 19 dorsal soft rays, 3 anal spins and 9 -10 anal soft rays and 17-19 pectoral fins - Has 5-7 gill rakers on the upper limb, and 13-16 rakers on the lower limb.

Spawning: Fertilization occurs external, has a protogyny mode.

Color: Yellowish to reddish brown base body with small round dark spots and pale blotches. small elongated dark brown spot distributed over the whole body and head, in horizontal elongation thus its poses short lines, the blotches were irregular pale black distinct in the head.

Feed: Feeds on fishes predominantly.

Source: Heemstra and Randall, 1993; Craig et al., 2011.

Table 4.5 Continued: Morphological characterisitics, No. 10 of 30.

Species: *E. areolatus.*

Body Shape: Body depth equivalent to 2.8–3.3 times the SL with a head length equal to 2.4–2.8 times the SL with concaveness at the interorbital area.

Fin:

Spines: Total of 11 dorsal spines, 15–17 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 17–19 pectoral fin rays. 8–10 gill rakers on the upper limb and 14–16 on the lower limb.

Spawning: One-male-to-multiple-female spawning manner in a respective ratio of 1:6 in a restricted period and builds aggregation with pelagic eggs. Maturity occurs at female length equal to 19.5 *cm* TL and at 29 *cm* TL for males.

Color: Pale head, body, and fins with close, dense, and large brown or brownish-yellow or greenish-yellow spots with pale pectoral fins and a white margin at the caudal fins.

Feed: Fishes, prawns, and crabs as primary benthic invertebrates.

Source: Craig et al., 2011.

Table 4.5 Continued: Morphological characterisitics, No. 11 of 30.

Species: *E. bleekeri.*

Body Shape: Elongate body with depth equal to 3 -3.5 times of standard length, and 2.4-2.7 times of standard head length, maximum length 76 *cm*.

Fin: Straight to slightly convex.

Spines: Has 11 dorsal spines, 16-18 dorsal soft rays, 3 anal spines, 8-9 anal soft rays. Has 17-19 pectoral fins rays, rounded truncate caudal fin, also the pelvic fins were short.

Spawning: Matures at 36 *cm* total length.

Color: Greyish brown body base with dark reddish brown to black spots, where the fins are darker than body. In addition to narrow pale yellow or white margins occurred in the anal fins. the dark reddish brown spots are well –distributed over the body, the spots are smaller than the pupil and elongate horizontally, also the small dark spots projected on the median fins.

Feed:

Source: Almukhtar et al., 2012; Heemstra and Randall, 1993; Craig et al., 2011.

Table 4.5 Continued: Morphological characterisitics, No. 12 of 30.

Species: *E. chlorostigma.*

Body Shape: Body depth equal to 2.8-3.3 of standard length, and 2.4-2.7 head length on standard.

Fin: A truncated or emarginate caudal fin with a white posterior margin.

Spines: Has 11 dorsal spines, 16-18 dorsal soft rays, 3 anal spines, 8 anal soft rays; also 17-19 pectoral fin rays.

Spawning: Spawning period varies and prolonged among this species, show a protogynous mode, where female matures at 25cm total length, at 34 *cm* to 56 *cm* the sexual changes occurs, but not all female experienced sex changes. Fertilizations are external in the aggregation form of matures.

Color: Whitish body base colour with dark brown spots. the spots are small, dark brown and scattered over all body and head in irregular close set network form. White line is projected on the posterior margin of caudal fin.

Feed: Main food is fishes and invertebrates.

Source: Jefri et al., 2015; Heemstra and Randall, 1993; Craig et al., 2011; Randall, 1983.

Table 4.5 Continued: Morphological characterisitics, No. 13 of 30.

Species: *E. coeruleopunctatus.*

Body Shape: Body depth contained 2.9-3.4 times of standard length, head length equal 2.3-2.5 times of standard length. Maximum length 76 *cm* total length.

Fin: Rounded.

Spines: Has 11 dorsal spines, 15-17 dorsal soft rays, 3anal spines, 8 anal soft rays, and 17-19 pectoral fin rays.

Spawning: Matures at 42 *cm* of total length, has a protogyny mode and fertilization occurs externally.

Color: Adults body base is brownish grey with pale spots and blotches, while juveniles has a dark grey to black body base with white spots and dots. the adults' body has distributed small white pale spots and large white pale blotches; also there are five black blotches in the base of dorsal fins, while the juveniles covered with small white spots and dots.

Feed: Fish and crustaceans.

Source: Almukhtar et al., 2012; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No. 14 of 30.

Species: *E. coioides.*

Body Shape: Elongated body with a length equivalent to 2.9–3.7 times the SL, which is equal to 10–78 *cm*, a head length equal to 2.3–2.6 times the SL, and a maximum length of 120 *cm*. The mature female TL is 25–30 *cm*.

Fin: Rounded.

Spines: Has 11 dorsal spines, 14–16 dorsal soft rays, 3 anal spines, 8 anal soft rays, 18–20 pectoral fin rays, and a rounded caudal fin.

Spawning: They spawn in aggregation regions in a specific period (probably from March to June). The successful and surviving larvae need 30°C water temperature conditions.

Color: Tan color on the dorsal part of the head and body, with a white shade in the ventral region and small scattered orange or reddish-brown spots. Small orange or reddish-brown spots distributed on the body, head, and fins in the middle, with two dark spots on the interopercle in addition to two junctions in the sub- and interopercles. It also has five unique, random, ventrally fork slanting and pale dark rods; the first rod is located in the lower region of the dorsal fin spines, and the far rod is located on the caudal peduncle. Meanwhile, note that the orange spots convert to brown in air-exposed conditions.

Feed: Feeds on small fishes, shrimps, and crabs.

Source: Almukhtar et al., 2012; McIlwain et al., 2016; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No. 15 of 30.

Species: *E. epistictus.*

Body Shape: body depth is 3.0-3.3times standard length, and the head length is 2.2-2.25 standard length, maximum total length is 80 *cm*.

Fin: Low or medium roundness.

Spines: Has dorsal spines, 14-15 dorsal soft rays, 3anal spines, 8 anal soft rays, 17-19 pectoral fin rays. 7-10 gill rakers in the upper limb, and 15-19 on the lower limb.

Spawning: protogyny, the Fertilization occurred external.

Color: a pale brownish to greenish body base, with brownish black spots, also has a second pattern where the colour body base is brown to olive with brownish black spots. the spots were small scattered in the dorsolateral part and disappeared in the posterior part of head and median fins, also, has brownish pectoral fin rays, has three faint dark brown band radiant from the eye and extend to the operculum end, where the juveniles have dark spots on the head and body perform three longitudinal rows

Feed:

Source: Almukhtar et al., 2012.

Table 4.5 Continued: Morphological characterisitics, No. 16 of 30.

Species: *E. fasciatus.*

Body Shape: Body depth equivalent to 2.8-3.3 times of standard length, with 2.3-2.6 times head length, maximum total length 40 *cm*.

Fin: Moderately rounded to truncated shape.

Spines: Has 11 dorsal fin spines, 15-17 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 18-20 pectoral fin rays.

Spawning: Performed hermaphroditism at juveniles' phase, and in the older stage it deprived of female functions, and performed male function only, matures at 24 *cm* of total length., and fertilization occurred externally.

Color: A body colour base ranged from greenish grey, to pale reddish yellow to scarlet, with varied dense dark bars, the median part of body is pale where the rear part is darker, the dorsal area if head and upper jaw has a darker reddish or reddish brown colour, while the other parts are pale orange. there is no spots appears in such species, but have a distinguish black triangle projected on the incised interspinous of dorsal fin, with 5-6 conspicuous dark bars.

Feed: Brachyuran, crabs, stomatopods, fishes, ophiuroids, and octopus, feeds predominantly on fishes and some crustaceans (mainly crabs).

Source: Randall, 1983; Heemstra and Randall, 1993; Craig et al., 2011.

Table 4.5 Continued: Morphological characterisitics, No. 17 of 30.

Species: *E. fuscoguttatus.*

Body Shape: Body depth equvilant to 2.6-2.9 times of standard length with head length equal 2.3-2.5 times of standard length, maximum total length is 120 *cm*.

Fin:

Spines: Has 11 dorsal spines, 14-15 dorsal soft rays, 3 anal spines, 8 anal soft rays and 18-20 pectoral fin rays. The 3rd and 4th dorsal spines are the longest compared with dorsal spines and shorter than the longest dorsal fin rays. However, it has an incised interspinouse membranes. Has 10-12 gill rakers on the upper limb, and 17-21 on the lower limb.

Spawning: Spawning season starts from November to January; form large spawning aggregation, exhibits protogyny hermaphrodite, where female changes sex at 68 *cm* total length, with external fertilization.

Color: Pale yellowish brown body base, with dark brown blotches, brown spots, and darks bar at side of the jaw. the small dark brown spots distributed in close set irregular form over the 5 irregular bars performed by dark brown blotches, and 2-3 faint bar on the jaw.

Feed: Fishes, crabs, and cephalopods.

Source: Randall, 1983; Heemstra and Randall, 1993; Craig et al., 2011.

Table 4.5 Continued: Morphological characterisitics, No. 18 of 30.

Species: *E. geoffroyi.*

Body Shape: Elongated body 2.9 times the SL depth, head length 2.7 times the SL, and small eyes. The lower jaw is projected, and the maxilla extends to the central vertical eye line.

Fin: Various anal fin shapes, which can be pointed or round.

Spines: Has 8 gill rakers on the upper limb and 17 on the lower limb. 11 dorsal fins, 17 dorsal rays, 3 anal spines, 8 anal soft rays, and 17 pectoral fin rays.

Spawning:

Color: Beige over the whole body with large, dense, dark brown spots. The spots are scattered over the whole body in a close-set manner, with pale spots in the lower part that are nearly orange in color and one single dark spotted bar at the caudal fins.

Feed:

Source: Golani et al., 2010; Randall et al. 1971.

Table 4.5 Continued: Morphological characterisitics, No. 19 of 30.

Species: *E. lanceolatus.*

Body Shape: Body depth contained 2.4-3.4 times in standard length, and head length 2.20-2.70 times of standard length, slight convex on the flat interorbital area, head is convex at the dorsal. Maximum total length is 270 *cm*.

Fin:

Spines: Has 11 dorsal spines, 14-16 dorsal soft rays, 3 anal spines, 8 anal soft rays, 18-20 pectoral fin rays; also 8-10 giller rakers in the upper limb and 14-17 on the lower limb for juveniles.

Spawning: Matures at 129 *cm*, exhibits protogyny mode, not known if it from spawning aggregation, but it potential.

Color: Changes based on the age, the base body colour of juveniles is yellow with black bar, while the body colour of adults is yellow to greenish to dark brownish with yellow, white, black spots. the juvenile's bars characterized as: three irregular wide bars, first extend from spinous dorsal fin to the belly, until reach head, the second bar extend from base of soft dorsal rays to the anal fin, the third bar projected on the base of caudal fin. However, the spots at adult body distracted irregularly, the yellow and white spots distributed over the darker part of body, while the black spots occurrs on the fins.

Feed: Has various foods such as spiny lobsters, fishes, small sharks, batoids, and juvenile turtles and crustaceans.

Source: Heemstra and Randall, 1993;

Table 4.5 Continued: Morphological characterisitics, No. 20 of 30.

Species: *E. latifasciatus.*

Body Shape: Body depth 2.9-3.4 times of standard length, and head length of 2.3-2.6 times of standard length, maximum total length 157 *cm*.

Fin:

Spines: Has 11 dorsal spines, 14-16 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 17-19 pectoral fin rays. However, The interspinous dorsal incised sharply. 8-11 gill rakers on the upper limb, and 15-18 rakers on the lower.

Spawning: Matures at 86 *cm* total length, exhibit protogyny mode, also fertilization occured external.

Color: Lavender –grey or pale brownish, where juveniles has whitish shades at median, with 2 black longitude edge bar, white bars and black spots, adults have not white bars, just dark edges. the black bar on juveniles started from the eye and extend edgy to upper dorsal fin rays, and lower to the caudal fins, also, black spots and streaks distributed at caudal and dorsal fin. At adults, the dark edges were breaking into dashes and spots.

Feed:

Source: Heemstra and Randall, 1993; Craig et al., 2011.

Table 4.5 Continued: Morphological characterisitics, No. 21 of 30.

Species: *E. malabaricus.*

Body Shape: Elongated body, with a body depth equal to 3.0–3.7 times the SL and 2.3–2.6 times standard head length.

Fin: Rounded.

Spines: Has 11 dorsal spines, 14–16 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 18–20 pectoral fin rays. 8–11 gill rakers on the upper limb and 14–18 rakers on the lower limb.

Spawning: Matures at 64 *cm* SL. Sex reversal is likely to occur after 10 years of age or between 97 and 113 *cm* TL. The spawning period is from September to February.

Color: A brownish body and head with blackish-brown spots, irregular white spots and blotches, and dark brown bars. Dark brown oblique bars, with small, well-separated blackish-brown spots scattered on the body (even the lower part and mouth roof) and small black spots on the fins, with white spots and blotches on the head and body.

Feed: Feeds equally on fishes and crustaceans and rarely on octopuses.

Source: Almukhtar et al., 2012; Choat, et al., 2008; Heemstra and Randall, 1993; Craig et al., 2011; Gaspare and Bryceson, 2013.

Table 4.5 Continued: Morphological characterisitics, No. 22 of 30.

Species: *E. marginatus.*

Body Shape: Body depth of 2.6-3.1times standard length, and head length is 2.3- 2.5 standard length. Maximum total length 143 *cm*.

Fin:

Spines: Has 11 dorsal spines, 14-16 dorsal fin rays, 3 anal spines, 8-9 anal soft rays, and 17-19 pectoral fin rays. 7-10 gill rakers on the upper limb, and 14-16 rakers on the lower.

Spawning: Exhibits protogynous hermaphrodite forms spawning aggregation, spawning occurs on December, where females mature at 45 *cm*, sexual changes occur after various year of maturity exceed ten years.

Color: Has dark reddish brown body base, with yellowish projection ventrally and greyish dorsally, distributed white, pale greenish yellow or silvery grey blotches. the blotches perform vertical series.

Feed: Fishes and invertebrates.

Source: Craig et al., 2011.

Table 4.5 Continued: Morphological characterisitics, No. 23 of 30.

Species: *E. microdon.*

Body Shape: Body depth of 2.7-3.2 of standard length and head length of 2.4-2.5 of standard length.

Fin: Rounded.

Spines: Has 11 dorsal spines, 14-15 dorsal soft rays, 3 anal spines, 7-9 anal soft rays (commonly 8 rays) and 16-17 pectoral rays. 14 -18 gill rakers in the lower limp and 8-10 on the upper limp.

Spawning: Its protogynous hermaphrodite species, the spawn season represents in two to three month per year, the sex inversion occurs at the resting period after spawning.

Color: Has a brownish body bases, with dark brown spots, and dark blotches, small dark spots but much larger than pupil eyes, covers the whole body, with dark blotches such as the one at the caudal peduncle.

Feed: Feeds on fishes and crustaceans.

Source: Randall, 1964; Morgans, 1982; Heemstra and Randall, 1993; Brusle'-Sicard et al., 1992; Rhodes et al., 2011.

Table 4.5 Continued: Morphological characterisitics, No.: 24 of 30**.**

Species: *E. morrhua.*

Body Shape: Body depth 2.8-3.1 times in standard length, and head length 2.3-2.5 times in standard length. Maximum total length is 100 *cm*.

Fin:

Spines: Has 11 dorsal spines, 14-15 dorsal soft rays, 3 anal spins, 7 -8 anal soft rays, and 17-18 pectoral fin rays. 8-10 gill rakers on the upper limb, 15-18 rakers on the lower limb.

Spawning: It exhibits protogyny.

Color: Has a tan body base with brawn bands and blotches. the brown bars radiant bifurcately from the edge of eye, the upper one extend to the brown blotches on the posterior part of dorsal spins, the lower band forked in other sub-bars curving to the upper 3^{rd} to 7^{th}, and last 4 dorsal fin rays, also to 5^{th} to 9^{th} dorsal spines, last band extend breaking in blotches curving to caudal fin.

Feed: Benthic fishes and large invertebrates.

Source: Craig et al., 2011; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No.: 25 of 30.

Species: *E. multinotatus.*

Body Shape: Body depth equal to 2.6-2.9 of standard length, and the head length reaches 2.4-2.7 of standard length, maximum total length is 100 *cm*.

Fin:

Spines: Has 11 dorsal spines, 15-17 dorsal soft rays, 3 anal spines, 8 anal soft rays and 18-20 pectoral fin rays. 9-11 gill rakers on the upper limb, and 15-17 on the lower limb.

Spawning: Matures at 41 – 50 *cm* of total length, it is protogynous hermaphrodite. However, it is spawning aggregately over the entire year but the high activity season represent from August to October.

Color: Olive to dark purplish gray body base of adults, while the juveniles has a dark greyish blue body base with yellow part cover the rear edge of caudal fin, peduncle, soft dorsal fin, and anal fin, with pale whit spot and blotches. the spot distributed irregularly on the body and head, these spots and blotches were be developed as gets larger, corresponding of yellow coloration losing.

Feed: Small fishes and crabs, also, the juveniles imitate the herbivorous damsel fish approaching to their unsuspecting prey.

Source: Craig et al., 2011; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No.: 26 of 30.

Species: *E. poecilonotus.*

Body Shape: Body depth equivalent to 2.6-3.1 times of the standard length, and head length of 2.3-2.5 of standard length, maximum total length is 65 *cm*.

Fin:

Spines: Has 11 dorsal spines, 14-15 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 17-19 pectoral fin rays. 8-10 gill rakers on the upper limb, and 15-18 rakers on the lower.

Spawning: Female matures at 41cm of standard length, exhibits protogyny mode.

Color: The juveniles have a faint yellowish grey with oval black blotches, and pale white, brown and brown black semicircular bands, the rear phase of juveniles has series black spots breaking from the blotches and dark brown bands, while the black spots in the adults were disappeared completely, and the bands colour be more faint. the juveniles have three bandsdescribed as: first bands its dark brown starts from the nape and divided into upper brand curving dorsally and extended broadly over the basal half of the dorsal fin between the 9^{th} spine and 4^{th} dorsal soft rays, while the lower extend to last 4 dorsal fin rays, the second band is brown band corresponding to the first band, start from the interorbital area extending dorsally to black saddle spot on the caudal peduncle. The third band isdark brown start from the lower edge of the eye expanding as a series of dark dots reaching the base of caudal fin. On the adults the bands were pale and fins become yellowish brown, and dorsal fin margin will be orange –yellow, while the other fins part shading to blackish ending with bluish white edge.

Feed: fishes and crustaceans

Source: Craig et al., 2011; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No.: 27 of 30.

Species: *E. polylepis.*

Body Shape: Body depth 2.6-3.3 of standard length, and head length of 1.8-2.4 of standard length, maximum total length is 75 *cm*.

Fin: Straight to slightly concave.

Spines: Has 11 dorsal spines, 16-17 dorsal soft rays, 3 anal spines, 8 anal soft rays and 18 -19 pectoral fin rays. 9-10 gill rakers on the upper limb, and 17-18 on the lower limb.

Spawning:

Color: Has a pale grey body base with dark spots. spots intensely distributed on the head and dorsal part of body and appeared in smaller close –set scattered pattern compered to those distributed on the ventral, with white margin projected at the edge of caudal fins - spawning: exhibits diandric protogynous hermaphrodite.

Feed:

Source: Almukhtar et al., 2012; Craig et al., 2011; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No.: 28 of 30.

Species: *E. polyphekadion.*

Body Shape: Body depth equal to 2.7–3.1 times the standard depth with a head length equal to 2.3–2.5 times the head length, flat interorbital area, and rounded caudal fin. Its maximum length is 90 *cm* SL.

Fin:

Spines: Has 8–10 gill rakers on the upper limb and 15–17 rakers on the lower limb. The maxilla extends past the rear edge of the eye. 11 dorsal spines with 14–15 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 16–18 pectoral fin rays.

Spawning: Matures at 27–30 *cm* SL. Spawning occurs on full-moon nights between February and April and sometimes between January and February. There are separate colonies for each sex, where the female releases hundreds to thousands of eggs, and then the male spreads smoky sperm for fertilization. Throughout spawning activity, the background body color of the fish becomes lighter.

Color: Pale brown basis for the body with dark brown and white spots and dark blotches. Small dark brown spots cover the whole body, even the inner part of the mouth, with irregular dark blotches over small spots and a large black distinguished saddle blotch on the caudal fin in addition to small white spots scattered on the head, body, and fins.

Feed: Primarily feeds on fishes and crustaceans, in addition to cephalopods and gastropods.

Source: Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No.: 29 of 30.

Species: *E. radiates.*

Body Shape: Body depth 2.6-3.0 of standard length, and 2.1-2.3 of standard length is head length. maximum total length is 70 *cm*.

Fin:

Spines: Has 11 dorsal spines, 13-15 dorsal soft rays, 3 anal spines, 8 anal soft rays and 17-18 pectoral fin rays. 8-9 gill rakers on the upper limb and 16-18 on the lower limb.

Spawning: Exhibits protogynous mode.

Color: The colour various based on size and age, for juveniles it is tan body bases with dark brown and black edged pale bands and black spots, for small adults, it is tan body base with dark edge pale bands and dark brown spots, while the large adults it is tan body bases with only dark spots. small adults have five curved and bifurcated oblique edged pale bands with small black spots scattering in addition to pale blotches on the dorsal. The large adults have series of dark spots disappeared on the third ventral part, also small dark spots covered the dorsal fin and caudal fin. While juveniles have dark brown with black edged pale brown bands confined black spots.

Feed:

Source: Craig et al., 2011; Heemstra and Randall, 1993.

Table 4.5 Continued: Morphological characterisitics, No.: 30 of 30.

Species: *E. stoliczkae.*

Body Shape: body depth of 2.8-3.3 of standard length, and head length equals 2.3-2.6 time of standard length, with maximum total length is 38 *cm.*

Fin: Rounded.

Spines: Has 11 dorsal fin spines, and 16-18 dorsal soft rays, 3 anal spines, 8 anal soft rays, and 17-19 pectoral fin rays.

Spawning:

Color: Yellowish grey bases with dark orange spots, dark grey bars and dark oval semicircular blotches. the orange spots were scattered intensely on the posterior part of head and body until ventral, the bars project under the posterior of dorsal fin spines, and two under soft dorsal fin and on the caudal peduncle. The blotches presented on the pectoral fin. The spinous of the dorsal fin is yellowish, with dark red spots at bases, while the median fins have yellowish in the posterior area.

Feed:

Source: Almukhtar et al., 2012.

4.4.3. Morphological analyses

The grouper is one of the most commercially significant tropical and subtropical marine fish, fetching high prices at live seafood markets around the world (Heemstrac and Randall, 1993). There have been multiple reports of grouper classification utilizing classical taxonomic techniques in Indian waters (Roy and Gopalakrishnan, 2011; Kirubasankar, 2013). Color patterns and morpho-meristic traits are commonly used to identify groupers (Heemstra and Randall, 1993).

The morphological investigation of the species in this study revealed a difference; while some species are almost identical in appearance and size, the examination of mitochondrial DNA is highly useful in correcting genetic distance between species, particularly within each species. *E. merra* is distinguishable from other reticulated groupers, according to Heemstra (1993), by its pectoral-fin pattern of noticeable black dots that are primarily restricted to the fin rays. *E. chlorostigma*, which has brown spots and a truncate or emarginate caudal fin with a white posterior margin, is sometimes confused with *E. areolatus*. The color pattern of E. *ongus*, which is sympatric with *E. coeruleopunctatus*, is similar, but the caudal and anal fins have only a few white spots (confined mainly to the proximal part of these fins). Both the genetic distance and the phylogenetic tree demonstrated a close relationship.

Despite the fact that Grouper species are classed based on morphological traits, species identification is difficult due to morphological similarity, varying color patterns, and the likelihood for species to interbreed. As a result of their nearly comparable physical traits, *Epinephelus* species are frequently misidentified in the field. Taxonomic

misunderstanding is common when groupers are identified morphologically (Chatla et al, 2019).

The taxonomy of groupers has changed a lot over the years and is still inconsistent at several levels of the taxonomic hierarchy, from species to families. A number of molecular phylogenetic studies utilizing a range of markers across several taxonomic levels have greatly contributed to clarifying the relationships between groupers and providing a categorization system over the last two decades (Lakra et al., 2009; Craig et al., 2011; Zhuang et al., 2013; Schoelinck et al., 2014; Ma et al., 2016; Basheer et al., 2017; Ghosh et al., 2017; Iswarya et al., 2018). To study the molecular link between *E. hexagonatus* and *E. fuscoguttatus*, Baharum and Nurdalila (2011) used cytochrome b (cyt b) as a molecular marker. Partially sequenced mtDNA cyt b of *E. fuscoguttatus* were found to be 99 percent identical to *E. hexagonatus* using the BLAST database. Nevertheless, in the classification of several common grouper species, the use of the links shown by these research in grouper taxonomy has remained inconsistent and ambiguous. As a result, six species of the genus *Epinephelus* from Indian waters were evaluated for genetic divergence and phylogenetic signal.

4.4.4. Genetic barcoding

Fish identification has traditionally relied on morphological characteristics. However, physical characteristics alone are sometimes insufficient for identifying fish and their various developmental phases. Technologies for detecting molecular DNA have been developed and proven to be analytically successful. Because it is a standardized and uniform technique, DNA barcoding corrects an error in grouper categorization based on morphological assessment (Zhang and Hanner 2012). Besides morphological approaches,

fish can be identified based on significant features such as anatomical, behavior, and habitat, and molecular genetics such as Allozymes (1966), Mitochondrial DNA (1979), Microsatellites (1990), Single nucleotide polymorphisms (SNPs) (2000), Population genomics (2010) (Helfman et all., 2009; Rohde et al, 2009).

By focusing investigation on a short, defined portion of the genome, DNA barcoding is a major diagnostic and taxonomic tool that offers fast, accurate, and automated species classifications (Hebert et al., 2003). The amplification of these DNA fragments using polymerase chain reaction (PCR), followed by sequencing and analysis using international genetic databases such as Barcode of Life (BOLD) and GenBank, has revolutionized the traceability and authenticity of finfish and shellfish species in global markets. Over 6000 different fish species have been identified using DNA barcodes (Lakra et al., 2011). It also found that the fish filets had various levels of fraud (Galal-Khallaf et al., 2014; Di Pinto et al., 2015; Almerón-Souza et al., 2018; Hu et al., 2018; Do et al., 2019). This genetic identification is typically supplemented with DNA barcoding gap analysis. The barcoding gap is the relationship between the highest intraspecific distance within a species and the lowest interspecific distance with its closest neighbor (Pandey et al., 2020). The accuracy of using certain genetic markers as a DNA barcode is particularly reliant on the occurrence of large discordance between genetic distances "inside" species on one side and genetic distances "between" nearby species on the other (Meyer and Paulay, 2005; Abdalwahhab et al., 2020).

The protection and use of Grouper genetic resources require a thorough understanding of fish genetic characterisation. The goal of this research was to determine the genetic diversity and phylogenetic relationships among several Grouper species.

Hassanien and Al-Rashada (2021) used two molecular marker systems, inter simple sequence repeat (ISSR) and microsatellite (SSR) markers, to study the eastern Saudi Arabian coast. A total of 219 grouper specimens (Epinephelus tauvina, Epinephelus coioides, Epinephelus bleekeri, Epinephelus malabaricus, and Epinephelus areolatus) were genotyped using 10 ISSR and 11 SSR primers. The ISSR generated 94 DNA fragments, 44 of which were polymorphic, and each primer produced an average of 2.13 fragments. Although SSR primers produced 107 alleles, they were all polymorphic, with an average of 9.72 per primer. ISSR and SSR approaches revealed a high amount of gene diversity and genetic distances between grouper species, as shown by UPGMA dendrograms. The findings demonstrated that SSR markers were very informative and effective in detecting genetic diversity and connections in Epinephelus spp.

DNA barcoding, or the sequencing of a small standardized piece of DNA, has been regarded as a revolutionary way for identifying animal species (Hebert et al., 2003). The technique uses universal primers to amplify a 650-bp segment of the mitochondrial cytochrome c oxidase I (COI) gene. This region is sequenced to create a DNA barcode for the specimen, which is compared to barcodes from source specimens to provide genetic analysis. Within-species variation for this gene is small when compared to between-species variation. As a consequence, a single sequence or a set of closely related sequences is usually used to differentiate species (Moftah et al., 2011). The frequency of synonymous nucleotide variations is largely responsible for DNA barcoding's capacity to distinguish closely related species, which has enabled it to discriminate 98–99 % of fish species investigated thus far (Ward and Holmes, 2007).

The mitochondrial genome proved to be highly useful in identifying species, especially by using COI and cytochrome b. Also, it is an effective method regarding to its low costs and its abundant source of DNA which make it easy to isolate the DNA and analyze it (Martinsohn, 2011).

Zhuang et al. (2013) gives a comprehensive description of Mitochondrial Genomes for 22 Grouper, and it provides significant new molecular resources for the species identification. wang et al. (2020) proved that it is possible to discover cryptic species based on DNA barcoding. The study reported two new cryptic species: a cryptic species in the T. minxianensis population and a cryptic species in the T. robusta population. Also, this studied to shed light on the importance of combining traditional taxonomies with molecular methods to accurately identify species.

Allozymes are gene products of one of the various alleles that have the same function but vary in the sequence of their amino acid and thus in their physicochemical properties so that they migrate different distances in an electrophoretic assay (Martinsohn, 2011). This approach was first applied by Lewontln and Hubby (1966).

It can discover sibling species that are morphologically identical but different genetically, (e.g., Shaklee and Tamaru, 1981) had distinguished two distinct species of bonefish (*Albula neoguinaica*, *Albula glossodonta*) off the coast of Hawaii, which were considered as a single species of bonefish (*Albula vulpes*).

”Mitochondria” are subcellular organelles, creating energy for cellular activity by aerobic respiration. Mitochondria contain their own genome, a single circular molecule of around 16 000 base pairs (Martinsohn, 2011)." The first two groups who published the first reports of genetic variation in mtDNA from natural populations are Avise et al. (1979) and

Brown and Wright (1979). Mitochondrial DNA provided a variety perspective of the genetic structure of natural populations because of its maternal inheritance, and lack of recombination (Allendorf, 2017).

Among the morphologically similar species, the 18S sequences of only *C. ireneae* and *C. buri* are available. Molecular analysis based on partial sequence of the 18S gene shows that the highest percentage of similarity (97.8%) was observed with *C. buri*. This similarity was also confirmed in the phylogenetic tree, where *C. buri* and the new species form an individual cluster supported by bootstrap values of 100%. The genetic distance between the two *Ceratomyxa* is, however, sufficient to separate them into two different species. The two sequences differ by 97 nucleotide substitutions and 34 insertion/deletion events. In this regard we noticed that, of the sequences we analysed, we tended to observe quite a high percentage of similarity between different species, as for example between *C. ireneae* and *C. diamenti* (99.6%), between, *C. dennisi* and *C. moseri* (99.8%) etc. This tends to support the contention that *C. buri* and the present *Ceratomyxa* species from hamour are different.

Table 4.6: Genetic distances between grouper species. (Govindaraju and Jayasankar, 2004; Chatla et al., 2018).

No.	Species ID	E. dia	E. are	E. chl	E. ble	E. coi	E. tau	E. mal	E. lon	E. fas
1	E. diacanthus		0.6387	0.7707	0.6748	0.7238	0.6377	0.5910	0.153	0.161
2	E. areolatus	0.4483		0.7921	0.7099	0.7580	0.6422	0.6966		
3	E. chlorostigma	0.2604	0.2331		0.8451	0.8098	0.6788	0.6323	0.136	0.149
4	E. bleekeri	0.3934	0.3426	0.1683		0.7779	0.6313	0.5685	0.112	0.138
5	E. coioides	0.3233	0.2771	0.2109	0.2512		0.8576	0.7993	0.018	0.017
6	E. tauvina	0.4500	0.4428	0.3875	0.4599	0.1537		0.8181		
7	E. malabaricus	0.5259	0.3615	0.4584	0.5648	0.2240	0.2008			
8	E. longispinis	0.017		0.016	0.015	0.163				0.018
9	E. fasciatus	0.018		0.016	0.016	0.157			0.157	

4.5. Conclusion

Fish come in a wide variety of shapes, sizes, and colors. The definition and identification of types of fish are important not only for taxonomists and systematists, but also for natural history and ecology research, fishery control, monitoring the dispersal patterns of eggs and larvae, estimating recruitment, and spawning areas, and food product authentication.

Historically different strategies have been used to identify species of fish. "Historically important contributions to ichthyology were made by Linnaeus, Peter Artedi, Georges Cuvier, Achille Valenciennes, Albert Günther, David Starr Jordan, B. W. Evermann, C. Tate Regan, and Leo S. Berg, among many others. “Taxonomy” deals with describing biodiversity (including naming undescribed species), arranging biodiversity into a system of classification, and devising identification keys. Rules of nomenclature govern the use of taxonomic names.”

Generally, morphological characteristics have been used to identify fish. Nevertheless, because of their high diversity and physical flexibility, fish and their various developmental phases are often difficult to recognize just based on morphological traits. DNA-based recognition technologies have been developed and have been shown to be analytically effective DNA barcoding recognition technologies have been extensively recommended in recent years as a uniform and universal method for identifying species and uncovering biological diversity.

The morphological characteristics and color patterns of groupers are used to identify them, although the variety of these color patterns sometimes leads to taxonomic misunderstanding. The evolutionary connection of groupers has been well clarified (Ding

et al., 2006; Ma et al., 2016), and various research about DNA barcoding of groupers have also been published, thanks to the widespread use of molecular genetic tools over recent decades (Alcantara and Yambot, 2016; Aziz et al., 2016). Nevertheless, widespread species' intraspecific diversity, putative cryptic species, and probable synonyms have yet to be completely identified. In this chapter, both morphological and genetic characteristics associated with Grouper species are highlighted.

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