Necessity of DNA barcoding to identify prospective marine organisms in Bangladesh

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Abstract:

Marine biodiversity is more critical than terrestrial. Marine species identification is difficult because of their variation. DNA barcoding is the scientific process where a short DNA fragment is used to identify species. The DNA sequence of unknown species is compared to the reference library of such DNA markers. Various gene regions are used in DNA barcoding. Cytochrome c oxidase I (COI or COX1) is most commonly used in species identification which is found in mitochondrial DNA.16S rRNA gene is widely used in prokaryotes. On the other hand, the 18S rRNA gene is used for microbial eukaryotes. Recently COI sequences were identified from 29 species of threatened fish in Bangladesh. Recently, 237 freshwater fish species DNA was sequenced, and 12 new species have been identified by DNA barcoding. Bangladesh has a maritime area of about 207000 square kilometers which is full of potential marine organisms. However, no systemic approach has been taken so far to identify these vast marine resources of commercial importance that may contribute to the blue economy. This article depicts the necessity of DNA barcoding for molecular identification and characterization of different potential marine organisms.

1. Introduction:

DNA barcoding is a new and exciting tool for the quick identification of any species of all life forms using a short nucleotide sequence. Paul D. N. Hebert et al. from the University of Guelph, Ontario, Canada, suggested current DNA barcoding as a method for identifying species in 2003 [1]. DNA barcoding has two basic steps, which is creating DNA barcode library of known species and identification of unknown samples against the barcode library using barcode sequence. Biodiversity monitoring (e.g., ecological, taxonomic, and conservation studies) and forensic science are examples of fields where DNA barcoding can be used. Furthermore, DNA barcoding could be used to track illegal wildlife trade, such as endangered or protected species [2, 3], or to

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determine the species of food produced commercially [4, 5]. Because of food safety issues, such as inaccurate food labeling, food replacements [6, 7], or recent food contamination [8], the use of DNA barcoding for food authentication has lately gained traction. Consumers must be protected from potential food adulteration, ingredient mislabeling [4], GMOs [9], and food poisoning [10] by correctly identifying species found in food.

Oceans contain two-thirds of the earth's surface; therefore, identifying marine biodiversity is the most challenging task. Due to global population growth, there is a tendency to exploit marine resources for food, energy, and other requirements. Traditional taxonomic methods for identifying marine organisms are sometimes challenging by taxonomic controversies. This molecular technique could be beneficial in assessing cryptic species that are common in the marine environment, as well as correlating the many life cycle stages to the adult, which is difficult to do in the marine ecosystem. Currently, CMarZ , CeDAMar, MarBOL ,SHARK-BOL, and other global DNA barcoding projects are underway for identification of marine habitat and marine ecosystem. DNA barcoding, especially combined with newer and faster techniques like high-throughput sequencing, can be an effective modern tool for assessing and conserving marine biodiversity [11].

Bangladesh is situated between the biologically varied Indo-Burma and Eastern Himalaya areas and is crisscrossed by three of Asia's greatest rivers, the Ganga, Brahmaputra, and Meghna, all of which fall into the Bay of Bengal [12]. Bangladesh's economic growth can be accelerated by making sustainable use of the sea resources [13]. The Blue economy concept, which involves the use of sea and marine resources, has opened a new horizon for Bangladesh's economic development. The blue economy is defined as any economic activity that relies on ocean resources. Thus, the blue economy and sustainable development are interrelated. Marine biotechnology will contribute significantly to the growth of this blue economy which explores the oceans to develop novel pharmaceutical drugs, chemical products, enzymes, and other industrial products and processes. It also plays a vital role in developing biomaterials, medical, health care diagnostics, fisheries, aquaculture, seafood safety, bioremediation, and bio-fueling. So, to ensure the proper use of marine resources, accurate identification is the first priority, which can be done by DNA barcoding.

This article delineates the importance and necessity of DNA barcoding for new species and organism identification, thus contributing to the blue economy of Bangladesh.

2. What is DNA Barcoding

DNA barcoding is a modern standard method to discover the unknown species. Some marker DNA are used to detect species. DNA barcoding is a DNA based technique where a small sequence of DNA is used to species identification. Marker DNA can be collected from nucleus, mitochondria and chloroplast. Cytochrome oxidase c subunit 1 is the ideal marker for eukaryotic species identification and *18S rDNA*, *28S rDNA*, and internal transcribed spacer are commonly used DNA barcodes [14–16]. Internal transcribed spacer (ITS) rRNA used for fungi and RuBisCO used for plants [17, 18]. Real-time DNA barcoding opens new chapter on biodiversity assessments and to understand the variation in genes, species, and ecosystems [19]. DNA barcoding provides a easy, simple and automatic qualitative method. Already this technique has been applied to different species [20–22]. Marine mollusks can be studied by this method [23].

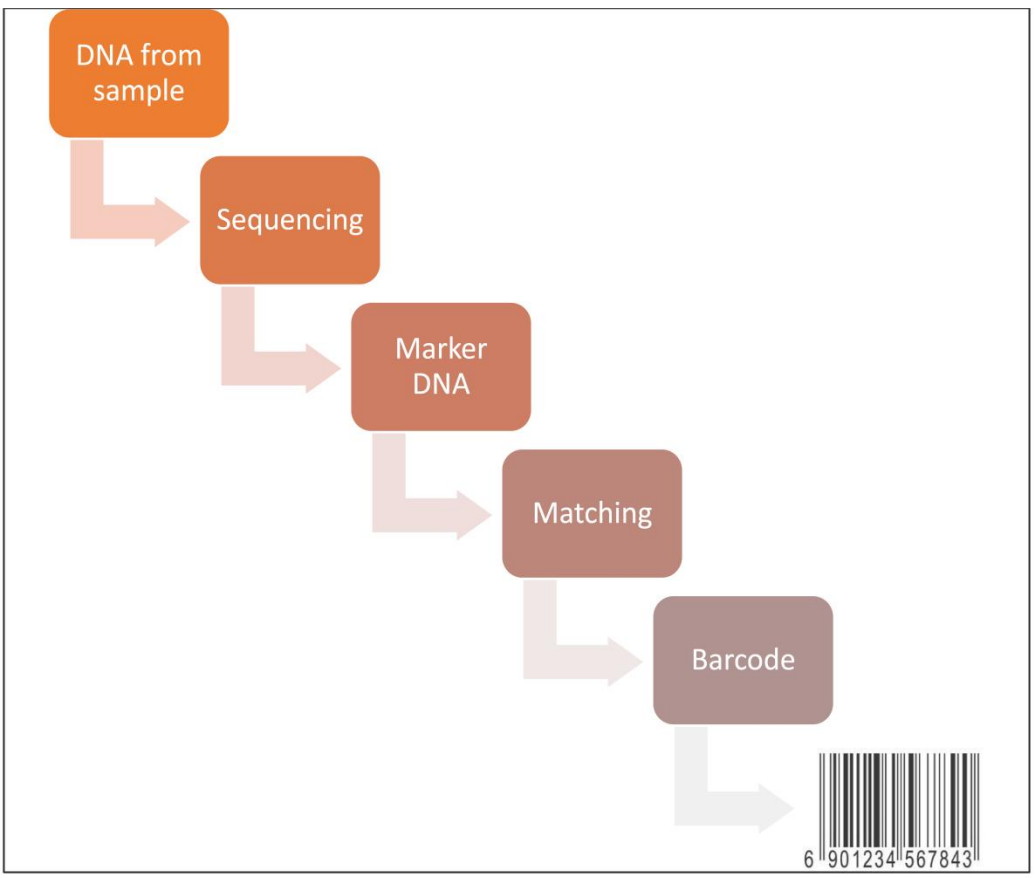

Figure 1: DNA Bar coding

3. How DNA Bar coding is done

DNA barcoding is the molecular technique for species identification. This technique is performed by few steps like i). Sample collection ii) Tissue sampling iii) DNA processing iv) PCR v) DNA amplification and sequencing vi) Bioinformatics (Blast) vii) Sequence similarity and species identification [24–26].

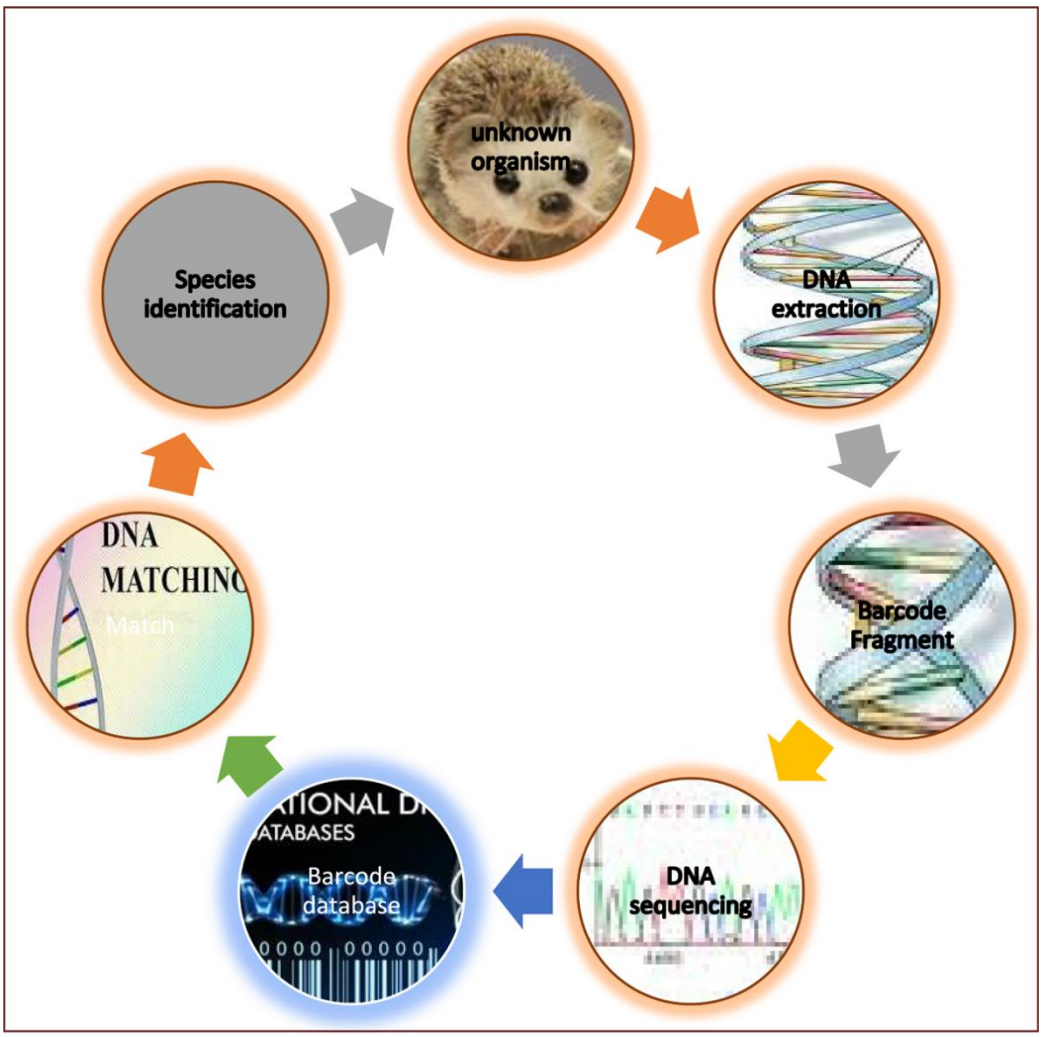

Figure 2: DNA barcoding workflow [27].

4. Applications and advantages of DNA bar coding

i. Species identification

DNA barcoding is the ideal tool for species discovery and identification. Which species are difficult to identify they also identified easily [28]. Barcoding and metabarcoding helps to increase number of world's species by discovering biodiversity [29]. Fish, mollusk, algae, plant, animal species can easily identified by this molecular method.

ii. Identifications of species where morphology is ambiguous

Ambiguous species are uncertain and difficult to understand. Genetic and morphological identification of species is very important for safe food chain and food industry. Molecular identification is significant for the alpha and beta diversities [30]. Hidden biodiversity can be discovered by DNA barcoding [31].

iii. Forefront technology

DNA barcoding is the latest and advanced molecular technique for identification. In oncology research advanced level diagnosis is done by sequencing of tumor DNA for better understanding of disease [32].

iv. Evolutionary perspectives

Tigers are one of the endangered species. The molecular evolution of tiger diversity can be analyzed through DNA barcoding [33]. Biodiversity of marine mollusk are measured by this molecular method [34]. DNA Barcoding is the advanced technique for the assessment of biodiversity of species of coral reef [35].

v. Ensuring food authenticity

There are some people who fraud the people through food. Food has a great impact on food chain and economic status of a country. By applying DNA barcoding food authenticity can be prevented [36].

5. Limitation of DNA Barcoding:

Low resolutions in the case of recently developed species, species complexes, and hybrids are some of the limitations of DNA barcoding. Collins and Cruickshank (2013) identified seven flaws and suggested ways to address each of them. The following are seven flaws in the experimental design that these researchers addressed:

a) Failure to test clear hypotheses.
b) Inadequate a prior identification of specimens.
c) The use of the term 'species identification'.
d) Inappropriate use of neighbor-joining trees.
e) Inappropriate use of bootstrap resampling.
f) Inappropriate use of fixed distance thresholds.
g) Incorrectly interpreting the barcoding gap.

Finding a universal barcode for all biological forms is more challenging than previously thought, according to several research.

6. Solving the Identification and authentication problem of fishes

Food identification and authentication is the rising demand of food industry for a safe food chain. It is very important to maintain a safe food chain for better growth and development. Some unscrupulous businessmen bamboozle us by selling the food product that is not the real one. Example: Hilsa is the most precious fish in our country. It has a great market value all over the world, but some people try to fraud with this asset. This problem can be solved by identification through DNA barcoding. Bangladesh is an economically developing country but there some problems in food security on food industry. DNA barcoding can protect Fraudulence in fish market and can prevent future incidences of mislabeling in Bangladesh fish market. Genetic diversity of Hilsa species from different source is analyzed with bio-molecular tools [13, 37].

7. Food Adulteration

Food adulteration is a punishable act. It is a common problem in food industry around the world. Some fraud people add another item of food to increase the quantity of food item, but the quality of these food materials is not same. As a result, this food fails to meet the legal standards. That affects food value in the world market. That's not a good sign for a developing country. In quality control process DNA barcoding is used to identify the contaminants in processed food [38]. Herbs and spices are substances derived from plants that add flavor to any food item. These are vulnerable in case of food adulteration. DNA metabarcoding is the modern tool for quality control of fresh food items [39].

8. Identification of Marine resources of Bangladesh (Fish, flora, fauna, algae, sponges etc.)

8.1 Marine Fish

Fisheries resources play an important role in meeting the country's demand for animal protein as well as its socioeconomic development. Bangladesh is endowed with large marine, coastal, and inland water resources with great fisheries production potential, accounting for 3.69 percent of the country's GDP and nearly one-fourth (22.60 percent) of agricultural GDP. Bangladesh's marine fisheries resources play an important role in the country's economy, accounting for around 17% of overall fisheries production of 3.55 million MT in 2013-14 [40].

In the lack of an expert-based taxonomy, DNA barcoding appears to be a significant component in biological conservation and management of biodiversity and fisheries of Bangladeshi freshwater fishes, given the economic importance of inland fisheries and the projected richness of the fish species.

9. Marine Fauna

Based on limited records, Bangladesh's maritime fauna includes roughly 1,600 vertebrate species and about 1,000 invertebrate species. There are around 22 species of amphibians, 708 species of fish, 126 species of reptiles, 628 species of birds, and 113 species of mammals in the vertebrate fauna. Bangladesh has over 1,600 vertebrate species in its marine biodiversity [41].

30 species of aphids, 20 species of bees, 178 species of beetles, 135 species of flies, 400 species of spiders, 150 species of lepidopterans, 52 species of decapods, 30 species of copepods, 2 species of starfish, and some species of sand dollars, sea cucumbers, and sea urchins make up the invertebrate fauna.

Therefore, DNA barcodes can play an important role for the identification of unidentified marine fauna in Bangladesh.

10. Marine Flora

Microflora (such as bacteria, actinobacteria, cyanobacteria, and fungus), microalgae, macroalgae (such as seaweeds), and flowering plants (mangroves and other halophytes) are all included in the category of "marine floras". The ocean covers over 71% of the Earth's surface and is home to an abundance of species; more than 90% of the ocean's biomass is made up of microscopic flora and algae [42]. Several minerals found in marine flora have been shown to have therapeutic value. They are a good

source of protein, carbohydrates, fats, vitamins, minerals, and antioxidants such flavonoids and phenolic acids. Using marine floras over the course of a lifetime can alter the follicular phase of the menstrual cycle, where a woman produces the most estrogen, hence reducing her lifetime risk of developing breast cancer. Chemicals with biological activity and therapeutic potential are abundant in marine flora. A large proportion of chemicals, including polyphenols and polysaccharides, can be used for antioxidant and anticancer purposes [42, 43]. There are many different types of marine flora, but the most common ones are the brown, blue, green, blue-green, and red algae. Brown algae are the seaweeds that have the widest range of applications [43]. All along Bangladesh's coastline, particularly on St. Martin Island, Coxs Bazar, and in the Sundarbans Mangrove forest, seaweeds can be found. There are a total of 193 species of seaweed, including 19 that are commercially important, spread across 94 genera [44]. Algae's outward appearance and morphology can shift depending on their habitat and the surrounding environment. Several phenotypes can be shown by the same genotype. This results in incorrect identifications being made occasionally, even by experts. Female reproductive architecture and the post fertilization event make red algae differentiation much more challenging [45]. There has been a steady decline in sea grass, and the largest mangrove forest in Bangladesh is losing its distinctive floral species variation [46].

DNA barcoding has the potential to be a useful identifier in such situations, both for species identification and preservation and for providing phylogenetic information for constructing a unified mangrove management strategy.

11. Marine Sponge

A total of 4851 compounds have been isolated from marine sponges of the phylum Porifera (Metazoa), making them the most abundant source of new marine natural products among all marine creatures studied. This accounts for over 30% of all marine natural products found to date. In the five years between 2008 and 2012, 1499 of these compounds were isolated for the first time. Anticancer, antiviral, antibacterial, antifungal, antiprotozoal, anthelmintic, antiinflammatory, immunosuppressive, neurosuppressive, neuroprotective, and antifouling are only some of the biological properties that have been discovered for these substances through extensive research. Additionally, marine sponges provide innovative leads against bacterial, fungal, and viral illnesses, which is especially important since infectious microbes change and develop resistance to existing medications [47]. Quader et al reported 3 marine sponges found in Bangladesh [48]. In marine ecosystems, marine sponges play a crucial role in

the intake, processing, and release of (in)organic nutrients due to their high filtering capacity and intimate interaction with different symbiotic bacteria [49]. Sponge taxonomy and systematics are intricate, and morphological identification can be time consuming and inaccurate because of phenotypic convergence, secondary losses, and other factors. DNA barcoding offers biologists studying sponges a quick and easy way to determine the phylogenetic affiliation of samples that have previously eluded classification [50].

12. Application of DNA bar coding across the world

12.1 DNA barcoding of marine microbes

Biodiversity assessment in the microbiological world has always been a difficult issue. Microorganisms must often be identified quickly and accurately to avoid the spread of diseases caused by microbes. Protists are eukaryotic microorganisms with a fast generation period and the ability to reproduce asexually. Dinoflagellates, which function as primary producers, coral symbionts, and induce red tides, are an ecologically significant group of protists. Dinoflagellate diversity was found by DNA barcoding of marine ecological samples [37].

13. DNA barcoding of mangroves and seagrasses

Mangroves, which are found at the confluence of the terrestrial, estuarine, and near-shore marine ecosystems, are extremely important ecologically and economically. Mangrove forests provide ecological benefits worth at least US$1.6 billion per year globally [38].

This dynamic and unique environment is becoming increasingly endangered and degraded. There has been a reported loss of evolutionary distinct species in the mangrove environment, and DNA barcoding has provided phylogenetic information for constructing a global mangrove management strategy [39].

The Sunderbans is the world's biggest tidal halophytic mangrove forest and has been designated as a UNESCO World Heritage Site. (http://whc. unesco.org/en/list). It is known as the world's largest natural nursery, as it attracts a significant number of marine and estuarine species to breed, and the juveniles stay back to exploit its rich natural resources [51].

DNA barcoding molecular approaches based on the rbcL subunit of the RuBisCO enzyme were utilized to identify phytoplankton groups smaller than 10 lm in the Sunderbans mangrove ecosystem [52].

Seagrasses are essential submerged flowering plants that have a significant ecological impact on the coastal environment due to their high primary productivity and ability to recycle nutrients. Aside from that, they contain beneficial secondary chemical compounds like as phenolic acids, which are employed in traditional medicine. Seagrass-derived rosmarinic acid and zosteric acid are commonly applied as antioxidants and antifouling agents, respectively. so, seagrass assessment and conservation are needed for our sustainable development.

DNA barcoding can be beneficial as an identification technique in such a case. Seagrasses have been identified using a variety of markers like nuclear ITS for Halophila [53], trnK introns and rbcL for Zostera [54], ITS1, 5.8S rDNA and ITS2 for Halophila [55]. It was discovered that DNA barcoding for seagrasses may be developed utilizing the rbcL and matK sequences [56].

14. DNA barcoding of marine algae

Using morphological methods, different species of red marine macro algae are typically difficult to distinguish. Although COI proved successful in identifying species, due to a lack of universal primers, not all species produced successful amplicons. Gracilariaceae is a red algae family that is commercially valuable for its usage as a phycocolloid agar in biotechnology and microbiology research. Gracilaria species are difficult to distinguish morphologically, and DNA barcoding has the ability to assist in species identification [57]. Harmful Algal Bloom (HAB) species can be detected using DNA barcoding, which is a fast, sensitive, and reliable tool for monitoring programs in marine and coastal ecosystems.

15. DNA barcoding of marine zooplanktons

Zooplanktons are important ecologically and represent 15 different animal groups (phyla). Consequently, zooplankton DNA barcoding is a significant part of modern ecological research. CMarZ has DNA Barcoding Centers in UConn (USA), Bremerhaven (Germany), ORI (Japan), Qingdao (China), and Goa (India). A barcode study utilizing the COI gene of 52 specimens from 14 species of chaetognaths was able to successfully distinguish between various chaetognath species across the phylum. Within species, the average K2P distance was 0.0145. Copepods are one of the most

systematically complex and ecologically relevant groups of marine zooplanktons, having about 2500 species. Because it can be difficult to distinguish between chaetognath species based on physical characteristics, especially when specimens are kept in alcohol, DNA barcoding can be a useful tool for resolving this issue (Jennings et al., 2010b) [58].

16. DNA barcoding of marine mammals

Mammalia Barcode of Life (http://www.mammaliabol.org) is a site dedicated to mammalian barcoding, especially marine mammals. A study along the French Atlantic coast found that DNA barcoding combined with a stranding network may be used to monitor marine mammal diversity [59].

16. DNA barcoding of sea birds

In September 2005, All Birds Barcoding Initiative (ABBI) was founded with the goal of barcoding approximately 10,000 recognized species of world birds. Netherlands, which is ornithologically one of the best covered countries, had 387 individuals of 147 species of birds (including seabirds) subjected to DNA barcoding research [60]. More efforts are needed for DNA barcoding of sea birds and their conservation due to the degradation of mangrove forests, ocean acidification, increased salinity, and sea level elevation.

18. DNA barcoding of marine reptiles

With 468 specimens from Madagascar's biodiversity hotspot, the first large-scale DNA barcoding of reptiles (including Squamata and Testudines) was done. According to a study conducted on Brazilian sea turtles, species-specific COI barcode tags can be utilized to identify each of the marine turtle species studied [61]. A different study looked at DNA barcoding for globally endangered marine turtles. DNA barcoding is not only a useful method for identifying species, but it can also help with wildlife forensics and conservation genetics [62].

19. DNA barcoding of marine fishes

The marine fisheries sector contributes significantly to food security and economic quality of life. Protein, vitamin D, vitamin B12, iodine, selenium, and omega-3 fatty

acids are all abundant in marine fish. Identification of fish species is essential for fisheries management and food product verification. Fish identification can be done quickly and efficiently using DNA barcoding. FISH-BOL (http://www.fishbol.org) and SHARKBOL (http://www.sharkbol.org) are two of the most widely used global fish barcoding initiatives. Not only may DNA barcoding be used to identify whole fish, but it can also be used to identify larvae, eggs, fillets, fins, and other body pieces that are difficult to identify purely on morphology. Shark fins recovered from illegal fishers in Australia were identified using this molecular technique [63]. Ornamental fish demand is continuously rising over the world. The majority (98%) of 391 ornamental fish species from 8 coral reef locales belonged to different barcode groups, according to COI gene analysis [64].

20. DNA barcoding of marine invertebrates

Because of their vulnerability to ocean acidification, pteropods, which belong to the phylum Mollusca and class Gastropoda, are of special research interest. Barcoding of Diacavolinia pteropods was discovered that the Atlantic specimens belong to a single monophyletic species with likely species-level divergence between Atlantic and Pacific populations [65]. To economic assistance species discovery and conservation, DNA barcoding projects for megadiverse groups like mollusks should be developed [66]. DNA barcoding can be quite effective in species delimitation, according to a study using 315 specimens from roughly 60 venerid species [67].

Oysters from the sea are economically important bivalves. Due to taxonomic issues, identifying oysters primarily based on phenotypic features such as shell form is difficult Habitat has a significant impact on shell morphology, which is regarded as a main distinguishing characteristic [68].Molecular identification may very helpful in these situations. Echinoderms are only found in the sea. A total of 191 echinoderm species from five groups were DNA barcoded. 97.9% of specimens were assigned to known species based on shallow intraspecific versus deep congeneric divergences [69].

Inside the body, sponges have a canal system and pharmacological qualities. The Sponge Barcoding Project (http://www.spongebarcoding.org) is a worldwide initiative. DNA barcoding method capable of analyzing huge sponge samples [50]. Nematodes are well-known in the marine ecosystem for their ability to detect anthropogenic stress. The 18S gene in nematodes was able to amplify across numerous species and had a 97 percent identification success rate [70].

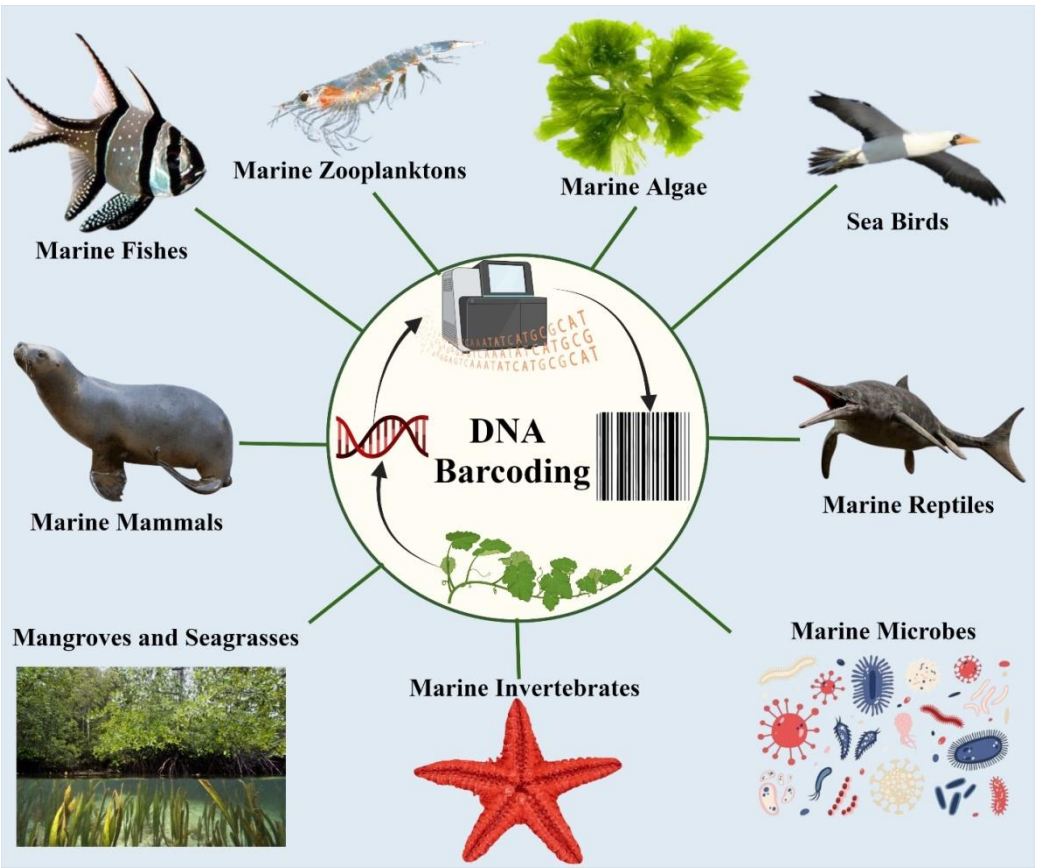

Figure 3: Application of DNA barcoding on Marine Ecosystem.

21. Necessity of implementation of DNA bar coding technology in Bangladesh

Water covers two-thirds of the earth's surface and Bangladesh is a riverine country as well. In this sense, the blue economy may make a significant contribution to the advancement of economic development and social welfare. The 'Blue Economy' was explored during the RIO + 20 United Nations (UN) Conference on Sustainable Development, which took place in June 2012 in Rio de Janeiro, Brazil. The sea is one of the most efficient energy sources. Currently, a huge number of countries are attempting to assure the most efficient use of the sea for long-term development [51]. The blue economy refers to the economic activity based on ocean resources. Bangladesh has 710 km long coastline with 200 Nautical Miles of an exclusive economic zone inside the Bay of Bengal. It includes all economic exercises involving

seas, ports, coastal zones, and other ocean-related activities. It has a strong commitment to the socio-economic sector and is associated with ocean-borne trade and commerce, maritime science, and other economic activities. It benefits countries that use marine resources both nationally and internationally. The purpose of this economy is to create a plethora of shortages around the country. Food, fuel, natural, economic, and currency crises have devastated the globe. A blue economy can deal with these problems in a systematic way. It's a one-of-a-kind view of what's possible in terms of practical economics. Our country's blue economy plays an important role. Since the sea protects over 70% of our globe and accounts for about 90% of global product trade, it is a natural choice. The fact that our country has been awarded a vast marine border. It has created enormous scopes for exploring mineral resources from the seabed without encountering any barriers. Under the seabed and in the water area, there is an abundance of living and non-living assets. In any event, skilled labor to identify availability and investigate assets is in short supply. It’s probably time for Bangladesh to make use of its ocean riches and empower its workforce with learning and creativity in order to achieve national success.

22. Conclusion

DNA barcoding a molecular tool for identification or mapping of species to prevent fraud in market which have a great negative impact on our social economy and health. Eukaryotic organism can easily be identified by COI sequence. DNA barcode data base could serve the platform for further research on species identification in Bangladesh. The DNA barcoding is a genetic tool in detecting food fraud, particularly in fish and meat markets. This type of research work has provided an initial overview of mislabeling in fish markets of Bangladesh. This will be a powerful tools of market monitoring in Bangladesh.

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