Thesis for the Degree of Master of Fisheries Science

The complete mitochondrial genome of Giant African threadfin, *Polydactylus quadrifilis* (Curvier, 1829) (Perciformes: Polynemidae) from Liberia

by

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Pukyong National University

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(라이베리아 연안에 서식하는 Polydactylus quadrifilis (Curvier 1829) 의 전장 미토콘드리아 유전체 분석)

Advisor: Prof. Kim, Hyun-Woo

by

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List of Abbreviations

ATP	Adenosine triphosphate
Bp	Base pair
COI	Cytochrome c oxidase subunit I
CR	Control region
Cytb	Cytochrome B
CZ	Contiguous zone
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphate
EEZ	Exclusive economic zone
FAO	Food and Agriculture Organization of the United Nations
GCLME	Guinea current large marine ecosystem
GPS	Global positioning system
IEZ	Inshore exclusive zone
IUCN	International Union for Conservation of Nature
Kb	Kilobyte
L_C	Common length
L_{Max}	Maximum length

ME	Minimum Evolution
mtDNA	Mitochondrial DNA
NaFAA	National Fisheries and Aquaculture Authority
ND1---ND6	NADH dehydrogenase subunit 1 to 6
ND4L	NADH dehydrogenase subunit 4 L-strand
NGS	Next-generation sequencing
NM	Nautical mile
O_L	Origin of L-strand
PBS	Phosphate Buffer Saline
PCG	Protein-coding genes
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
SDS	Sodium Dodecyl Sulfate
TE	Tris EDTA
TL	Total length
tRNA	Transfer ribonucleic acid
TW	Territorial water

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Abstract

Fish species identification is crucial and equally important for fisheries resource management and its sustainability, which provide the basic criteria for biodiversity monitoring and conservation of the species stock biomass. Using a molecular technique such as sequencing the complete mitochondrial DNA to provide an accurate and fast result for species identification as an alternative method to morphological identification method is emerging. In this research, the complete mitochondrial DNA sequence of Giant African threadfin, *Polydactylus quadrifilis* (Curvier, 1829) was acquired through the MiSeq platform. The accumulated size of *P. quadrifilis* complete mitochondrial genome

was 16,849 bp in length (GenBank accession number: MH995529), it contained 37 genes, including 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and 2 non-coding regions (an origin of light strand replication, O_L and *D-loop* control region, CR). The overall G+C content (45.80%) was less than the A+T content (54.20%), indicating high purine and pyrimidine. Eleven protein-coding genes commenced with a typical start codon (ATG). Unusual start codons include GTG (COX1), and ATC (ATP6). Six protein-coding genes terminated with typical TAA, two genes (ND5 and ND6) terminated with TAG stop codons. An unusual stop codon (AGA) was identified in COX1 and incomplete stop codon (TA-/T--) was identified in 4 genes including ND2, COX2, ND3, and ND4. Transfer RNAs genes were interspersed on each strand of the mitogenome structure, 8 tRNA genes on the L-strand whereas, 14 tRNA genes were on H-strand. The phylogenetic tree analysis showed six species in Polynemidae family including *P. quadrifilis* were grouped together. Among them, *P. quadrifilis* was most closely related to *Polydactylus* sextarius (GenBank No. NC027088) with 80% nucleotide sequence identity. To discriminate between the two species of *Polydactylus* genus, both morphological and molecular characteristics can be used for distinction by taking into account the thread-like filaments on the pectoral fin of both species, and comparing the size of the complete mitochondrial DNA sequences for the reliable taxonomical classification.

1 Introduction

Fish has a significant role in the growing populations of the world; contributing directly to the growth and development of every human depending on it as a source of animal protein and other essential health benefits. Fish and fisheries are cardinal to every human development in terms of food security, livelihood or income generations, and poverty alleviation mostly in the rural parts of the World. Liberia as a coastal state in Sub-Sahara Africa, approximately half of the coastal settlers is actually depending on fishing and fisheries related activities for self-empowerment, job creation, livelihood generation, and food sustainability (Wehye et al., 2017). The increase in fish production to alleviate food insecurity has increased tremendously over the years at an accumulative percent of 3.2 more than the World populations growth from 1961-2013 at 1.6 percent (FAO, 2014, Wehye et al., 2017). It was reported in 2011; 28.8 percent of the global fish

stocks are overfished; 71.2 percent of the caught fish stocks were fished at biological sustainable level far lower than 90 percent reported in 1974; nevertheless, the fully fished stocks were reported at 61.3 percent whereas the under fished at the level of 9.9 percent of the global fish stock biomass (FAO, 2014, Wehye et al., 2017). The fish stock biomass is threatened by unsustainable harvestings such as increased in fishing efforts on the stock without knowing the status of the stock biomass; illegal, unreported and unregulated (IUU) fishing activities, and over-explorations. If these unsustainable methods of harvesting continue on the fish stock biomass only to meet the high demand for human food and cultural fish feed and meal without careful identification of the species within the stock, some species are likely to be extinct. Systematically, fish are the most diverse species found within the aquatic ecosystem (Ward et al., 2005, Moftah et al., 2011); and also they are the most species-rich group of vertebrates that requires careful monitoring of environmental health within the aquatic ecosystem (Hutchings, 2000). In 2015 according to FishBase data statistic (FishBase, 2015),

approximately over 33,200 fish species are being identified morphologically constituting roughly about 50 percent of all vertebrates species including Polynemidae species such as the Giant African threadfin, *Polydactylus quadrifilis.* However, molecular identification of those species in comparison with morphological characteristics would be key to substantially discriminate between synonymous species within the same family or region and would provide classical information for taxonomy studies and sustainable management of all important fisheries resource fish species.

2 Background and Literature Review

2.1 Overview of the species *Polydactylus quadrifilis*

The species *"Polydactylus quadrifilis"* belongs to Polynemidae family. The Polynemidae species are diverse and distributed Worldwide with the exception of the Red and Mediterranean Seas, and it makes up economic important species from the Order of Perciformes. The Polynemidae family comprises of 8 genera and 42 species; they occur in marine, brackish and freshwater habitats. The species in this family have so many usages, some are used for aquaculture, aquariums, recreation, and commercial fisheries purposes (Motomura, 2004). The genus "Polydactylus*"* is the most abundant in the family; it constitutes approximately over 47% of the total species of Polynemidae (Motomura, 2004).

2.2 Classification of *Polydactylus quadrifilis* (Curvier, 1829)

- ❖ Kingdom: Animalia
  - o Phylum: Chordata
    - ▪ Class: Actinopterygii
      - Order: Perciformes
        - o Family: Polynemidae
          - ▪ Genus: Polydactylus

✓ Species: *Polydactylus quadrifilis* (Curvier,1829)

Figure 1. Giant African threadfin, *Polydactylus quadrifilis*

The Giant African threadfin, *Polydactylus quadrifilis* (Figure 1) is one of the most important economic and commercial species of the Polynemidae family. It is exclusively found within the Eastern Atlantic Ocean regions from Senegal to Congo, extending southward to Angola as well, (Fischer et al., 1981, Daget, 2003, Motomura, 2004) and it is also reportedly found in Mauritania (Mohamed Fall, 2005) (Figure 2). The species "*Polydactylus quadrifilis*" is a tropical demersal species that inhabit marine, brackish and freshwater usually at the depth range of 15-55 m (Motomura et al., 2002); in a tropical range of 22°N-5°S, 26°W-13° E (Motomura, 2004). The species has a maximum and a common total length of L_{Max} = 200 cm TL; L_C = 150 cm TL respectively (Daget, 2003, Fischer, 1978); and a maximum weight of 75 kg. The species has 2 dorsal fins, the first dorsal fin contained 9 spines whereas the second dorsal fin contained 12-13 rays; 3 spines within the anal fin and 11-12 rays; the pectoral fins contain 4 threadlike filaments which served as a most distinctive character for identification, and the pectoral fin is lowly inserted on the body which may be sometimes be longer than the upper part

of the fin (Daget, 2003). *Polydactylus quadrifilis* inhabits nearshore coastal shallow waters with sandy and muddy bottoms; and it also occurs in brackish aquatic environments (Motomura et al., 2002, Motomura, 2004). It is an amphidromous species; are caught occasionally in freshwater (Daget, 2003, Motomura, 2004), and occurs abundantly in marine waters. They prey on crustaceans and other smaller fishes (Motomura et al., 2002, Sylvain et al., 2018); and are fleshy as well as delicious for consumption (Daget, 2003).

Figure 2. Africa's map, showing the coverage of *Polydactylus quadrifilis* within the Eastern Atlantic region in pale-yellow (**www.iucnredlist.org/species/21132355/ 42691854**), retrieved: 2018/11/02

Polydactylus quadrifilis is less abundant but valuable for trade and consumption and are mostly caught as bycatch in trawl fisheries, artisanal fisheries, industrial fisheries; and it also targeted by beach seiners (Motomura, 2004). Some studies have been conducted in

the past by researchers that included *Polydactylus quadrifilis* such as the spatial and seasonal distribution of the ichthyofauna (Lalèyè et al., 2003), threadfins of the world (Motomura, 2004), length-weight relationships and condition factors (Kumolu-Johnson and Ndimele, 2010), growth patterns, diet composition and sex ratios (Lawson and Olagundoye, 2011), morphological and trophic status of three Polynemid fishes (Edema and Osagiede, 2011), and feeding habits of Polynemids (Sylvain et al., 2018). However, for sustainable and ecological fisheries management practices more scientific research on *Polydactylus quadrifilis* such as molecular studies are also relevantly important. Although the species status is the least concern according to the IUCN red list of threatened species, (www.iucnredlist.org/species/21132355/42691854), the actual status of the species in Liberia fisheries is yet to be established. In Liberia, to differentiate between *P. quadrifilis* from other related or similar species within the same family such as *Galeoides decadactylus* and *Penntanemus quiquarius* remain challenging and on numerous occasions, these species have only been reported as *Galeoides decadactylus.*

3 Significant of the study

3.1 Thesis statement

Accurate species identification in Liberia's fisheries is one of the numerous challenges faced by the sector. Species misidentification is dangerous for any sustainable fisheries management practices. *Polydactylus quadrifilis* is one of the numerous species in Liberia that are usually being misidentified for other species morphologically, and it requires an accurate identification in order to establish the proper status of this economically important species within the territorial waters of Liberia. *Polydactylus quadrifilis* is an important commercial fishery resource species in Liberia that has data deficiency due to inaccurate morphological identification. In order to sustainably manage this important fishery resource species, it requires some accurate identification techniques both morphologically, through observation of the species external characteristics carefully, and molecularly, through

mitochondrial DNA sequencing, and construction of a phylogenetic tree to provide the evolutionary history of *Polydactylus quadrifilis* within Polynemidae family.

3.2 Objective

This study aimed at sequencing the complete mitochondrial genome of Giant African threadfin, *Polydactylus quadrifilis* from Liberia for the first time. The construction of its genetic structure, a phylogenetic analysis that would provide useful information for an accurate and effective species identification which will enable sustainable and scientific management of this commercial fishery resource species.

3.3 Limitation of the study

The field study lacks basic morphometric and meristic measurements for the body parts such as head length, eye diameter, standard length and body depth that could be used to compare with other closely related species of Polynemidae for differentiation.

Moreover, there were no sequence-specific primers designed for *Polydactylus quadrifilis* in order to obtain large PCR product for sequencing.

4 Materials and Methods

4.1 Area of study description

Liberia is a tropical country, relatively small, and a coastal state on the continent of Africa; specifically located on the west coast of Africa; Eastern Atlantic or within Guinea Current Large Marine Ecosystem (GCLME) region with the geographic coordinates of 06.4281°N; 09.4295°W. Liberia shares border with three countries and the Atlantic Ocean: Sierra Leone (West), Guinea (North), Cote D'Ivoire (East) and the Atlantic Ocean (South) (Figure 1 & 2). Its total area is 111,369 km^2, which contained 96,320 km^2 of land areas, and 15,049 km^2 of water areas.

Liberia has a coastline approximately 579 km long that stretches from east to west where most fish and fisheries related activities occur; with a continental shelf width of 34 km that comprised of moderately warm water with minimum nutrient contents. The

fisheries waters of Liberia is further delimited into zones such as inshore economic zone (IEZ) 6 nautical miles (NM) from shore preserved purposely for artisanal/small-scale fishers, and used as a breeding habitat for some species; the territorial waters (TW) is12 nautical miles from shore where all other related fisheries activities occur; contiguous zone (CZ) 24 nautical miles offshore, and the entire water area of Liberia or area of national jurisdiction “the exclusive economic zone” (EEZ) 200 nautical miles offshore (Figure 3) (Sherif, 2014). Liberia also has 2 main Lakes and 6 major rivers that drained down southward into the Atlantic Ocean creating a rich biodiversity ecosystem for both freshwater and marine or brackish water fish species (GOL, 2014, Wehye et al., 2017).

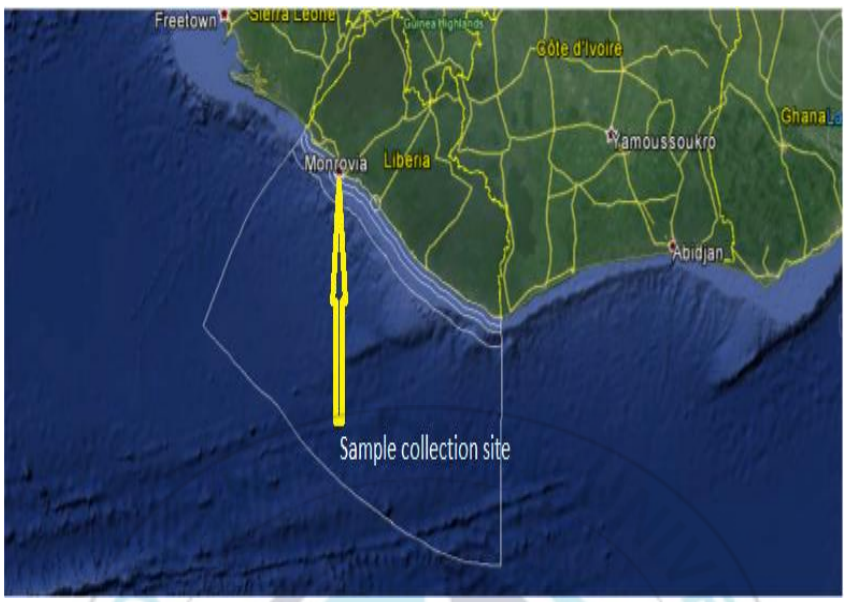

Figure 3. Map of Liberia indicating the sample collection site and various fishing zones (Sherif, 2014)

4.2 Sample collection and preparation

The sample of *Polydactylus quadrifilis* (Figure 1) was collected from the artisanal fishers' fish landing harbor Popo Beach Monrovia (Figure 3), Liberia (GPS location: 06°21.796´ N; 010° 47.595´ W). The sample collection was conducted in May 2018 with the consent of the fisheries managing authority of Liberia. The species was identified based on its morphological

characteristics and other morphometric measurements, which was described in the fish identification guide (Fischer et al., 1981, Strauss and Bond, 1990). After collection of the sample, it was directly preserved in 96 % ethanol and stored in a freezer (-20 ℃) in within the laboratory of the National Fisheries and Aquaculture Authority (NaFAA) Headquarter in Liberia.

4.3 Genomic DNA extraction

The extraction of genomic DNA from the specimen of *Polydactylus quadrifilis* was conducted with the DNeasy® Blood and Tissue Kit (Qiagen, Germany) in accordance with the manufacturer's protocols. Briefly, around 200 mg muscle tissue extracted from the abdominal part of the fish sample in 2.0 mL tube; it was dissected and mixed with 1X lysis buffer which was further homogenized by a motorized Tissuelyser II (Qiagen, Hilden, Germany). Then 50 µL of Proteinase K and 60 µL of Sodium Dodecyl Sulfate (SDS) were added, mixed using vortex mixer (DAEIL TECH, Co. Korea), and incubated at 60 ℃ for 12 hours (overnight) until the tissue was completely lysed. The tube

containing the solution was taken from the incubator and briefly spin down to remove drops from inside the lid; 600 µL of Binding buffer (GC buffer) was added, mixed using vortex mixer, and then incubated at 60 ℃ for 10 minutes. The supernatant was transferred into a new 2.0 ml tube. In order to extract proteins and lipids away from the DNA and help DNA cling to the glass fiber of the Binding column tube, 300 µL of Isopropanol (BIOSESANG, Co. Korea), was added to the solution and mixed well. The solution was spin down briefly, 750 µL (2 times) of the lysate was carefully transferred into the upper reservoir of the binding column tube without wetting the rim, and it was centrifuged at 13,000 rpm for 1 minute. The solution from the binding column was poured, 500 µl washing buffer-1 was added, centrifuged at 13,000 rpm for 1 minute, then another 500 µL washing buffer-2 was added and centrifuged at 13,000 rpm for 1 minute. The binding column was transferred to a new 1.5 mL tube for elution; 100 µL of TE buffer was added into the binding column tube. It was incubated at 60 ℃ for 10 minutes and centrifuged at 13,000 rpm for 2 minutes to elute. The NanoDrop Spectrophotometer, ND-1000 (Thermo

Scientific, Waltham, MA, USA) was used for the quantification of the extracted DNA. The extracted DNA product was kept at -20 ℃ for further analysis (Knebelsberger and Stöger, 2012).

4.4 Polymerase chain reaction and sequencing

A pair of universal PCR primer sets, BCL and BCH (Folmer et al., 1994), was used to obtain a partial sequence targeting cytochrome c oxidase I (COI) region and amplification for species identification. The primers' integrity and quality used in this experiment was analyzed by OligoAnalyzer 3.1 (http://sg.idtdna.com/calc/analyzer) and synthesized commercially (Bioneer Co. Korea). The PCR reaction was prepared in 0.5 mL PCR tube; 20 µL PCR mixture contained 11.2 µL ultrapure water, 2.0 µL 10X Ex Taq buffer, 2.0 µL deoxynucleotide triphosphate (dNTPs) mixture (2.5 µM, Takara, Japan), 1 µL primer (0.5µM forward and reverse), 0.6 µL Dimethyl Sulfoxide (DMSO), 0.2 µL Ex Taq DNA polymerase (Takara, Japan) and 2.0 µL genomic DNA as template. PCR was conducted under the following conditions: the initial denaturation step at 94 ℃ for 3 minutes;

followed by 35 cycles of denaturation at 94 ℃ for 30 seconds; annealing at 50 ℃ for 30 seconds; and extension at 72 ℃ for 45 seconds (COI target sequence). The PCR products were separated by 1.5% agarose gel electrophoresis after staining with the loading star (Dynebio, Sungman, Republic of Korea). The PCR products for targeting the COI partial sequences were further purified using the AccuPrep® Gel purification kit according to the manufacturer's protocol (BIONEER, Korea). The purified PCR products were sent for direct sequencing (BIONEER, Korea); then the partial COI gene sequence was matched with the National Center for Biotechnology Information (NCBI) database and confirmed the species name as *Polydactylus quadrifilis.*

4.5 Library construction and next-generation sequencing

The full mitochondrial DNA sequence of *Polydactylus quadrifilis* was determined by the next-generation sequencing (NGS) platform. The mitochondrial DNA was extracted from the fish sample using

the mitochondrial DNA isolation kit (Abcam, USA) followed by the manufacturer's instruction. Briefly, 200 mg of the fish tissue sample was taken, 3 times wash with ice-cold Phosphate Buffer Saline (PBS), then centrifuged at 600 x g for 5 minutes at 4 ℃ and removed the supernatant. Re-suspend the tissue sample in 1.0 mL of 1X Cytosol Extraction Buffer, and incubated on ice for 10 minutes. The sample was homogenized in an ice-cold Dounce tissue grinder, the efficiency of the homogenization was checked by pipetting 2 µL of the homogenized suspension onto a cover-slip and was observed under a microscope to determine whether cells are still intact. The homogenate was transferred to a new 1.5 mL microcentrifuge tube and centrifuged at 700 x g for 10 minutes at 4 ℃ in order to remove nuclei and intact cells. The supernatant was transferred to a new 1.5 mL tube and centrifuged at 10,000 x g for 30 minutes at 4 ℃. The supernatant was removed, and re-suspend the pellet in 1 mL 1X cytosol extraction buffer and centrifuged at 10,000 x g for 30 minutes at 4 ℃ again. The supernatant was removed and the pellet was isolated mitochondria. The mitochondria were lysed in 30 µL of mitochondrial lysis

buffer, and added 5 μL enzyme mix and incubated at 50 ℃ water bath for 90 minutes or until the solution became clear. Added 100 μL absolute ethanol, mixed and kept at -20 ℃ for 10 minutes. It was centrifuged in a microcentrifuge at top speed for 5 minutes at room temperature. The supernatant was removed, and the pellet was mitochondrial DNA. The pellet DNA was washed 2 times with 1 mL of 70% ethanol, then trace amount ethanol was removed using pipette tip, and air dried for 5 minutes. The DNA re-suspended in 20 μL TE buffer, and the extracted DNA stored at -20 ℃ for further analysis.

The purified mitochondrial DNA was further fragmented into smaller sizes (~350 bp) by Covaris M220 Focused-ultrasonicator (Covaris Inc., USA). For the construction of a library, TruSeq® sample preparation kit v2 (Illumina, USA) was used; and from the fragmented sequence, quality and quantity of the constructed library was measured using 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). The Illumina MiSeq platform 2x300 bp pair ends (Illumina, USA) was performed for NGS sequencing. For the mitogenome assembly, Geneious®

11.0.2 (Kearse et al., 2012) was used.

4.6 Species analyzed for phylogenetic comparison

To authenticate the phylogenetic relationship of *P. quadrifilis* in Polynemidae family, the phylogenetic relationship of *P. quadrifilis* within the family Polynemidae was analyzed. The currently reported mitogenome sequences from 5 species in Polynemidae, 4 species in Sciaenidae and a species in Istiophoridae were used for analysis (Table 1). The phylogenetic tree was reconstructed by Mega7.0 software with the minimum evolutionary (ME) algorithm distances computed by 1000 bootstrap replications (Kumar et al., 2016). The species analyzed in this research complete mitogenome length in bp and submitted GenBank accession numbers were gathered from the NCBI database, and they were combined along with the already-obtained mitogenome sequence of *P. quadrifilis* for the phylogenetic analysis.

Table 1**.** Fish species used for the phylogenetic study

Species	Mitogenome length (bp)	GenBank Accession Number	Reference
Family Polynemidae			
Polynemus dubius	16555	NC029710	(Li et al., 2016)
Polynemus paradiseus	16710	NC026236	(Unpublished)
Polydactylus sextarius	16836	NC027088	(Wang et al., 2016)
Poldactylus quadrifilis	16849	MH995529	Current study
Polydactylus plebeius	16765	NC026235	(Unpublished)
Eleutheronemas tetradactylum	16470	NC021620	(Zhang et al., 2014)
Family Sciaenidae			
Collichthys niveatus	16493	NC014263	(Xu et al., 2011)
Larimichthys crocea	16466	NC011710	(Cui et al., 2009)
Miichthys miiuy	16493	NC014351	(Cheng et al., 2010)
Subfamily Argyrosominae			
Nibea albiflora	16499	NC015205	(Cheng et al., 2011)
Family Istiophoridae			
Makaira indica	16526	NC012675	(Unpublished)

5 Results and Discussion

5.1 Results

5.1.1 Mitochondrial DNA structure

Polydactylus quadrifilis complete mitochondrial genome sequence was acquired through next-generation sequencing and the Geneious® 11.0.2 (Kearse et al., 2012) was used for the assembly. The mitogenome contained 37 genes, which include 13 protein-coding genes (PCG), 22 transfer RNA (tRNAs) genes, 2 ribosomal RNAs (rRNAs), which include the 12S rRNA and 16S rRNA genes, and two non-coding regions (origin of light strand replication, O_L and control region, *D-Loop*) (Figure 4). The mitogenome structural organization and translation direction were typically similar to that of other published vertebrates mitogenomes (Miya and Nishida, 1999, Boore et al., 2005). The mitogenome structure organization of *P. quadrifilis* revealed that

12 protein-coding genes were encoded on the heavy (H) strand with the exception of ND6 along with eight other tRNA genes ($tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, $tRNA^{Ser}$, $tRNA^{Glu}$, and $tRNA^{Pro}$) were encoded on the light (L) strand (Figure 4). The *Polydactylus quadrifilis* mitochondrial genome in comparison are most likely similar to other published bony fish species mitochondrial DNA sequence (Zhao et al., 2015, Wang et al., 2016). The mitochondrial genome of *Polydactylus quadrifilis* also contained 12 overlapping gene regions with the length of 44 bp ranging from 1-19 as well as 13 intergenic spacers regions ranging from 1-35, which accumulates the length of 68 bp (Table 2).

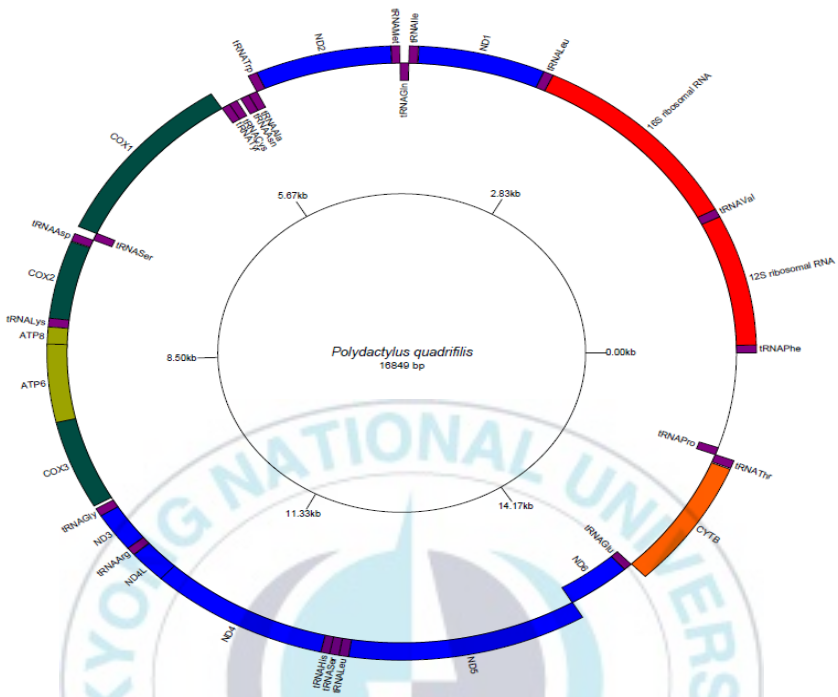

Figure 4. The Organizational structure of the complete mitochondrial genome of *Polydactylus quadrifilis.* The mitogenome was assembly using Geneious® 11.0.2 (Kearse et al., 2012), the heavy (H) strand genes are encoded outside the circle and the light (L) strand genes are encoded within the circle respectively.

5.1.2 Protein-coding genes

The assembled and identified mitochondrial DNA sequences were further annotated and analyzed. The protein-coding genes were

recognized using the NCBI ORF finder (http://www.ncbi.nlm.nih.gov/orffinder/), subsequently annotated by alignment of homologous genes of other published Telestos mitogenomes.

Table 2. Characteristics of *Polydactylus quadrifilis* mitogenome

Gene	Position		Size (bp)	Codon		Intergenic nucleotide (bp)	Strand
	Start	Stop	Nucleotide	Start	Stop		
tRNA Phe	1	70	70			0	H
12S-rRNA	71	1,232	1162			0	H
tRNA Val	1,233	1,305	72			0	H
16S-rRNA	1,306	3,029	1724			0	H
tRNA Leu	3,030	3,105	76			1	H
ND1	3107	4,081	975	ATG	TAA	4	H
tRNA Ile	4086	4,155	70			-1	H
tRNA Gln	4155	4,225	71			0	L
tRNA Met	4226	4,292	67			2	H
ND2	4295	5,340	1045	ATG	T--	0	H
tRNA Trp	5341	5,412	72			-1	H
tRNA Ala	5412	5,483	72			1	L
tRNA Asn	5485	5,557	73			0	L
O_L	5558	5,592	35			0	H
tRNA Cys	5593	5,662	70			-1	L
tRNA Tyr	5662	5,731	70			1	L
COX1	5733	7,286	1554	GTG	AGA	-4	H
tRNA Ser	7283	7,351	69			2	L

tRNA Asp	7354	7,423	70			6	H
COX2	7430	8,120	691	ATG	T--	-1	H
tRNA Lys	8120	8,196	77			0	H
ATP8	8197	8,364	168	ATG	TAA	-19	H
ATP6	8346	9,038	693	ATC	TAA	-1	H
COX3	9038	9,823	786	ATG	TAA	33	H
tRNA Gly	9857	9,924	68			-1	H
ND3	9924	10,272	349	ATG	T--	0	H
tRNA Arg	10273	10,342	70			3	H
ND4L	10346	10,642	297	ATG	TAA	-7	H
ND4	10636	12,019	1384	ATG	T--	0	H
tRNA His	12020	12,088	69			-1	H
tRNA Ser	12088	12,159	72			1	H
tRNA Leu	12161	12,233	73			0	H
ND5	12234	14,072	1839	ATG	TAG	-4	H
ND6	14069	14,590	522	ATG	TAG	1	L
tRNA Glu	14592	14,660	69			4	L
Cyt b	14665	15,807	1143	ATG	TAA	9	H
tRNA Thr	15817	15,889	73			-2	H
tRNA Pro	15888	15959	72			-1	L
D-Loop	15,959	16,849	891			0	H

* Negative (-) numbers indicate overlapping nucleotides between each gene, the positive (+) numbers indicate space/gap between each gene.

5.1.3 Ribosomal RNA and transfer RNA genes

Two ribosomal RNAs (12S rRNA and 16S rRNA) and 22 transfer

RNAs (tRNA) genes of *P. quadrifilis* mitogenome were identified as in any other bony fishes (Boore et al., 2005). The small 12S rRNA gene was situated between $tRNA^{Phe}$ and $tRNA^{Val}$; whereas the large 16S rRNA was situated between $tRNA^{Val}$ and $tRNA^{Leu}$. Two rRNAs 12S (1,162 bp) and 16S (1,724 bp), occupied 17.13% of the total mitogenome of *P. quadrifilis*.

Twenty-two tRNA genes were predicted from *P. quadrifilis* mitogenome, which was identical to other fish mitogenomes (Sektiana et al., 2017, Chen et al., 2016, Ma et al., 2013). The secondary putative structure was constructed based on the result of ARWEN (Laslett and Canbäck, 2007). The mitogenome tRNA genes appeared to be folded in classical clover-leaf secondary structures except for the $tRNA^{Ser}$, that revealed a two arm-like structure (Figure 5).

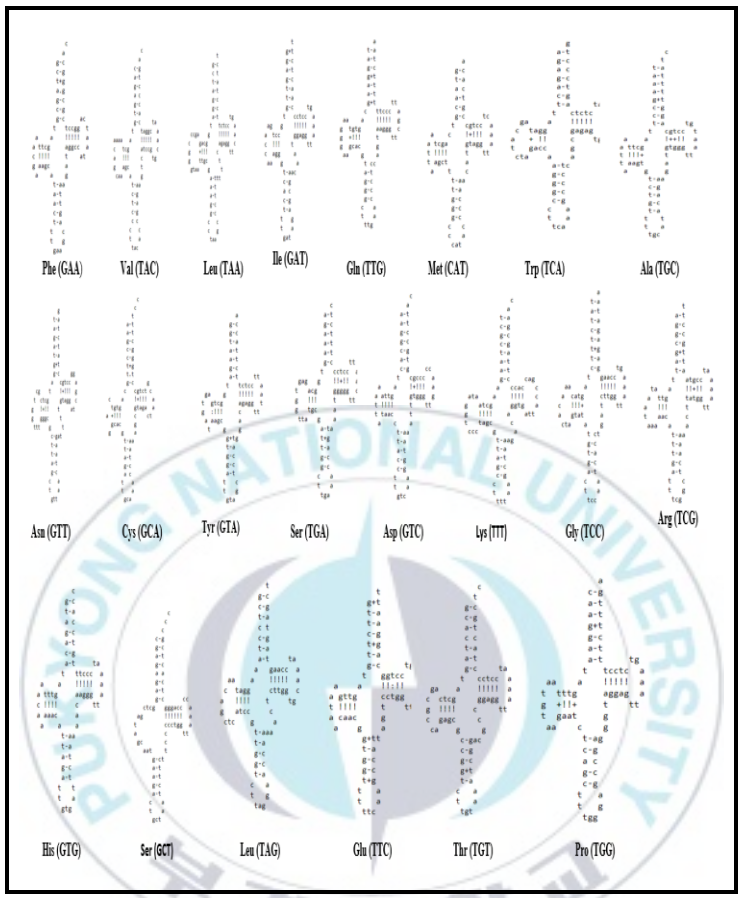

Figure 5. The Putative secondary structure of *Polydactylus quadrifilis* mitogenome twenty-two tRNA genes. The secondary structure for each tRNA was predicted by ARWEN software (Laslett and Canbäck, 2007).

5.1.4 Non-coding regions

The two non-coding regions (origin of light strand replication, O_L

and *D-loop* control region, CR) were found in the mitochondrial genome of *Polydactylus quadrifilis*. The origin of light strand replication, O_L was found between two tRNAs gene ($tRNA^{Asp}$ and $tRNA^{Cys}$) with 35 bp nucleotide, and the control region (*D-loop*) was located between two tRNAs gene ($tRNA^{Pro}$ and $tRNA^{Phe}$) with the calculated size of 926 bp, approximately 5.49% of the total mitogenome 16,849 bp (Figure 4). The control region of *P. quadrifilis* was larger compared to the reported control region size in the mitochondrial genome of *Polydactylus sextarius* (Wang et al., 2016).

5.1.5 Construction of Phylogenetic tree for Polydactylus quadrifilis

The phylogenetic tree of *P. quadrifilis* complete genome was constructed based on the complete mitochondrial genome of three different families including 6 species of Polynemidae, 4 species of Sciaenidae and *Makaira indica* from Istiophoridae family were used as an outgroup to root the evolutionary history.

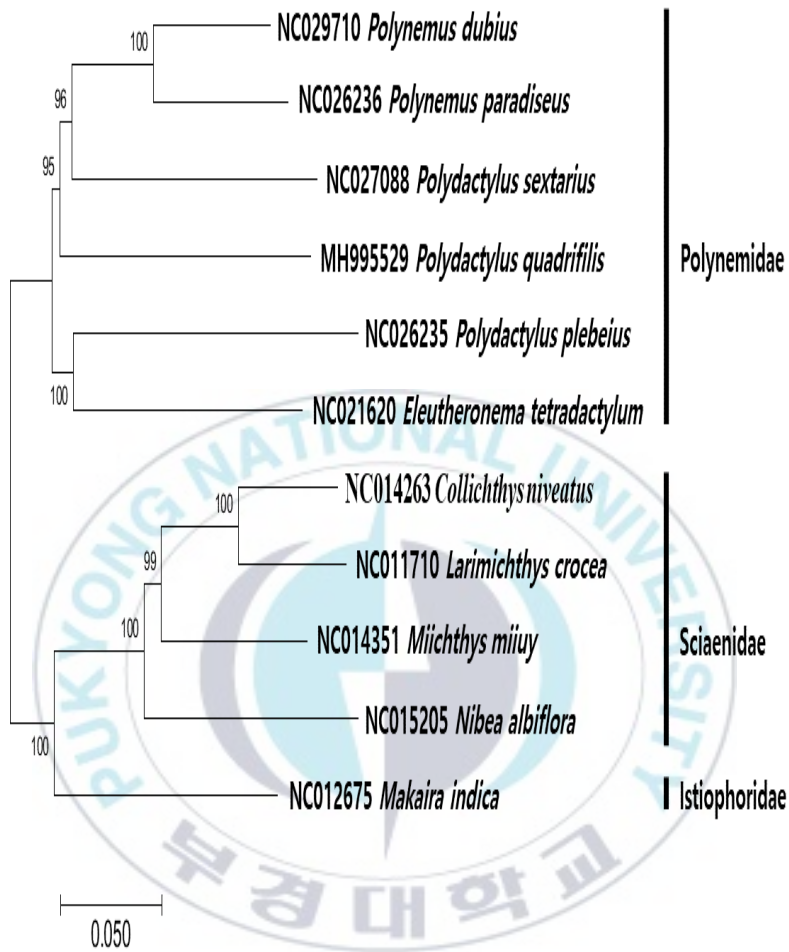

Figure 6. A phylogenetic tree of *Polydactylus quadrifilis* within the Family Polynemidae. The phylogenetic tree of *Polydactylus quadrifilis*, constructed using Mega7.0 software with the minimum evolutionary (ME) algorithm distances computed by (Kumar et al., 2016) with 1000 bootstrap replications.

5.2 Discussion

5.2.1 Role of species identification in fisheries management

Fish species identification is crucial and it is of paramount importance for fisheries resource management and its sustainability. Species identification is necessary, it provides the basic criteria for biodiversity monitoring and conservation of the species stock biomass (Strauss and Bond, 1990). Accurate species identification is necessary for conservation planning, regular stock assessment and to protect the exact ichthyofaunal biodiversity in a given area (Ahmed et al., 2019). Accurate identification of fish species will also provide an in-depth understanding of the species status relative to its abundance and exploitation rates; threatened, vulnerable or endangered; under fished, fully fished, overfished or fished at its sustainable biological levels based on a stock assessment report for sustainable management. Conversely, misidentification of fish species in a fishery is dangerous for fisheries management and its' sustainability. For instance, if a species within a stock is not properly identified, that species is

likely to be depleted unknowingly through over-exploitation or unsustainable harvesting practices. Therefore, the various techniques being instituted nowadays in species identification such as morphological and molecular techniques are significant for fishery resource management.

5.2.1.1 Morphological technique for species identification

The morphological technique for fish species identification is referred to as the traditional method which had been used over decades in species identification. This morphological technique examines the external characteristics of the species such as body parts (dorsal, anal, pelvic, pectoral and tail fins), color pattern, scale size and counts, the number of spines and rays, body shape, and all necessary body and parts measurement as well as any other external distinctive characters that can be used to differentiate between similar or almost identical fish species for identification purposes (Strauss and Bond, 1990). Other features such as the gill rakers are also used in the process of identification most especially between species with great similarity (Iff At, 2002), and if none of

the latter features are available, the otoliths are used for fish species identification, for instance, identifying a fossil or stomach contents. Morphological information on fish species identification is widely found on a website like (www.fishbase.org) and to be more precise like on fish otoliths (www.pescabase.org). The morphological technique has some limitations most especially in the identification and differentiation process of fish species early developmental stages like egg and larvae; or whenever fish species external features are being removed for processing and digestion (beheaded, gutted, filleted, scaled, skinned, etc.); morphological identification in these scenarios are much more difficult and complicated than identification of the whole specimen (Strauss and Bond, 1990, Ward et al., 2009).

5.2.1.2 Molecular technique for species identification

With all the complexities engulfing fish species morphological identifications, researchers are now using alternative measures such as the molecular technique to confirm species identification in line with its morphological identification (Teletchea, 2009,

Boidya et al., 2015).

Besides the traditional species identification based on morphological characteristics, the molecular technique using the mitochondrial DNA partial sequence of the cytochrome c oxidase subunit I (COI) region is widely using for the standardized and a potential tool for an effective species identification (Hebert et al., 2003, Ahmed et al., 2019). The partial sequence region of the cytochrome c oxidase I (COI) and cytochrome b (Cyt b) of mitochondrial DNA are two most frequently used genetic markers for the effective species identification (Palumbi, 1991). Nowadays many researchers are using this approach as a reliable tool for fish species identification effectively including all major taxa of marine and freshwater species (Griffiths et al., 2013, Knebelsberger et al., 2014). Another molecular technique involved the use of genetic information such as the complete mitochondrial DNA sequencing (mtDNA) to provide an accurate and fast result for species identification as an alternative method (Moftah et al., 2011). The DNA sequencing method is emerging as an effective species identification tool to avoid the challenges with the morphological

identification process (Hebert and Gregory, 2005). The increased in taxonomy uncertainties in fish identification is now being resolved through the construction of phylogenetic relationships of a species based on its molecular data in conformity with qualitative morphological information (Moftah et al., 2011). For acquiring a clear understanding of the species biogeographical or ancestry information, sequencing the complete mitochondrial genome of a species will produce that evolutionary information instead of fragmental sequences.

Moreover, the complete mitochondrial DNA of a species also provides vital information that can be used for genetic and biological studies as well as fisheries and aquaculture potentials of the species (Okumuş and Çiftci, 2003, Chauhan and Rajiv, 2010, Yáñez et al., 2014). However, within the GenBank database system approximately over 5,000 mitochondrial genomes have been registered (www.ncbi.nlm.nih.gov) and according to FishBase (www.fishbase.org), over 33,500 fish species being identified morphologically (Sektiana et al., 2017).

5.2.2 Mitochondrial DNA structure and genes arrangement

In this study, the complete mitochondrial genome sequence structure and genes arrangement were observed as a circular molecule that ranged in sizes from 14-20 kb and contained 37 genes (Anderson et al., 1981, Boore, 1999). *Polydactylus quadrifilis* complete mitochondrial genome sequence was 16,849 bp length, it had been submitted to the GenBank with an accession number MH995529 (Figure 4). The mitogenome was slightly longer than the closest species (*Polydactylus sextarius*) mitogenome length 16,836 bp (Wang et al., 2016) which indicate molecular differentiation between the two species (Table 2).

The mitogenome of *Polydactylus quadrifilis* overall base composition were predicted as: A = 4,680 (27.78%), T = 4,453 (26.42%), G = 2,831 (16.80%), and C = 4,844 (29.00%); the overall G+C content (45.80%) was less compared to the A+T content (54.20%), indicating high purine and pyrimidine. The mitogenome structural organization and translation direction were typically similar to that of other published vertebrates mitogenome

(Miya and Nishida, 1999, Boore et al., 2005). Except for ND6 and 8 tRNAs ($tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, $tRNA^{Ser}$, $tRNA^{Glu}$, and $tRNA^{Pro}$), all the other genes were encoded on the H-strand (Figure 4). The gene organizational structure of *P. quadrifilis* mitochondrial was highly similar to those of the published bony fish species (Zhao et al., 2015, Wang et al., 2016). The overlapping regions were identified in 13 genes, which range from 1 to 19 bp (Table 2).

5.2.3 Protein-coding genes

The gene arrangement of the 13 PCGs of *P. quadrifilis* (Figure 4) revealed 12 genes were encoded on the heavy strand (H) whereas a gene (ND6) was encoded on the light strand (L). The protein-coding genes base pair varied in sizes 168 bp (ATP8) to 1839 bp (ND5), which represents 67.93% of the mitogenome of *P. quadrifilis*. The majority of all the protein-coding genes commenced with a typical start codon (ATG). The unusual start codons include GTG (COX1), and ATC (ATP6). Six protein-coding genes terminate with typical (TAA), and two PCGs

terminate with (TAG) stop codons. An unusual stop codon (AGA) was identified in COX1, and incomplete stop codons (TA-/T--) was shown in 4 genes including ND2, COX2, ND3, and ND4 (Table 2). The protein-coding genes termination codons varied in the mitogenome of fish species (Chen et al., 2016, Kim et al., 2004). The incomplete termination codons of protein-coding genes in a vertebrate mitochondrial genome are rare among species (Ojala et al., 1981). Six PCG genes COX1, COX2, ATP6, ATP8, ND4L, and ND5 contained overlapped nucleotides ranging from 1-19, whereas four PCGs (ND1, COX3, ND6, and *Cytb*) contained intergenic spacers that ranged in nucleotides (1-33), and ND2, ND3 and ND4 zero nucleotide (Table 2).

5.2.4 Features of the transfer RNAs genes

The twenty-two tRNA gene of *Polydactylus quadrifilis* complete mitochondrial genome (Figure 5) is foldable into a secondary classical clover-leaf structure with the exception of one tRNA gene ($tRNA^{Ser}$) that showed missing arm structure. This particular $tRNA^{Ser}$ structure had 72 bp in length, the structure was also

revealed in the mitogenome of other organisms such as *Sardinella jussieu* (Sektiana et al., 2017), *Brama japonica* (Chen et al., 2016), and *Scylla paramamosain* (Ma et al., 2013). The twenty-two tRNA genes (Table 2) of *P. quadrifilis* mitogenome were interspersed among the genes, contained 1565 bp diverged in sizes from 67 bp to 77 bp in length, approximately 9.28% of the total 16,849 bp. The tRNA genes were interspersed on each strand of the mitogenome structure, 8 tRNA genes on the L-strand whereas, 14 tRNA genes were on H-strand (Figure 4). Seven tRNAs genes have overlapped nucleotides ($tRNA^{Ile}$, $tRNA^{Trp}$, $tRNA^{Cys}$, $tRNA^{Gly}$, $tRNA^{His}$, $tRNA^{Thr}$, and $tRNA^{Pro}$) that varied from 1-19 nucleotides, whereas nine tRNAs contained intergenic spacer ranging from 1-35 nucleotides, and the remaining six tRNAs genes contained zero intergenic nucleotides.

5.2.5 Phylogenetic analysis of Polydactylus quadrifilis

The phylogenetic tree was constructed using Mega7.0 software, with the minimum evolutionary (ME) algorithm distances computed by (Kumar et al., 2016) with 1000 bootstrap replications.

The species analyzed (Table 1) complete mitochondrial genome sequences used were taken from the GenBank database with the Accession Numbers, and scientific names were shown followed by GenBank accession numbers of each species. As shown in (Figure 6), *P. quadrifilis* form a clade with five species in Polynemidae, and *P. sextarius* (from the Indian Ocean and the Western Pacific Ocean regions) showed the highest identity to *P. quadrifilis.* Morphologically, *P. quadrifilis* can be easily discriminated from *P. sextarius* with its four threadlike filaments within the pectoral fins (Daget, 2003). For the molecular identification to discriminate between the two species, the mitogenome length would be useful for its high degree of nucleotide sequence variation. It is also important to compare the size of the complete mitochondrial genome sequences of the two species for reliable taxonomical classification. In this regard, the species with the smallest mitochondrial genome sequence character in the Polynemidae family could be used as an efficient character to differentiate between the two species.

6 Conclusion

The complete mitochondrial DNA (mtDNA) sequence of *Polydactylus quadrifilis* from Liberia's waters was obtained for the first time in this research. The mtDNA sequence was 16,849 bp in length and contained 37 genes (13 PCGs, 22 tRNAs, 2 rRNAs) and 2 non-coding control region (origin of light strand replication, O_L and *D-Loop* control region, CR). Its mitogenome length was slightly longer than the reported mitogenome length of its sister species *Polydactylus sextarius*. The gene organization of *P. quadrifilis* was identical to other published mitogenome of its relatives such as *Polydactylus sextarius* (GenBank number: NC027088), *Polynemus dubius* (GenBank number: NC029710), *Polynemus paradiseus* (GenBank number: NC026236), *Polydactylus plebeius* (GenBank number: NC026235), and *Eleutheronems tetradactylus* (GenBank number: NC021620). The complete mitochondrial DNA sequence of *P. quadrifilis* was

compared with the complete mitogenome of the ten species from three different families. *Polydactylus quadrifilis* form a clade with five relative species in the family Polynemidae and was most closely related to *Polydactylus sextarius* with 80% sequence identity. The relationship between these two species is very complicated and need further studies and analysis.

In this research, *P. quadrifilis* was identified as a true origin of the Eastern Atlantic region and a native species that inhabit the territorial waters of Liberia along with other species of Polynemidae such as *Penntanemus quiquarius* and *Galeoides decadactylus.* Although *P. quadrifilis* is less abundant compared to other Polynemidae species in the region especially in Liberia, more assessments need to be conducted to know the actual status and spatial distribution of the species in Liberia. The reported complete mitochondrial genome sequence of *P. quadrifilis* collected from Liberia in this research would help to provide accurate information for sustainable and scientific management of this commercially important fishery resource species. For more accurate species identification and sustainable fishery resource

management, sequencing of the complete mitochondrial genomes of other unreported Polynemidae species such as *Galeoides decadactylus, Penntanemus quiquarius,* and as well as other commercially important species within Liberian waters would provide useful information to distinguish between synonymous species.

As the world is being modernized through the advancement of technologies, the application of molecular technique in fisheries management for species identification would be helpful to circumvent morphological uncertainty. The molecular technique could contribute to accurate identification of fish species, and its usefulness for taxonomical classification and sustainable management of fish species resource.

7 Recommendations

Sustainable fisheries management and practices are cardinal for the existence and credibility of any fisheries management authority in a particular country, especially countries that are solely depending on fisheries resources for economic growth and development, food production and livelihood earning. To adequately manage the diverse, complex and synonymous fish species in the coastal waters of Liberia, accurate species identification is key for sustainable fisheries management, conservation and exploitation of economic commercially important species in Liberia for the current and future generations. Therefore, the following recommendations should be highly considered in order to sustainably manage the fish resources of Liberia:

1) Comprehensive stock assessment should be conducted to establish the status of *Polydactlyus quadrifilis* and others

important commercial species within the territorial waters of Liberia.

2) Train data collectors such as Fisheries Observers, Inspectors, and Enumerators on species identification morphologically; taking into consideration the various external features used to discriminate between synonymous species.

3) Since the National Fisheries and Aquaculture Authority of Liberia is lacking research laboratory for molecular and others emerging studies for species identification, collaboration with others well-equipped research institutions are highly encouraged and as well as providing training for Research Officers in this area of studies.

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