UNIVERSITY OF CAPE COAST

GENETIC CHARACTERIZATION OF ATLANTIC CHUB MACKEREL (*SCOMBER COLIAS*) IN GHANA

EUNICE OSEI-YEBOAH

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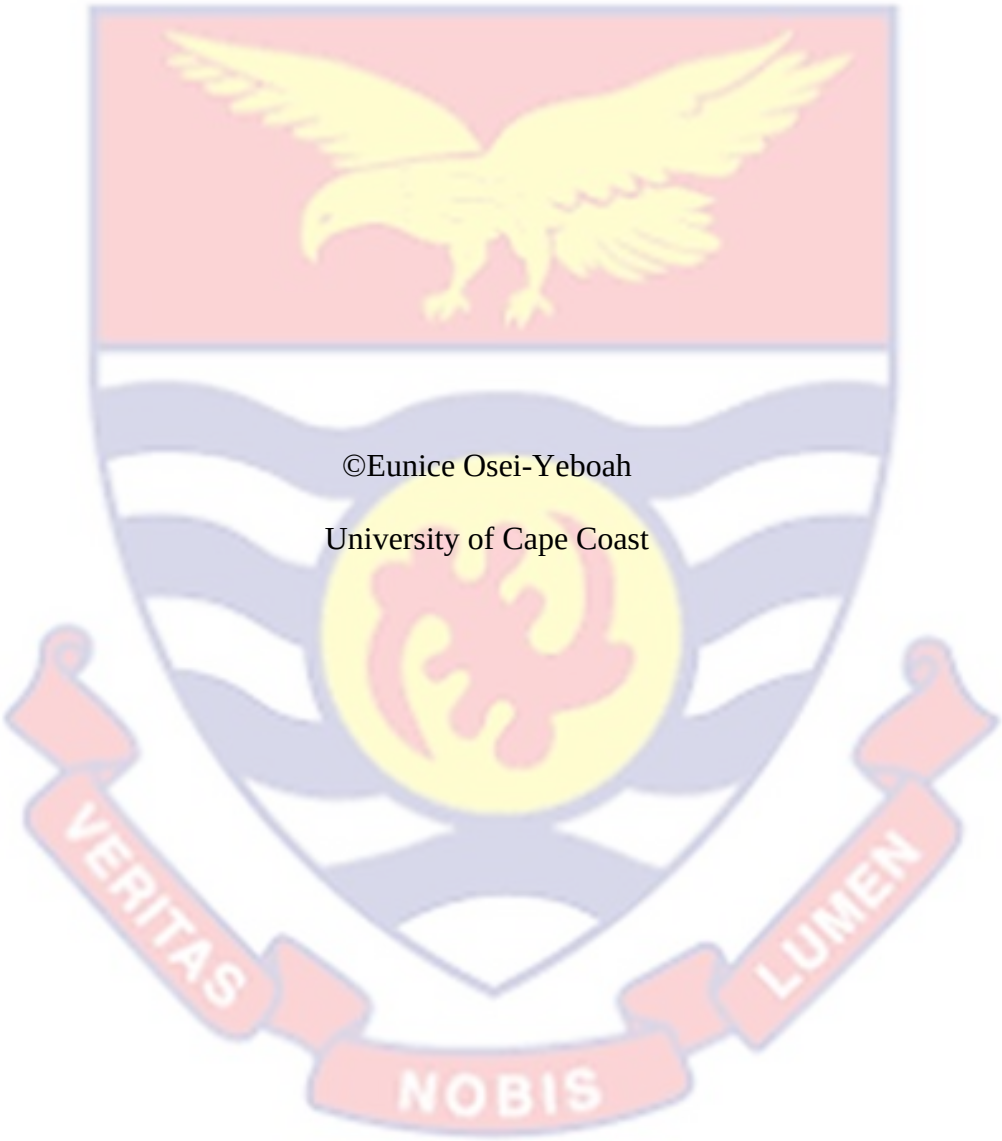

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GENETIC CHARACTERIZATION OF ATLANTIC CHUB MACKEREL (*SCOMBER COLIAS*) IN GHANA

BY

EUNICE OSEI-YEBOAH

Thesis submitted to the Department of Fisheries and Aquatic Sciences of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Fisheries Science

AUGUST 2021

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: ..... Date: .....

Name: Eunice Osei-Yeboah

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: ..... Date: 20/12/2021

Name: Dr. Foster Kyei

Co-Supervisor's Signature: ..... Date: .....

Name: Professor John Blay

ABSTRACT

The Atlantic chub mackerel *Scomber colias* is a small pelagic coastal migratory species belonging to the family Scombridae. Available scientific data reveals a gradual decline of *S. colias* species within Ghana's fisheries waters as a result of growing fishing efforts. Despite their economic value and wide distribution in Ghana, there is lack of information on their genetic diversity. This study aims to genetically characterize *S. colias* along the coast of Ghana using microsatellite and mitochondrial DNA cytochrome *b* markers to ascertain their genetic diversity. *S. colias* specimens were characterized using 8 microsatellite loci and 1 mtDNA cytochrome *b* gene. All 8 microsatellite loci were polymorphic with 3 to 22 alleles per locus however, the effective number of alleles (Ne) per locus and the mean number of different alleles (Na) were 1.466 and 2.054 respectively. Appreciable genetic diversity exists in the population of *S. colias*. The mean observed heterozygosity (Ho) within the population was 0.152 whilst the mean expected heterozygosity (He) was 0.244, which means the *S. colias* population has decreased in size. The mean gene diversity and PIC were 0.58 and 0.54 respectively. Growth in all the samples exhibited isometric. The nucleotide sequences of cyt-*b* gene for samples demonstrated the existence of divergence within and among the nucleotide sequence of the *S. colias* population. There was high genetic divergence (F_{ST} = 0.240) within the *S. colias* population. However, 14% of the variance existed in the population. Nei's distance between the samples ranged between 0.0614 and 0.4695, with an overall gene flow (Nm) of 1.706 and Shannon Information Index 0.406. A tanglegram generated similarities and divergences between the genotypic and phenotypic dendrograms.

KEYWORDS

Cytochrome *b*

Depletion

Growth

Microsatellite

Mitochondrial DNA

Polymorphic

Scomber colias

Species

Tanglegram

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DEDICATION

To my parents, Mr. and Mrs. Yeboah.

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LIST OF ACRONYMS

% -	Percentage
AFLP -	Amplified fragment length polymorphism
AP-PCR -	Arbitrary Primed-Polymerase Chain Reaction
CO I -	Cytochrome Oxidase Subunit
cSNP -	coding Single Nucleotide Polymorphism
CSIR -	SARI – Council for Scientific and Industrial Research
Cyt-*b* -	Cytochrome b
eSNP -	expression Single Nucleotide Polymorphism
DNA -	Deoxyribonucleic acid
EST -	Expressed Sequence Tag
FAO -	Food and Agriculture Organization
Indel -	Insertion-Deletion Polymorphisms
IUCN -	International Union for Conservation of Nature
kb -	1000 nucleotide base pairs (kilobase)
MGDw -	Molecular Grade Distilled water
MoFA -	Ministry of Food and Agriculture
mtDNA -	mitochondrial Deoxyribonucleic Acid
nDNA -	nuclear Deoxyribonucleic Acid
PASA -	Polymerase Chain Reaction Amplification of Specific A Alleles
PCR -	Polymerase Chain Reaction
PCR-RELP -	Polymerase Chain Reaction Restriction Fragment Length Polymorphism
PIC –	Polymorphism Information Content

RAPD - Random amplified polymorphic DNA

RFLP - Restriction Fragment Length Polymorphism

SNP - Single Nucleotide Polymorphism

SSR - Simple Sequence Repeat

STR - Short Tandem Repeat

UPMGA - Unweighted Pair Group Method with Arithmetic Mean

CHAPTER ONE

INTRODUCTION

This thesis seeks to bridge the knowledge gap on the genetic diversity of *Scomber colias* along the coast of Ghana. There is a paucity of knowledge about the genetic diversity of *Scomber colias*. Studies in relation to that will however add up to the literature available providing up-to-date scientific information on the population diversity of *S. colias* population in Ghana waters which can be used for their sustainable management. Findings will help enlighten policymakers and stakeholders in executing enhanced management practices for rational exploitation of the declining small pelagic species in the coastal waters of Ghana. Additionally, information gathered will help broaden understanding of the species for academics, students, interested stakeholders and also for the management of the species

Background to the Study

The Atlantic chub mackerel *Scomber colias* (Gmelin, 1789) is a small pelagic coastal migratory species belonging to the family Scombridae. Their distribution varies periodically which is linked to the periodic variability of the fishing fleets' movement and the availability of the resources (Abdallah & Gaamour, 2004). In Ghana, it is known to school with other small pelagic species (Hernández & Ortega, 2000) such as sardinellas (*Sardinella maderensis and Sardinella aurita*), scads (*Decapterus spp.*) and horse mackerels (*Trachurus spp.)*

According to Osei and Thordarson (2008), sardinellas and chub mackerel are the most valued species with regards to abundance and quality. The significance of chub mackerel to the fisheries sector resulted in the

establishment of a pilot cannery in Accra years ago (Koranteng, 1995). In Ghana, the Atlantic chub mackerel is locally known as "saman" (Ga), "Awukongula" (Fante) and "Ablotsikpokponkuvi" (Ewe) (Kwei & Ofori-Adu, 2005).

Ghana is a powerful fishing nation and its fishing sector accounts for an estimated Gross Domestic Product (GDP) of 1.2 percent (Atta-Mills et al., 2004; Ward, Smith & Tran, 2016). The sector shares in the economic development goals connected to livelihood, food security, foreign exchange income, sustainability of resources and poverty reduction (Chauvin, Mulangu & Porto 2012; World Bank, 2011; Center, 2008).

It has been reported that fish stocks within Ghanaian waters have declined as a consequence of high fishing pressure and inappropriate fishing practices (MOFAD, 2015). Over the years, catches of marine species in the coastal waters of Ghana have experienced wide fluctuations amounting to 250,000 tonnes yearly (Marine Fisheries Research, 2007). There is therefore the need to establish more effective fisheries management, as well as safer and more environmentally friendly procedures of production.

According to Hutchings (2000), lack of information on characteristics of population units to confirm identity of the species and hence definition of the stocks could result in local overfishing and eventually to depletion of the resource. It is therefore essential to examine the genetic structure, diversity and gene flow of the fish populations to obtain baseline information to guide in fisheries management (Utter, 2006; Cha et al., 2010). The need for proper approaches towards the management and sustainability of fisheries has brought about numerous genetic studies employing diversified genetic markers.

Numerous genetic markers which include allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite, SNP, and EST have been applied in population studies to determine genetic variation, the commonly used ones being, single nucleotide polymorphisms (SNPs), restriction site-associated DNA sequencing (RADSeq), insertion-deletion polymorphisms (Indels) and microsatellites. Microsatellite and mitochondrial DNA markers will be used in this study.

Statement of the Problem

The Ghana Sustainable Fisheries Management Project (SFMP) and United States Agency for International Development (USAID) in July 2018 expressed concerns that the depletion of the fish stocks in Ghanaian coastal waters could leave food security, economic development objectives and rate of poverty in fishing communities at risk. Also, the scientific report indicates a drastic decrease of the small pelagic in Ghanaian fishery and is even on the verge of collapse (Lazar et al., 2018). The decline of the fish population is basically caused by human-induced factors. The anthropogenic factors such as over-exploitation of the natural fishery from open water, modification of the aquatic environment by pollution load and damming of aquatic bodies have led to continuous depletion of the marine population and these have genetic and evolutionary consequences (Wohfarth, 1986). There is therefore the need for genetic information for the effective management, sustainable utilization and exploitation of the stocks.

Atlantic chub mackerel is a highly exploited species worldwide (Cheng, Yanagimoto, Song, & Gao, 2015). The large fluctuations in its abundance have been reported around the globe of which Ghana is no exception (Goode, 1887;

Hernández & Ortega, 2000). There is limited information on the genetic diversity of chub mackerel despite their economic significance and prevalence in the marine waters of Ghana all through the upwelling period.

Scomber colias is an oceanic species with an oceanic migratory habit. It can move across latitudes as well as between inshore and coastal areas for feeding and reproduction (Hernández & Ortega, 2000). Its broad distribution, ocean current and lack of oceanographical or physical boundary in the marine terrain may pave the way for extensive gene flow among their population (Palumbi 1994), leading to multiple disparate populations. Several studies of local fishing grounds in the Southwest of the Atlantic Ocean have underlined diverse traits related to the biology of the species (Pajaro, 1993; Perrotta, 1992, 1993; Hansen et al., 1997; Perrotta & Christiansen, 1993) and the fishery (Perrotta et al., 1998b; Perrotta & Pertierra, 1993).

Monitoring genetic stock diversity is vital in improving fish stocks and establishing fisheries management legislation (Bentzen & Thodesen, 2005; Haughton, Carryl & Bissember, 2006). Also, genetic monitoring displays previous and current genetic variation within managed fisheries where data gathered will give a deeper understanding of species composition and history of this populace. The data can also help develop informed management strategies to protect this important species.

Research questions

The questions to be addressed at the end of the study are;

a) Is the *S. colias* population in Ghana morphologically different?

b) Is there genetic variability in *S. colias* population in Ghana?

c) Is there genetic variability across the genome of *S. colias* population in Ghana?

d) Is there genetic divergence of *S. colias* in the different geographical environments in Ghana?

Research Objectives

Main Objectives

The main goal of this work was to characterize *Scomber colias* along the coast of Ghana and assess their genetic diversity.

Specific objectives

The specific objectives of the study were to:

a. Determine the length and weight relationship of *S. colias* population along the coast of Ghana.

b. Assess the molecular genetic variability in the *S. colias* population.

c. Characterize the mtDNA cytochrome *b* cyt-*b* gene across the genome of *S. colias* population to assess genetic variation.

d. Investigate genetic divergence of *S. colias* population in the different geographical environments using molecular patterns.

Significance of Study

A major challenge in the management of small pelagic species in the Ghanaian fisheries has been the identification of stocks for utilization and conservation. The population structure of fish stocks is fundamental for effectual fisheries management because it decreases the spatial boundaries of the species related to its periodic migration and its long-term stability within its distinct genetic makeup. Most of the work on pelagic fisheries management has centered on biological and morphological characteristics such as age

determination, reproduction and growth and this calls for the need for genetic characterization of the Ghanaian stock. Morphological detection of stocks is highly confused by the vast intraspecies variation of the features used for classical determination. Therefore, using genetic procedures as additional tools is commended to address those obstacles (Ollivier, Chevalet & Foulley, 2000). These approaches are also employed to propose management plans of stocks (Ambali & Malekano, 2004; Brinez, Caraballo & Salazar, 2011; Palumbi, 1994).

Genetic characterization of fish populations is geared towards knowing the genetic diversity of populations and it is aimed at providing data to inform the development of sustainable, conservation and utilization strategies and programs. Maintaining genetic variation is widely approved as a common long-lasting aim in wild populace management (Ward, 2000; Koljonen, 2001; Laikre, Palm & Ryman, 2005).

Despite their economic value and wide distribution of chub mackerel along the coast of Ghana, there is lack of adequate information on their genetic diversity. Since molecular markers (molecular information) have become significant for genetic diversity characterization (Groeneveld et al., 2010), the microsatellite and mitochondrial DNA markers (cyt-*b*) will be used to examine the genetic diversity of *S. colias* in the Ghanaian coastal waters. The importance of using diverse types of molecular markers for examining species help in the acquisition of the population history data along a continuum of 'time slices' derived from the genetic variation of each marker and the molecular method employed, and that will give a significant validation of the patterns uncovered (Dudgeon et al., 2012). The significance of this study is therefore to characterize

S. colais to ascertain their genetic diversity in the coastal waters of Ghana and propose a road map for its sustainable management and utilization.

Delimitations of the Study

Studies centered on fish landing sites from the Central and Western coasts of Ghana, where Atlantic chub mackerel are abundant and mostly landed (Kassah, 2020). The Eastern coast of Ghana was not involved because of the comparatively low landings of chub mackerel along that range. Six major landing beaches (Tema, Jamestown, Apam, Elmina, Sekondi and Half Assini) were selected for sampling on the basis of practical factors based on the availability of species. Acquiring data from the field was based on a onetime random sample from commercial artisanal fishes obtained from purse seine landings ("poli/watsa") gears immediately fishermen offloaded their catches at the various fish landing sites.

Limitations of the Study

The lunar cycle affected the landings of small pelagic species. Artisanal fishers did not often go to sea during periods where there was the appearance of the full moon or gibbous. According to them, nets and canoes were more visible to fish during those times and hence evaded capture at those times. Sampling around those times were therefore difficult to obtain samples. The reliance on commercial landings also meant that, the researcher had no control over the exact time at which fish were harvested. The lack of a research vessel meant that data was fisheries dependent. However, it gave an accurate reflection of the species as landed commercially.

The coronavirus disease 2019 (COVID 19) pandemic had impact on delivery of items from South Africa for the molecular work. The ordered items took more months than they were expected.

Due to challenges with equipment, some laboratory analysis was carried out using manual methods. Despite the fact that these limitations were beyond the control of the researcher, they do not undermine the results and inferences made from this study as scientific methods and procedures were followed. They also reflect the major challenges associated with research where extrinsic determinants including weather, climate and logistics play an important role in data collection.

Organization of Study

This study is structured into six chapters. Chapter one unveils a general overview of the study. It briefly describes the Atlantic chub mackerel, the problem under study, application and importance of the study. The aims to be achieved and the hypotheses to be tested are also outlined. Chapter two mainly focuses on the literature review. Chapter Three details the materials and methods employed in the course of the study. A description of the study sites, field data collection methods, equations and statistical packages used, as well as analyses carried out are outlined. The results of the study based on the procedures outlined in Chapter Three, are presented in the forms of relevant graphs and tables in Chapter Four with brief descriptions of trends observed. Chapter Five deals with the discussion which expands on the observations made from the results. The key findings are compared with information reported on the species from earlier studies on the species globally. The trends in the results are discussed in detail and the relevant information that can be gleaned from the

study is put forward. Chapter Six is the Summary, Conclusions and Recommendations. The main outcomes of the study are outlined and recommendations based on the outcome of the research are put forward.

Chapter Summary

Available scientific proof shows a continuous decline in the stocks of fish within Ghana's fisheries waters as a result of increasing fishing efforts. This points to the need to develop more effective fisheries management frameworks and environmentally friendly methods of production. Despite their economic value and wide distribution of chub mackerel in Ghana, there is no adequate information on their genetic diversity. Most of the work on pelagic fisheries management has centered on morphological characterization which is highly complicated by the extensive intraspecific variation. Therefore, the application of genetic methods as additional tools is commended to address these obstacles. In genetics research, the amount of genetic information is measured by the quantity and quality of genetic markers. The study aims to genetically characterize *S. colias* species along the coast of Ghana using microsatellite and mitochondrial DNA cytochrome *b* markers to ascertain their genetic diversity. The relevance of the study is stated, and the organizational framework of the thesis is also noted.

CHAPTER TWO

LITERATURE REVIEW

This chapter deals generally with the genetic characterization of fisheries resources using molecular markers with emphasis on the two most commonly used markers in fish population genetic studies. It also examines relevant literature on description, migration and distribution, economic and ecological importance and studies conducted so far on the species in the context of this study that is Atlantic chub mackerel.

Fishery resources and their importance to the world

Literature available shows that oceanic organisms have lately been estimated as species that surmount 30,000 and with a yearly representation of about 300 new species (Eschmeyer, Fricke, Fong & Polack, 2010; Weigt, Driskell, Baldwin & Ormos, 2012). Among all the oceanic organisms, fishes constitute the most precursory and specious group of vertebrates, pervading more diverse aquatic environments around the world (Torres & Artoni, 2019).

Fisheries resources perform a substantial role in the growth of a nation's economy and thus need not be undervalued. They are generally characterized by the populace of organisms that are from animal and plant expended by a human being, particularly to provide fishmeal for animal husbandry, aquaculture products along with crafts and decoration of distinctive types (Tursi, Majorano, Sion & D'Onghia, 2015). Fisheries resources also contribute to reliable earnings and reduce the rate of hardship in numerous households and communities, due to their significance, research in the conservation of their genetic diversity has greatly increased (Ekelemu & Zelibe, 2006). Without a doubt, the fisheries' resources contribute hugely to national advancement.

However, fisheries resources are getting difficult to manage due to human-induced factors. The anthropogenic factors such as over-exploitation from open water, modification of the aquatic environment by pollution load and damming of aquatic bodies have led to continuous depletion of their population and these have genetic and evolutionary consequences (Wohlfarth, 1986). Many essential pecuniary stocks are critically depleted, leading to a steady substitute of invaluable 'top predators' by species that were previously discarded (FAO, 2011; Pauly et al., 1998).

The roles of fisheries in National Development

Source of livelihood

Although there is a paucity of comprehensive understanding about the level of employment generated by fisheries globally (Teh & Sumaila, 2013), many people globally depend on resources from fisheries for livelihood and economic benefits. In developing countries, more than 500 million people depend directly and indirectly on fisheries resources for their livelihood (Brander, 2010) and are counted on as a fallback by some of the world's poorest, providing sustenance mostly during times of financial hardship (Teh & Sumaila, 2013). In Ghana, most people depend on fisheries as their source of livelihood.

Guarantee food security and well-being of a nation's populace

Food insecurity still remains a crucial global worry as billions of people suffer from starvation, under-, and malnutrition (Sasson, 2012). Food security is indicated in the most basic form as an individual's use of food required for a healthy living at all times (Unit & WHO, 1992). Healthy life requires that, food intake is nutritively sufficient pertaining to quantity, quality and variety and that it is allowable within a given culture. A populace is therefore deemed to be

"food secure" when all these factors are present (Hotta, 2000). Capture fisheries and aquaculture occupy an essential place in the socio-economic development of a nation and also contribute significantly to food security.

FAO has noted that returns from fisheries resources are predominantly fish supply and remain important to the world's food security, producing beyond 15 % of the total protein acquired from animals in addition to a minimum of half a percentage of animal protein and minerals to 400 million people in undeveloped countries (Rabo, Zarmai, Jwanya & Dikwahal 2014; Brander, 2010).

Enhances the nutritive and health condition of a nation's populace

Over 2 billion people in the world are malnourished in virtue of deficiency in essential vitamins and minerals (Allison, 2011). Fish, as a component of fisheries resources, forms a substantial source of protein and essential nutrients; an eminent origin of lean protein, fatty acids, and essential micronutrients that can impede undernourishment and dietary deficiencies. On account of that, there is a rising realization of their dietetic and health-promoting qualities (Bennett et al., 2018; High Level Panel, 2014).

International trade and foreign exchange earnings

One of the most commonly marketed foods is fish products with over 37% (by volume) of the global production been traded internationally. International fish trade has swiftly been increasing in recent decades and approximately 45% of the world catch is now traded internationally (FAO, 2001-2021).

Fish according to study comprises a paraphyletic group classified into three main types based on their mouth structure and skeleton types. These are

the jawless, cartilaginous and bony fishes. The jawless fishes are known to be the earliest vertebrates. As the name implies, this group of fishes lacks jaws. They are void of scales, they do not have pairs of fins and their skeleton is made up of cartilage. The only living examples of this group of fish are the hagfishes and lampreys. Cartilaginous fishes are the second group of fishes made up of sharks and rays. This group of fishes has jaws and skeletons made up of cartilage. The third group of fishes is the bony fishes, which are the most familiar fishes that inhabit both salt and fresh water. Bony fishes are classified into two; the Sarcopterygii (lobe-finned fishes) an example is the lungfish and the Actinopteriygii (ray-finned fishes) where Teleosts belong (Grigg, 2009). An example of teleost is the chub mackerel.

Atlantic chub mackerel belong to the Scombridae family. They consist of 15 genera and about 51 epipelagic species. Species that fall within the family Scombridae are mostly migratory and are one of the most rapidly developing group of all fishes (Collette, Reeb & Block, 2001; Juan-Jordá, Mosqueira, Freire & Dulvy, 2013). According to study, Atlantic chub mackerel mature at early stage and can obtain a total length of 50-62 cm and 13-20 years of age (Alves, 2016; Navarro et al., 2012). However, its lifespan in Ghana is estimated to be 5-6 years (Osei & Thordarson, 2008). Currently, there are fluctuations in the abundance of chub mackerel which may be partly attributed to environmental factors (Goode 1887, Hernández & Ortega 2000).

Description of *Scomber colias*

Scomber colias is classified as a small pelagic fish, with a fusiform body and a narrow caudal peduncle that is void of lateral keel between superior and inferior oblique keels (Carpenter & De Angelis, 2016c; Alves, 2016) It has a

distinctive bluish to greenish posterior and silvery white ventral color with dotted lines (Collete & Nauen, 1983; McEachran & Fechhelm, 2005). The initial dorsal fin emanates below a short pectoral fin bases with 19 or 21 rays and 9 or 10 spines. The subsequent dorsal fin also has 11 or 12 rays preceding 5 finlets. It has a single lateral line which is slightly arched anteriorly. *S. colias* have small scales that are easily lost, however, those around the gular region are bigger and more recognizable (Collete & Nauen, 1983; Alves, 2016) (Figure 1).

Figure 1: Exterior features of S*comber colias* (Alves, 2016)

Scomber colias shares few resemblances with its close relative in the Atlantic Ocean, *Scomber scombrus* which has a more northerly distribution (Carpenter & De Angelis, 2016c). A few of the taxonomic distinctions between the two species are the presence of dusky markings on the belly and the presence of a swim bladder, which are absent in that of *S. scombrus* (Collette & Nauen, 1983; Hernández & Ortega, 2000)

Migration and distribution

Scomber colias are found across the temperate and warm coastal waters on the continental shelf to depths of about 250-300 meters throughout much of the eastern and Atlantic Ocean, and also southern Black and the Mediterranean Sea (Hernández & Ortega, 2000; Collette & Nauen 1983) (Figure 2).

Its periodic variability of the distribution is connected to the periodic variation of the operation of the fishing fleets activity and resources availability (Abdallah & Gaamour, 2004). Like most scombrids, *S. colias* is a highly migratory fish. According to Allaya et al. (2016), there is a reformation of chub mackerel to vertical daily migrations driven by feeding activities. It stays near the bottom during the day and moves to open waters to feed at night. However, in the northern hemisphere, chub mackerel migrate between northern areas in warmer months and southern areas in cooler months (McEachran & Fechhelm, 2005; Collette & Nauen 1983). The evolution of fish from the juvenile to the adult immature stage is followed by an offshore movement from shallow coastal waters to the incline area and a reverse movement is seen in the time of the spawning period (Castro, 1993; Hernández & Ortega 2000).

Figure 2: Map showing the distribution of resident populations of Scomber colias in the world. Image credit: (Collette et al., 2011)

Economic and ecological value

Species that belong to the Scombridae family like; chub mackerel, true mackerels, bonitos and tunas have high commercial interest. Scombrid fish to date has been one of the most economically important fishery resources globally due to their worldwide distribution (Collette, 2003). Mackerels that belong to the genus *Scomber*, according to Catanese et al. (2010), are placed fourth of the worldwide captures and are highly valued by consumers for the excellent characteristics and quality of the meat. Ecologically, chub mackerel plays the role of both forage species and predators of other forage species (Okey, Cisneros-Montemayor, Pugliese & Suaila, 2014).

Status of the World Fisheries and Need for Genetic Studies

The consumption of fishery products has been in higher demand in recent times and this has caused the global exploitation of the fishery products to gradually increase so as to satisfy the worldwide rising request for seafood (Anderson, 2009; Rabo et al., 2014). The FAO has declared that production from fish will continue to expand globally in years ahead in spite of the fact that the current number of captured fishes in the wild have evened off and the previously explosive growth for aquaculture is now slowing down. This points to the need to develop more effective and economic management of the fisheries resources together with safer and more environmentally friendly methods of production.

Research indicates that by 2030, produce from aquaculture and capture fisheries will grow to 201 million tonnes. This implies that growth in the time to come will entail perpetual advancement in building up management plans in

fisheries, cutting down loss and waste, and addressing issues such as illegal fishing, pollution of aquatic environments, and climate change (FAO, 2018).

Genetic studies on chub mackerel

The chub mackerel was noted for years as Pacific chub mackerel *Scomber japonicus* based on some morphological features. (Infante et al., 2007; Catanese et al., 2010). This motion was then disputed by Matsui (1967), who indicated phenotypic variation between chub mackerels in the Atlantic and Pacific Oceans in terms of their coloration, scale size, tooth crenulation and number of gill-rakers on the lower first arch. It has been noted that identification specifically based only on morphological traits is unreliable due to significant geographical and ecological divergence (Tsigenopoulos & Berrebi, 2000; Siraj, Esa, Keong & Daud, 2007). This was then further proved by genetic analysis determined from nuclear and mitochondrial DNA markers and have now recognized a conclusive light on the two species as genetically distinct species (Catanese et al., 2010; Infante et al., 2007).

Studies have revealed that, due to dissimilarities in morphology, spawning seasons and size at maturation, sub-stocks of chub mackerel may exist (Chen, Li, Feng & Tian, 2009; Weber & McClatchie 2012; Cerna & Plaza 2014; Yasuda, Yukami & Ohshimo, 2014). Some other studies have also shown to be genetically uniform across wide areas (Scoles, Collette & Graves, 1998; Zardoya et al., 2004). As stated by Scoles et al., (1998), there is no significant genetic difference between chub mackerel from the eastern Mediterranean Sea, the Ivory Coast, and South Africa. However, they found significant genetic variation between chub mackerel from the eastern and western Atlantic.

Tzeng et al. (2007) proposed the presence of a single gene pool of chub mackerel in Taiwan waters after an experiment carried out by the application of mitochondrial DNA markers. On the other hand, supplementary studies based on the application of random amplified polymorphic DNA (RAPD) by Shao and Chen, (2008) showed the presence of two stocks in the Yellow and East China Sea. All these studies on chub mackerel have led to distinctive conclusions on the genetic structure and population distinctiveness at fine geographical scopes (Cheng, Yanagimoto, Song, & Gao, 2015).

However, there is no comprehensive study on the genetic diversity and structure of chub mackerel in the coastal waters of Ghana. Information available on this species however, focuses on biological and morphological studies. In view of the significant contribution of this species to the Ghanaian fisheries sector, there is a need for genetic studies to consolidate the already available information to confirm their status and to generate vital information for the effective management and exploitation of the stock.

Globally, there is a rise in human influence on the fishery population. Human-induced activities have an important effect on natural populations of a number of species, in some cases even threatening their existence (Olsson et al., 2016). Anthropogenic factors such as over-exploitation of natural fishery from open water, modification of aquatic environment by pollution load and damming of aquatic bodies have led to continuous depletion of the marine population and these have genetic and evolutionary consequences (Wohlfarth, 1986). Before a species would decline into extirpation, it might have long suffered a decline in genetic diversity within and among its populace (Kenchington, 2003). Decline in the size of a population is related to reduced

genetic variation, an effect to exploitation consequence considered destructive to recovery (Lande 1993)

Maintaining the genetic diversity of a population is a globally accepted common long-lasting plan in management (Ward, 2000; Koljonen, 2001; Laikre et al., 2005). It is also noted that managing the genetic diversity among the population will serve as a key component in any breed conservation program for keeping the animal resources in any country (Bjørnstad & Røed, 2002; Toro, Barragan & Ovilo, 2003). The need for conserving biodiversity has been identified by International Union for Conservation of Nature at three different levels. These are; genetic, species and ecosystem diversity. However, genetics is directly involved in genetic and species diversity (Frankham, 2018).

For a population to progress in repercussion to environmental reformations, such as global warming and new or altered diseases, genetic diversity is required (Frankham, 2018). Genetic diversity is therefore an indicator of population health for living organisms (de Vicente, Guzman, Engels & Rao, 2006).

Every organism is prone to mutations traceable to normal cellular operations or environmental interactions resulting in genetic variation (polymorphism) (Liu & Cordes, 2004). One of the issues of maintaining genetic diversity is detecting how variation is genetically distributed within a species (Kenchington, 2003). Genetic variation is the raw material in a populations and species, which provides the potential for them to conform to changes in their environment (Çiftci & Okumuş, 2002). Hence, enormous loss of genetic variability should be avoided to enhance the sustainability of fisheries resources. Scientists are however not only slanted in determining variation among and

between populations but also detecting similarities and differences between individuals so as to establish optimum conservation strategies (Dudu, Georgescu & Costache, 2015).

Genetic characterization in fisheries distinctly presents an improved power for determining variation that outreaches traditional approaches such as reproductive condition both temporally and spatially, breeding and feeding sites, population specific behaviors, and movement patterns to infer similarity or independence of gene pools (Nguyen et al., 2006). Characterization from a genetic perspective means identifying the variation caused by changes in either DNA sequences or specific genes or modifying components (de Vicente et al., 2006). According to research, characterization with molecular techniques contributes enormous power of detection than that of phenotypic methods. Those techniques serve as reliable tools which can be used together with quantitative methods and traditional breeding approaches for an effective design of preservation strategy (Dovc, Kavar, Sölkner & Achmann 2006). In addition, molecular procedures underline genotypic differentiation, particularly, in the optimum level of differentiation embodied by the DNA sequences of an individual and unagitated by the environment. On the contrary, variation showed by phenotypic methods are at the gene expression (proteins) level (de Vicente et al., 2006).

Biological variation between an individual species specifically, variation at the gene level is usually described as intraspecies variability (Dudu et al., 2015). Types of genetic variation at the DNA level comprise; insertions or deletions of nucleotide sequences (indels) within a locus, single nucleotide polymorphisms (SNPs) or base substitutions, inversion of a segment of DNA

within a locus and rearrangement of DNA segments around a locus of interest. Many different examples of each form of mutation should exist in any given species due to long evolutionary accumulation, and the number and degree of the many types of mutations characterize the genetic variation within a species. (Liu & Cordes, 2004). Information on the genetic composition and variation of species serves as the ultimate basis in avoiding ecological loss which contributes to effective and sustainable management of exploited stocks (Olsson et al., 2016).

Importance of Genetic Information

i. Genetic information can function as another biodiversity measure that can be used with aquatic habitat information.

ii. Genetic information can complement traditional habitat and fish assessment analysis to contribute an integrated, multidisciplinary method for assessment of aquatic diversity

iii. Genetic information provides measures of variation that can be allocated proportionally at individual, species and population levels

iv. Genetic information can be used with traditional ecological assessment information and landscape models to determine biodiversity hotspots, anticipate ecosystem impacts to anthropogenic effects, and determine impairments to underlying processes (Scribner et al., 2016).

Genetic markers

In population genetics research, the level of genetic information is determined by the quantity and quality of genetic markers (Kim, 2019). A "marker" in biology terminology is defined as any stable variation, that is

heritable and can be studied by a suitable technique (Ferguson et al., 1995). Genetic markers serve as basic tools for examining fish populace and species genetic variability (Rashed et al., 2008; Saad, Mansour & El-Naggar, 2009). The popular application of genetic markers in fisheries is to examine if samples from natural populations or cultural resources are genetically different from each other. They are also employed to distinguish between species in the event of a taxonomic disagreement, as well as to detect genetic introgression within a species. The detection of genetic differences would imply that the source groups are made up of different stocks and should be handled as such. (Carvalho and Hauser, 1995; Moritz, 1994).

Types of Genetic Markers

Variations are expressed at different levels; molecular, biochemical and morphological. Based on their levels of variation, genetic markers are classified into three kinds; morphological (phenotypic) markers, biochemical (protein) markers and molecular (DNA) markers (Ferguson et al., 1995). Morphological traits transferred by Mendelian inheritance were the first markers used for genetic investigation (Anne, 2006). One crucial shortcoming between both phenotypic and protein markers is that they may be restricted in number and are inveigled by environmental changes and factors (Winter & Kahl, 1995). Molecular markers by contrast can provide more fundamental information for checking relationships among populations and higher taxonomic levels. They are not complex by any possible outcome of the environment due to the fact that they are fixed at fertilization (Nguyen et al., 2006).

DNA (Molecular) markers

DNA markers are powerful tools for solving ecological, evolutionary, management and conservational related controversies of most fish taxa (Wilson et al., 2000; Thangaraj & Lipton, 2010). With these types of markers, differences in their DNA sequence determine their genetic diversity (Nguyen et al., 2006). Numerous molecular markers have been used to determine genetic variation in population studies in fish. Some of them include; restriction site-associated DNA sequencing (RADSeq), single nucleotide polymorphisms (SNPs), insertion-deletion polymorphisms (Indels) and microsatellites.

What to consider when choosing a marker

One of the questions that come to play at the onset of any genetic study is, what type of marker is most appropriate with regards to the work and the species under study? The choice of marker should precisely conform to the specific research question(s). The methodology should also be simple and affordable. The selected marker should be within the resource limitations that is, the cost and availability of equipment should be considered (Liu & Cordes, 2004; Vignal, Milan, SanCristobal & Eggen, 2002). In view of this, however, some research questions can best be solved by synthesizing information from different types of markers.

Types of molecular (DNA) markers

Primarily, DNA markers are grouped into two classifications. The first class is categorized as either type I or type II. Those that are linked with genes of known function are classified as Type I markers. Type II markers are markers linked with anonymous genomic segments (O'Brien, 1991) (Table 1). The type I markers include; RELP, allozyme, and EST. RAPD and AFLP are examples

of type II markers. However, some markers can either be described as type I or II based on their association, development and location. For instance, SNP markers are usually classified as type II markers except if developed from expressed sequences (eSNP or cSNP). Microsatellite markers are classified as type II markers except if related with genes of known function. Depending on the location in genes, the Indels can either be classified as type I or type II markers.

The second class of markers is based on their position in the cell. That is location in either the nuclear or mitochondrion genome, known as nuclear and mitochondrial DNA (mtDNA) markers. Mitochondrial and nuclear DNA markers render an edge on protein-based procedures because they do not rely on age of the individual, tissue source or sample damage (Irwin, Kocher & Wilson, 1991; Yang et al., 2014). These two classifications of markers can either be PCR-based or not. Regardless, the molecular markers detected by PCR are the most commonly used ones in determining genetic variation.

PCR-based markers are divided into two based on the primers used for their amplification. These are the; PCR markers for target sequences (two specific primers are employed to amplify the region of interest) and PCR markers for arbitrary sequences (one primer with an arbitrary nucleotide sequence is used).

Table 1: Classification of Genetic Markers and some examples

Function	Type I	Type II	Either I or II
	RELP, EST allozyme	RAPD, AFLP	Microsatellite, SNP, Indels
Location	Nuclear		Mitochondrial
Detected (PCR)	Minisatellite, microsatellite, PCR-based markers		Cyt-*b*, CO I Non-PCR-based markers
	AFLP, RELP		Allozyme
	For target sequence	For target sequence	
	PCR-RELP, PASA, SNP	RAPD, AP-PCR, AFLP	

The most widely used molecular markers in fisheries

All eukaryotic cells have at least a copy of the whole nuclear genome accommodated in their nucleus. Also, all cells contain so many several thousand mitochondria, based on energetic provisions. Mitochondria house a minute but vital part of a eukaryote's genetic material. (Saccone et al., 2000). Genetic investigations are commonly used to uncover diagnostic disparities between putative stocks in mtDNA haplotypes or nuclear allelic types (Danzmann & Ihssen, 1995)

Mitochondrial DNA markers (mtDNA)

Mitochondrial DNA markers are frequently used for population and phylogenetic analysis to genetically define the structure of populace and rectify unknown phylogenetic relations among marine organisms, among nearly related species within the same species or within the same family (Wu RX, & Zhuang, 2012; Sun, Yin, Shi & Peng, 2013; Yan et al., 2015; Cheng & Sha, 2017; Hartl & Clark 1997). Mitochondrial DNA constitutes a minute fraction of

organismal genome size (about 15-20 kb) and that makes mtDNA successful to recover from limited or degraded samples (Hubert et al., 2008). Animal mtDNA contains 36 or 37 genes; two for ribosomal RNAs, 22 for tRNAs and 12 or 13 for subunits of multimeric proteins of the inner mitochondrial membrane (Wan, Wu, Fujihara & Fang 2004).

Mitochondrial DNA has numerous distinct biological features which score it a suitable marker for genetics study. It is known for its typically maternal inheritance, relatively rapid base substitution rate (rapid mutation rate), absence of recombination, and easy isolation (Wolstenholme, 1992; Avise et al., 1987). Mitochondrial DNA markers over the last three decades have been the commonest marker for detecting molecular diversity in animals (Galtier, Nabholz, Glémin, & Hurst, 2009). Several mtDNA markers such as cytochrome *b*, cytochrome oxidase I, hypervariable region and ribosomal genes (12S and 16S rRNAs) have been used in species identification. One of the most useful mtDNA markers for phylogenetic analysis is cytochrome *b* (Bloomer & Crowe, 1998; Callejas & Ochando, 2000; Edwards & Arctander, 1997; Johns & Avise, 1998; Zardoya & Meyer, 1996). It shows adequate interspecies variation in nucleotide sequence to enable differentiation even between the nearly related species, examples include; bonito and tuna (Mackie et al., 1999; Bartlet and Davidson, 1991, Bartlet and Davidson, 1992)

Efficient conservation approaches do not rely on either maternal or paternal variation, but strongly on biparental nuclear genetic variability, depicting features integral to manage environmental conditions. But due to the fact that mtDNA markers have a uniparental inheritance, specifically, maternally inclined and this feature limits it to probing events at the maternal

perspective. However, mtDNA markers can serve as helpful markers to nuclear DNA making both markers auxiliary tools in conservation genetics (Wan et al., 2004).

Microsatellites

Microsatellites can also be noted as "simple sequence repeat" (SSR) or "short tandem repeat" (STR) DNA. Microsatellites fall under nuclear DNA markers and are generally seen along the eukaryotic genome. They are mostly used to determine the variation within and between the populace (Vigouroux et al., 2005). These markers are short dually arranged di-, tri-, or tetranucleotide duplicate sequences with a repeat size of 1-6 bp duplicated numerous times (Tautz, 1989).

In fish population genetic studies, microsatellites are used because of their multi-allelic nature, reproducibility, co-dominant inheritance, wide genomic distribution patterns and chromosome-specific location (Yonash et al., 2001).

A significant disadvantage of microsatellites is that distinguishing specific regions from a genomic library for a new species can be costly and time-consuming. Additionally, the presence of null alleles (alleles that do not amplify due to mutational changes in the priming site) can also make the method complex (Nguyen et al., 2006).

This points to the need of using multiple classes of molecular markers for efficient results. The significance of analyzing species with multiple types of DNA markers is that information on population history can be acquired along a continuum of 'time slices' based on the genetic diversity of each marker and

the molecular approach used, and that can give important confirmation of the patterns uncovered (Dudgeon et al., 2012).

The need to characterize fishery resources will help ascertain their genetic diversity and to propose a road map for their management. In view of that, many of genetic markers have been used in characterizing fishery resources. It should therefore be noted from the above that in genetic analyses, irrespective of the type of molecular markers used, they have their own challenges and limitations. Despite these difficulties, they can also be very informative. Therefore, our ability to resolve such problems and to draw concrete conclusions in the area of population genetics is always better served if alternative markers are used concurrently.

CHAPTER THREE

MATERIALS AND METHODS

This chapter brings into view information of the sites from which Atlantic chub mackerel, *Scomber colias* samples were obtained for the study; it also gives details on the sampling approaches, data collection and the respective statistical analytical tools employed.

Sampling Sites

Ghana's coastline stretches for 550 kilometers from the border with Cote d'Ivoire to the border with Togo, and it mostly consists of a low-lying area of 30 meters above sea level. Coastal zones exist in the Volta, Greater Accra, Central, and Western regions of Ghana (Quaatey, 1997).

Scomber colias were randomly collected from commercial catches from six landing sites in Ghana based on their geographical regions. Half Assini and Sekondi in the Western Region, Elmina and Apam in the Central Region and Tema and Jamestown in the Greater-Accra Region (Figure 3).

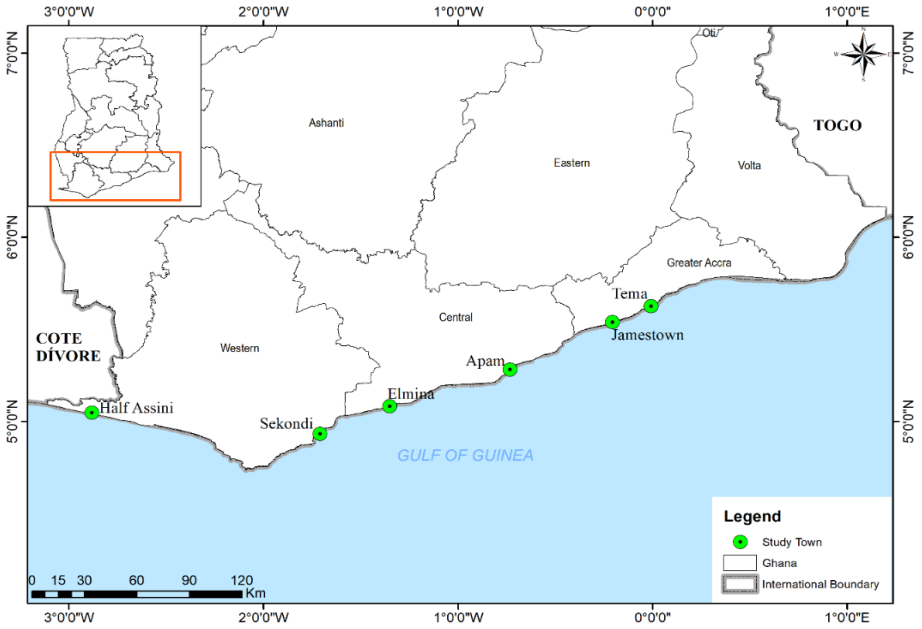

Figure 3: Map of Southern Ghana, the study area, showing the locations of the sampling sites for the study. Inset is a map of Ghana in the top left corner.

Half Assini is situated at an altitude of 70m above sea level. Jomoro district is the capital of Half Assini. The people of Half Assini are mainly into farming and fishing. However, trading and industrial activities also amount to their economy. Half Assini has a long stretch of sandy beach defined by a fishing community of migrant fishermen from the Volta and Central Regions of Ghana. Fishers who hail from the Volta Region engage in beach seining which exploits small pelagic species and juveniles of other fishes that utilize the shallow coastal waters as nursery and feeding grounds (Aryeh-Adjei, 2015; Kassah, 2020)

Sekondi-Takoradi is the capital city of the Western Region and is located 280 kilometers from La Cote d'Ivoire border to the West. It is the largest and the fourth densest city in Ghana. Sekondi is made up of generally Ahantas but in spite of that, all other ethnic groups in Ghana inhabit there. It has a harbor, Albert Bosumtwi-Sam Fishing Harbor where fish are normally landed. The

Albert Bosumtwi-Sam Fishing Harbor which is also known as Sekondi Fishing Harbor was commissioned by the late former president J.J. Rawlings on the 2nd of June 1999. The harbor has over 1000 artisanal fishermen. Infrastructure at the harbor comprise ice production units and cold stores (Adom, Sekyere & Yarney 2019; GSS, 2012; Gyan et al., 2020; Kassah, 2020)

Elmina is the Capital of Komenda-Edina-Eguafo-Abrem (KEEA) Municipality. It is a traditional fishing community and a major fish landing site in the municipality in the Central Region of Ghana. Elmina fish landing site is the third biggest landing site in Ghana after Sekondi and Tema harbor. The fish landing station was established along the Benya lagoon's bank to provide a decent landing spot for artisanal canoes and semi-industrial vessels. The shorelines of Elmina are known for their Ice-making plants and a cuttlefish processing infrastructure (Odotei, 2002; Kassah, 2020)

Apam, which is the capital of Gomoa West District is a coastal town in the Central Region with a total populace of 26,466 and a 2.5% growth rate. The people of Apam are actively involved in fishing. The main fishing period there is between August and December and fish that are usually exploited from their waters are “Akoonai, Eban (*Sardinella aurita*), Opeku, Apaa”. The fishermen in Apam fish in two groups; those that go to the sea in the evening and return in the morning and others that go in the morning and return in the evening between 5 pm and 7 pm. The night fishers often employ the light system of fishing to exploit more herrings and other small pelagics. (Akutse & Samey, 2015)

Jamestown is situated within the Asheidu Keteke Sub-Metro in the Accra Metropolis. It is a small and most densely populated community with a mixture of the indigenous Ga community and migrants groups from diverse

parts of Ghana. It is also a socioeconomically deprived community that depends typically on fishing. (Bain et al., 2020).

Tema has a fishing harbor that is situated in the south-eastern part of Ghana along the Gulf of Guinea and is 18 miles away from Greater Accra. The harbor was built in 1960 and has been functioning since 1962 after its commission. The harbor has 5 km of breakwaters, 12 deep-water berths, one oil-tanker berth, one dockyard, a warehouse and transit sheds. In the east of the ice breakwater is the fishing harbor with cold storage and marketing facilities that handle fish processing. Species that are normally landed there include; the anchovy (*Engraulis encrasicolus*), sardinellas *(Sardinella aurita and S. maderensis*), frigate mackerel (*Caranx hippos*), Atlantic chub mackerel (*S. colias*) and Atlantic bumper (*Chloroscombrus chrysurus*) (Assan, Akongyuur & Abarike, 2019; Botwe, 2018; Dovlo, Amador & Nkrumah, 2016)

Fish Sampling

Random samples of freshly-landed *S. colias* (~30 individuals) were collected from commercial catches from each of the selected landing sites (Figure 3). Individuals were identified using a taxonomic key. Also, frozen by-catch of the species from trawlers commonly known as "saiko" were randomly collected from Elmina (Table 2). The samples were kept on dry ice (-80˚C) and transported to the laboratory for subsequent analysis.

Table 2: Sample size for the various sampling stations

Sampling sites	Sample size for various sites
Apam	30
Sekondi	30
Elmina	30
Saiko	20
Tema	30
Half Assini	25
Jamestown	30
Total	195

Data Collection and Morphometric characterization

A total of 195 specimens *S. colias* were sampled (Table 2). Fishes were defrosted and for each specimen, the measurement for standard length (*SL*), fork length (*FL*) and total length (*TL*) to the nearest 0.1 cm using a measuring board (Figure 4). The body weight (*BW*) of each specimen was measured with an electronic balance (Ohaus R71MD15, China) to the nearest 0.1 g.

Figure 4: Atlantic chub mackerel (*Scomber colias*).

For each of the 195 specimens, snips of the pectoral fin, caudal fin and muscle tissue were excised and placed in separate 2 ml Eppendorf tubes with absolute ethanol. The tubes were marked to identify name, species, type of tissue, date and area fished. The tissues were held at -20˚C before DNA extraction.

Molecular characterization

DNA extraction

Extraction of genomic DNA was undertaken using the modified Quick-DNA Miniprep Plus Kit (Zymo Research Company, US) and protocol. A 25 mg piece of muscle tissue of each specimen was mechanically grinded in a mortar to a fine powder and then transferred into a 2 ml microcentrifuge tube. Five

hundred microliters of Genomic Lysis Buffer and 2.5 µl 2-Mercaptoethanol (Qualikems Fine Chemical Pvt Ltd, India) were added.

Samples were mixed in a vortex for 5 seconds and kept at room temperature for 10 minutes. The mixture was poured into a Zymo-Spin™ IIC Column (Zymo Research Company, US) in a Collection Tube and centrifuged at 10,000 x g for 1 minute and transferred into a new Collection Tube. Two hundred microliters of DNA Pre-Wash Buffer were added to the spin column and centrifuged at 10,000 x g for 1 minute. The spin-column was removed and placed in a 1.5 ml microcentrifuge tube. Fifty microliters of DNA Elution Buffer were added to the spin column and incubated at room temperature for 30 minutes. The sample was centrifuged at high speed for 30 seconds to elute the DNA and then kept at -20 ºC for subsequent use (Muhammad, (2019).

Agarose gel electrophoresis was used to test the integrity or quality of the isolated DNA. 1.0% agarose gel was prepared with 0.03 % ethidium bromide. 10 µl of the genomic DNA sample was pipetted and 2µl loading buffer was added. The samples were then loaded in the wells on gel submerged in 1 x TBE buffer. The samples were run at 90 volts, 120 AMP and 50 W for 45 minutes and photographed under UV light. The band's integrity and brightness were utilized to determine the quality of the DNA generated.

Primer Dilution

Eight microsatellite primers designed by Catanese et al. (2007) and one mitochondrial DNA cytochrome b primer (personally designed) (Sequences of primers used are listed in Table 3) were ordered from Inqaba Biotec West Africa Ltd, South Africa. All the primers were spun with the aid of the centrifuge to ensure that, the dislodged pellets that might have been caused by transportation

or shipping settled at the bottom of the tubes. A master stock (100 µM) of the primers were prepared using the formula; 100 µM= X nmoles lyophilized primer + (X × 10 µl molecular grade H_2O).

The master stock primers newly suspended in the molecular grade water were kept at room temperature for 10 mins and well mixed before they were used for working stock dilution. The primer master stocks were diluted with molecular grade water in the ratio of 1:10 to form the working solution (10 µM) and stored at 4 ℃.

To confirm robust amplification, the primer pairs were tested using gradient PCR at an annealing temperature range of 52-62 ℃ to determine the optimum annealing temperature.

Table 3: Sequences of Microsatellite and Mitochondrial DNA cytochrome B markers used

Name of Primer	Primer sequence (5'-3') Forward (F) and Reverse (R)	Repeat motif	Annealing Temp. (ºC)
Sco2-1	F: GAGGAACCAGATTACAGGTAGTCAGGTATTGGTG R: TGTGGAGCAACAACGAGTTTAACAACGTC	$(CCCT)_6(CCTT)_{11}(CCTC)_2$	58.8
Sco2-3	F: AAAAACGCCTTCGCCAGCCTGGAC R: CACTATGAGCTGTATTTTATGTATGAAAGGTGCTA	$(CCTT)_{12}$	58.0
Sco2-6	F: CACAGCTCCACAAGATGAACATGAACAGGATA R: TCACTGAGCACCTGCCCCCCAAAAT	$(CA)_{41}$	60.0
Sco2-25	F: CACTCCTCTTGTCCCTCATACTCCCATAACC R: GCAGAGCTGTGATCGATTCCCAGTTAAGAC	$(CT)_{23}$ GT $(AT)_8$	61.8
Sco2-36	F: TTAGGAGTGTGGCAGTCGCAGCAGA R: GTCCCTCATACTCCCATAACCCAGCATC	$(TA)_4$ CA $(GA)_{21}$	56.9
Sco2-39	F: CCCCGAGTGTGTAGAAAACCACCTCTG R: TATAAATAACCACGCCACTCATTTTGCTTCTG	$(CA)_9$ $(GA)_2$ $(CA)_{30}$	60.0
Sco2-43	F: CTGACATCAGAGACTGAACTGCACCACT R: GGTGTTGGCTGGTAAAAGGTTGATTG	$(CA)_{23}$	60.0
Sco2-48	F: CATTTCACATCTCGCCACGCTTTCA R: TCCTTCTCCCTCCAGGTTAGTGTGCAG	$(CA)_{16}$	56.1
Sco-CytB	F: AAAAACCCACCCACTGCTAA R: TTGTTTTCTACTCAGCCTGT	–	54.6

Table 4: Primers and their base pair range

Size range (bp)	120-180	120-150	200-250	100-400	80-180	80-400	90-280	100-200	1110
Primer names	Sco2-1	Sco2-3	Sco2-6	Sco2-25	Sco2-36	Sco2-39	Sco2-43	Sco2-48	Sco-Cyt*B*

PCR analysis

To amplify the DNA, a 20 µl reaction mixture was prepared. Each reaction mixture of 20 µl contained 2 µl each of reverse and forward primers, 10 µl of One Taq Quick-Load 2 x Master Mix with Standard Buffer from, "New England BioLabs, 2 µl of genomic or template DNA and topped with 6 µl of Molecular Grade distilled water (MGDw). PCR amplification was done using BIO RAN T100TM thermal cycler (USA). PCR conditions involved initial denaturation at 95 °C for 3 minutes, denaturation at 95 °C for 30 seconds, annealing at temperatures ranging from 54.6 – 61.8°C (Table 3) for 30 seconds and extension at 72°C for 45 seconds. This cycle was done 35 times with a final extension at 72°C for 5 minutes and held at 4°C after completion. The PCR products were visualized by running on horizontal 2% Agarose gel electrophoresis to separate and resolve the bands

Gel Electrophoresis and Scoring

The 2% Agarose gel was cast in a tray (27.5 cm x 24.5 cm) with barriers to retain gel and 15 well-forming combs were placed to create wells. 0.8 g of Agarose was dissolved in 40 ml of 1×TBE buffer to make 40 ml of Agarose gel. The mixture was stained with 5 µl of ethidium bromide. The mixture was poured into the tray and dispersed uniformly across the whole surface without trapping bubbles and the mixture was allowed to solidify. The whole assembly was placed into the electrophoresis tank submerged in 1×TBE buffer and the combs were gently removed. The PCR products were gently loaded into the wells to avoid puncturing the gel' skirt' of Agarose. Perfect DNA™ 100bp DNA Ladder (Novagen from EMD Millipore Corp. Billerica, MA USA) was used as a molecular weight-sized marker for each gel alongside the DNA samples. The

electrophoresis tank was covered with the lid. The PCR products were resolved for 45 minutes at 90 V, 50W and 120 Ma, after which the gels were visualized and photographed under UV light (Accuris™ UV Transilluminator, USA). DNA bands that corresponded to the marker's product size were scored present (1) and where no visible DNA band corresponded to the marker were scored absent (0) (Figure 10).

Preparation of Samples for Sequencing

A total of 22 *S. colias* specimens (selected across the sampling sites, Table 5) were prepared for sequencing. PCR reaction was done in a thermal cycler using 25 µl reaction that contained 3 µl of genomic DNA, 1.5 µl each of the reverse and forward primers, 10 µl of One Taq Quick-Load 2 x Master Mix with Standard Buffer (New England BioLabs), and topped with 9 µl of Molecular Grade distilled water (MGDw). PCR amplification was done using BIO RAN T100TM thermal cycler (USA). PCR conditions involved initial denaturation at 94 °C for 3 mins, denaturation at 94 °C for 30 secs, annealing temperature for primer at 54.6°C for 30 secs and extension at 68°C for 1 min. This cycle was repeated 35 times with a final extension at 68°C for 5 mins and held at 4°C after completion. 8 ul of PCR products were visualized by running on horizontal 2 % Agarose gel electrophoresis to separate and resolve the bands. The remaining products were well identified, packaged and transported to Inqaba Biotec West Africa Ltd, South Africa for DNA sequencing.

Table 5: Selected samples for genomic DNA sequencing

Sampling sites	Number of specimens	Specimen ID
Apam	2	A6, A13
Jamestown	1	J8
Tema	3	T10, T20, T24
Sekondi	3	S7, S12, S15
Elmina	5	E4, E6, E11, E18, E21
Elmina Saiko	3	ES2, ES8, ES15
Half Assini	5	H6, H7, H9, H11, H25
Total	22	

Data Analysis

Length-weight relationships

Scatter plots of data on fish body weight (*BW*) and total length (*TL*) were used to determine trends in the relationship between the two parameters for all samples collected from the different sampling stations. A regression analysis using the least-squares method was performed to establish a mathematical relationship between the two variables.

Principal Component Analysis (PCA)

The morphological data were subjected to Principal Components Analysis which was done using Minitab software to produce eigenvalues and eigenvectors which reveal both the percentages of total variation and cumulative variation. Also, biplots were generated to reveal associations among the variables.

Cluster Analysis

Morphological data among individuals of the seven populations were established in a Cluster analysis by using the UPGMA (Unweighed Pair Group

Method with Arithmetical average) to construct a phylogenetic tree using Power maker (version 3.25) and observed in Molecular Evolutionary Genetics Analysis 4 (MEGA 4).

Analysis of Molecular Data

In this study, one monomorphic marker and eight polymorphic microsatellite markers were used to characterize *S. colias* population in Ghana (Table 3 and 4).

Microsatellite data

The polymorphisms of all the populations were purported as a mean number of effective alleles (Ne), observed heterozygosity (Ho), number of alleles for each locus (Na), percentage of polymorphism bands (PPBs) and expected heterozygosity (He) using GenAlEx 6.512b2 software (Peakall & Smouse, 2012) and Population Genetic Analysis POPGENE Version 1.32 (Yeh, 1999). They were also used to assess Wright's F-statistics (F_{IS}, F_{ST} and F_{IT}). The number of migrants exchanged per generation (Nm) was calculated using the pairwise FST values. The PowerMarker Software v 3.25 was used to determine the polymorphism information content (PIC) for all primers based on allelic frequencies (Liu & Muse, 2005).

A test for conformation to the Hardy-Weinberg equilibrium (HWE) by a Markov chain approximation of the exact test (Guo & Thompson, 1992) was undertaken by the Genepop (version 1.2) (Raymond, 1995). To determine the genetic differentiation between populations, the GenAlEx 6.512b2 software was used to generate Shannon's information index (I) and molecular variance (AMOVA). The proportion of private alleles in the populations were also used to determine inter-population variability. The GenAlEx 6.512b2 software was

also used to construct genetic differentiation indices such as Nei's genetic distance (D) and Nei's genetic identity. UPGMA was used to construct a phenetic tree.

Mitochondrial DNA cytochrome b data

To investigate a pattern of overall mitochondrial cytochrome *b* homogeneity of *S. colias* with statistical significance, the gene (cytochrome-*b*) was selected for the DNA sequencing (Figure 5, Table 5).

Figure 5: DNA bands from PCR amplification products of Sco-Cyt*B*, resolved in 2 % Agarose gel stained with ethidium bromide. NT- Non template control, L- 100bp ladder (Lab data, 2021).

Joint analysis of genetic and phenotypic data

A tanglegram was generated to illustrate the similarities and divergences between the genotypic and phenotypic dendrograms using Power maker (version 3.25) and Molecular Evolutionary Genetics Analysis 4 (MEGA 4). The dendrograms were drawn to face each other and helper lines were used to connect similar populations to establish a network of interactions.

CHAPTER FOUR

RESULTS

The results presented in this chapter are based on onetime random sampling from the selected landing sites along the coast of Ghana, and laboratory analysis to assess the genetic diversity of *Scomber colias*. This chapter also reports the length-weight relationship of specimens from the bycatch of commercial trawlers.

Length-Frequency Distributions of *S. colias*

The size distributions of *S. colias* from the canoe fishery of Ghana are shown in Figure 8. The 195 specimens ranged from 13.5 cm *TL* to 41.0 cm *TL* with a mean value of 24.65 ± 0.25 cm for measurements that were taken (Appendix A). The measurements had modal length of 24 cm *TL* (Figure 6). Body weight ranged from 51.4 -337.4 g for all specimens, with a mean weight of 144.18 ± 4.82 g for all fish.

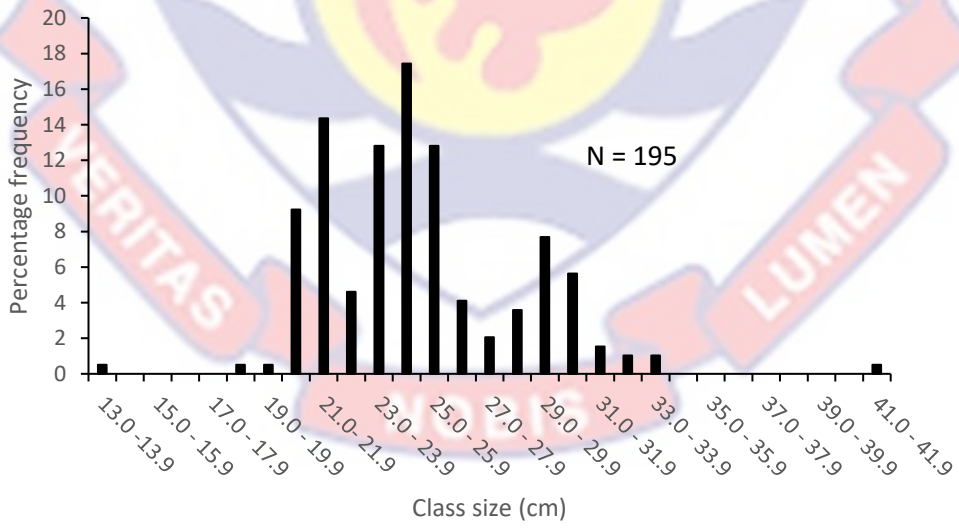

Figure 6: Length-frequency distribution of *Scomber colias* in the coastal waters of Ghana (N = sample size)

Length-Weight Relationships

Figure 7 represents relationships between body weight (*BW*, g) and total length (*TL*, cm) of all the fish collected from the selected landing sites along the coast of Ghana. The relationship was described by the power function: $BW = aTL^b$, where *a* is the antilog of the intercept value on the y-axis of the logarithmic transformed data and *b* is the regression coefficient. The total length (*TL*) and body weight (*BW*) relationship for all fish was described by the equation:

$$BW = 0.0114TL^{2.9242}$$

The exponent ($b = 2.92$) did not differ significantly from 3.0 ($t = 29.9$, $p>0.05$), suggesting isometric growth for the species in Ghana waters.

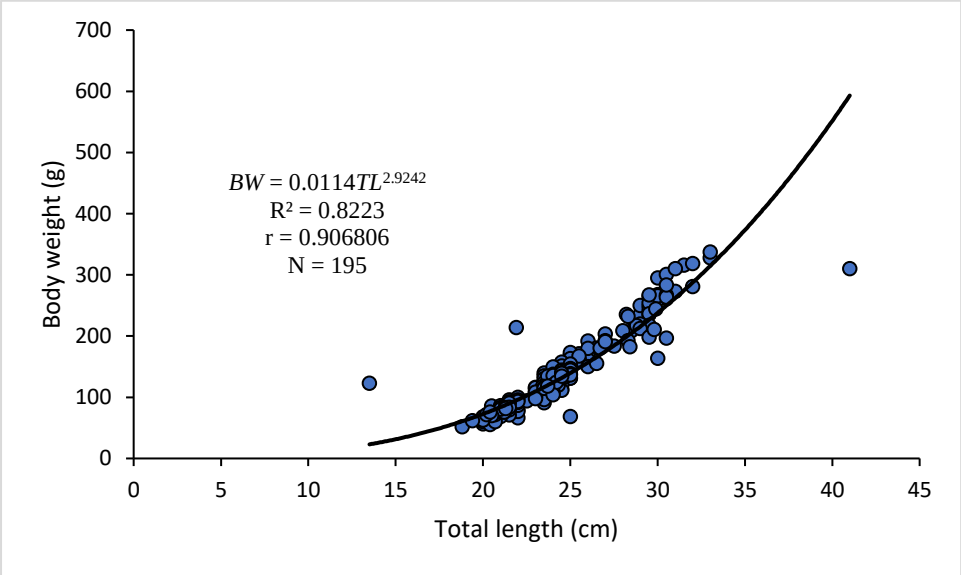

Figure 7: Relationship between body weight (*BW*) and total length (*TL*) of *Scomber colias* from all sites along the coast of Ghana (N = sample size).

Principal Component Analysis

The principal component analysis for the four morphological variables, standard length (SL), fork length (FL) total length (TL) and body weight (BW) were estimated as shown in Table 6. Four principal components explained 100%

of the total variation with a variation proportion of 95.8, 2.7, 1.2, and 0.2 % respectively. In the first component (PC1), all the variables thus, total length, body weight, standard length, and fork length had important contributions to variations observed with positive loadings Eigenvectors of 0.489, 0.500, 0.506, and 0.505 respectively (Table 6; Figure 8). The second component (PC2) was dominated by body weight, standard length, and fork length variables, all with positive loading impact. The total length contributed negatively with the loading of -0.872 (Table 6). In the third component (PC3), total weight and body weight dominated in the observed variations with positive loadings of 0.019 and 0.813. However, variables for the standard length and fork length had a negative loading impact of -0.400 and -0.423 respectively. Total length, body weight, and fork length were the highest contributors to the fourth component (PC4), having a loading of 0.026, 0.004, and 0.693 respectively (Table 6)

Table 6: Principal Component Analysis (PCA) of morphological variables among the *S. colias* population

Variables	PC1	PC2	PC3	PC4
TL	0.489	-0.872	0.019	0.026
BW	0.500	0.299	0.813	0.004
SL	0.506	0.254	-0.400	-0.721
FL	0.505	0.295	-0.423	0.693
Eigenvalue	3.8337	0.1088	0.0484	0.0091
Total variation (%)	95.8	2.7	1.2	0.2
Cumulative of variation (%)	95.8	98.6	99.8	100

TL- Total length (cm); BW- Body weight (g); SL- Standard length (cm); FL- Fork length (cm) (Field data, 2020-2021)

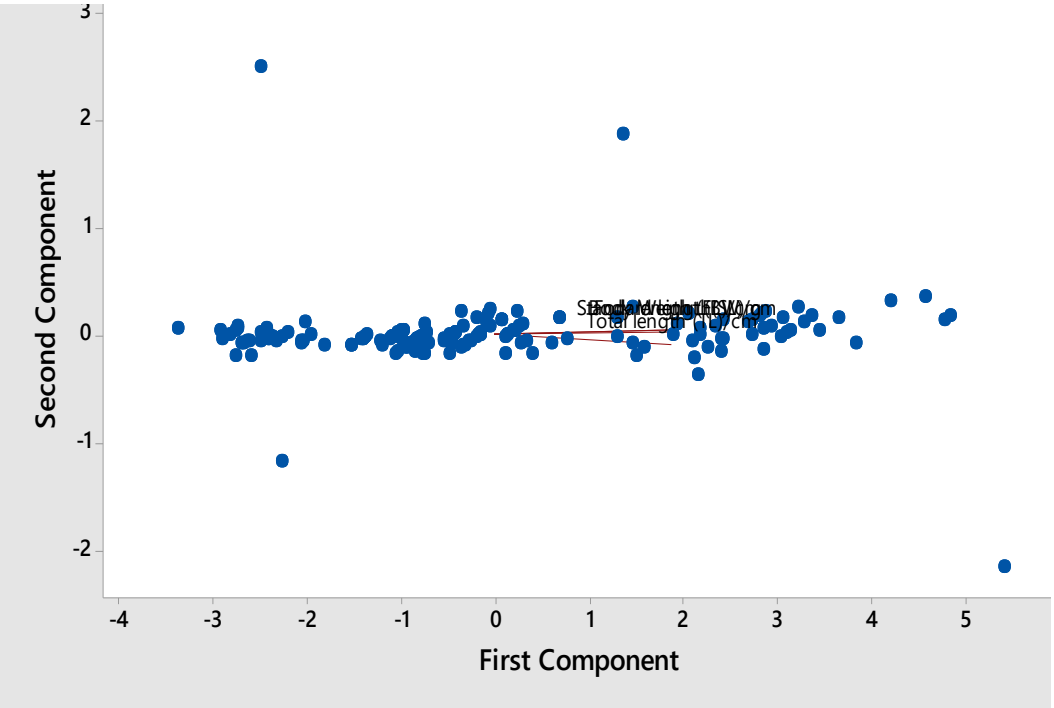

Figure 8: Biplot showing variable distribution, according to the first and second components.

Cluster Analysis

The Unweighed Pair Group Method with Arithmetical average (UPGMA) tree for the seven populations clearly shows two clusters, A and B (Figure 9) at approximately 0.5 dissimilarity co-efficient. Cluster B was made of Apam and Sekondi population. Cluster A, grouped Jamestown, Elmina Saiko, Elmina, Half Assini and Tema populations. Among the populations in cluster A, Jamestown and Elmina Saiko clustered together while Elmina, Half Assini and Tema also formed a different cluster.

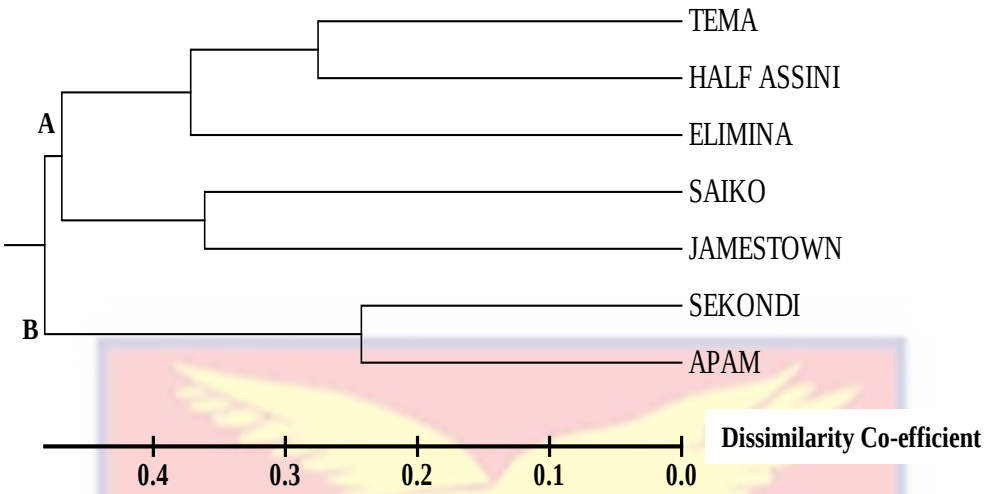

Figure 9: UPGMA trees of samples of *Scomber colias* from along the coast of Ghana based on phenotypic characteristics.

Diversity within the *S. colias* samples

The number of alleles ranged from 3 to 22 per locus however, the effective number of alleles (Ne) per locus and the mean number of different alleles (Na) and were 1.466 and 2.054 respectively (Table 7). Further analysis of alleles showed that Apam, Sekondi, Half Assini and Jamestown had 37.5, 12.5, 12.5 and 12.5% private alleles respectively. The mean observed heterozygosity (Ho) was 0.152 whilst the mean expected heterozygosity within the sites (He) was 0.244 (Table 7). Among the 7 sampling sites, Apam had the highest expected heterozygosity of 0.365 whilst Elmina had the least expected heterozygosity (0. 155). Moreover, Apam had the highest observed heterozygosity of 0.338 whilst the trawl fisheries bycatch (Saiko) showed the least observed heterozygosity (0.055). Also, Apam (APM) had the highest Shannon diversity index (I) of 0.586 whilst the Elmina had the least diversity (0.278). Fixation index (F_{IS}) ranged between 0.019 and 0.592 with a mean of

0.313. Fixation index (F_{IS}) ranged between 0.019 (Apam) and 0.736 (Elmina Saiko) with a mean of 0.313. All the population had positive F_{IS} values.

Locus variability

Among the 8 polymorphic loci examined, locus Sco2-36 generated the highest total observed heterozygosity with a value of 0.2405 and a polymorphism information content (PIC) of 0.4900, whilst Sco2-3 and Sco2-39 had the least observed heterozygosity (0.0000) (Table 8). With regards to the mean expected heterozygosity, Sco2-25 had the highest value of 0.452 whilst Sco2-3 had the least value of 0.013. All additional loci varied considerably from the HWE ($P < 0.05$) as revealed in Table 8.

Diversity between the *S. colias* samples

The mean F_{IS} ranged between -0.587 (Sco2-43) and 1.000 (Sco2-3 and Sco2-39) with an average of 0.290 (Table 9). The F_{IT} values ranged between -0.131 (Sco2-36) and 1.000 (Sco2-3 and Sco2-39) with a mean of 0.434 whilst the F_{ST} values ranged between 0.041 (Sco2-3) and 0.366 (Sco2-1) with a mean of 0.240. The mean number of migrants (Nm) per generation was 1.706 whilst the least genetic distance of 0.0468 was found between Tema and Elmina Saiko as shown in Table 9. The furthest distance of 0.4695 was found between Sekondi and Jamestown. Nei's genetic identity matrix produced values ranging from 0.6253 (Jamestown and Sekondi) and 0.9405 (Elmina Saiko and Elmina) (Table 10).

Elmina Saiko and Elmina are relatively close, as indicated by the pairwise F_{ST}. Apam also shows more closeness to the Sekondi rather than to Jamestown. Analyses of molecular variance (AMOVA) revealed that 14% of

the variance existed among the population, whilst among individuals within the population was 66% and variation within individuals 20% (Appendix B).

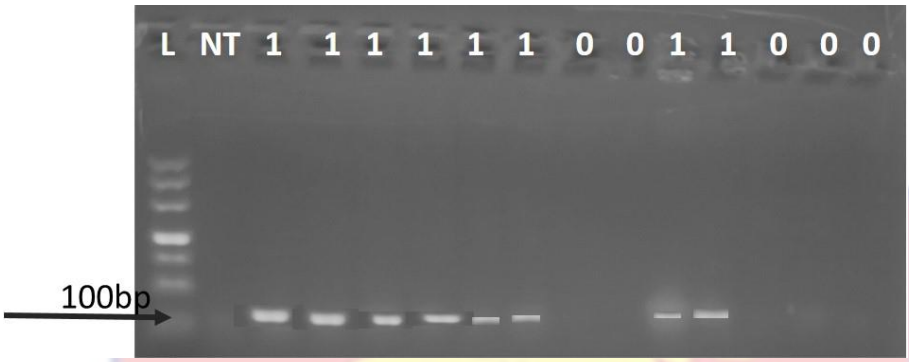

Figure 10: DNA bands from PCR amplification products of Sco2-48 for samples from Half Assini resolved in 2 % Agarose gel stained with ethidium bromide. NT- Non template control, L- 100bp ladder (Lab data, 2021)

Table 7: Genetic parameters of the 7 populations

Population		N	Na	Ne	I	Ho	He	uHe	Np	F_{IS}
APM	**Mean**	20.375	2.375	1.740	0.586	0.338	0.365	0.376	0.375	0.019
	SE	1.899	0.375	0.197	0.129	0.116	0.078	0.081	0.263	0.196
SEK	**Mean**	22.625	2.125	1.300	0.349	0.203	0.203	0.209	0.125	0.212
	SE	2.138	0.227	0.098	0.078	0.088	0.053	0.054	0.125	0.215
HAF	**Mean**	16.000	2.000	1.729	0.476	0.131	0.290	0.301	0.125	0.427
	SE	1.268	0.463	0.366	0.180	0.058	0.099	0.103	0.125	0.180
TEM	**Mean**	20.250	2.375	1.452	0.422	0.093	0.240	0.248	0.000	0.355
	SE	1.780	0.375	0.180	0.138	0.031	0.083	0.086	0.000	0.171
JAM	**Mean**	23.000	2.000	1.491	0.420	0.093	0.258	0.264	0.125	0.592
	SE	1.195	0.327	0.200	0.132	0.037	0.080	0.082	0.125	0.147
ELI	**Mean**	21.000	1.875	1.210	0.278	0.153	0.155	0.159	0.000	0.031
	SE	1.701	0.295	0.067	0.085	0.052	0.048	0.049	0.000	0.113
ESK	**Mean**	13.000	1.625	1.339	0.309	0.055	0.197	0.204	0.000	0.736
	SE	1.086	0.263	0.139	0.122	0.040	0.078	0.080	0.000	0.129
Total	**Mean**	19.464	2.054	1.466	0.406	0.152	0.244	0.251		0.313
	SE	0.737	0.126	0.076	0.047	0.027	0.028	0.029		0.069

Na = number of alleles; Np = number of private alleles; Ne = number of effective alleles; He = expected heterozygosity or gene diversity; I = Shannon's Information Index; Ho = observed heterozygosity; F_{IS} = fixation index (F_{IS}).

Table 8: The total heterozygosity, gene diversity, polymorphism information content, and Hardy–Weinberg genetic deviation probabilities

Marker	Major Allele Frequency	Ht (obs)	Ht (ave)	Allele No	Gene Diversity	PIC	Exact p-value
Sco2-1	0.38	0.1479	0.3423	8	0.7500	0.7100	0.0000
Sco2-3	0.65	0.0000	0.0130	4	0.4600	0.3700	0.0000
Sco2-6	0.53	0.2162	0.2849	4	0.6300	0.5800	0.0000
Sco2-25	0.35	0.2114	0.4519	22	0.8200	0.8000	0.0000
Sco2-36	0.62	0.2405	0.1850	3	0.5500	0.4900	0.0000
Sco2-39	0.45	0.0000	0.3648	20	0.7300	0.7000	0.0000
Sco2-43	0.52	0.2093	0.1420	8	0.6100	0.5400	0.0000
Sco2-48	0.59	0.2303	0.1686	5	0.58	0.54	0.0000
Mean	**0.51**	**0.1569**	**0.2441**	**9.25**	**0.64**	**0.59**	**0.0000**

Table 9: F-Statistics and estimates of Nm of all population for each locus

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
Sco2-1	0.546	0.712	0.366	0.433
Sco2-3	1.000	1.000	0.041	5.833
Sco2-6	0.201	0.309	0.135	1.597
Sco2-25	0.612	0.770	0.407	0.365
Sco2-36	-0.250	-0.131	0.095	2.372
Sco2-39	1.000	1.000	0.470	0.282
Sco2-43	-0.587	-0.110	0.300	0.582
Sco2-48	-0.198	-0.075	0.103	2.180
Mean	0.290	0.434	0.240	1.706
SE	0.211	0.175	0.058	0.660

Table 10: A matrix of pairwise Nei genetic distance (below diagonal) and Nei genetic identity (above diagonal) among the seven *S.colias* populations

MATRIX	APAM	SEKONDI	HALFASSINI	TEMA	JAMESTOWN	ELMINA	ELMINASAIKO
APAM	****	0.8151	0.7984	0.8179	0.7452	0.7133	0.7854
SEKONDI	0.2044	****	0.7540	0.8153	0.6253	0.7226	0.7282
HALFASSINI	0.2251	0.2823	****	0.8587	0.7735	0.9000	0.9088
TEMA	0.2010	0.2042	0.1523	****	0.9073	0.8756	0.9542
JAMESTOWN	0.2941	0.4695	0.2568	0.0973	****	0.7511	0.8758
ELMINA	0.3378	0.3249	0.1054	0.1328	0.2862	****	0.9405
ELMINASAIKO	0.2416	0.3171	0.0957	0.0468	0.1326	0.0614	****

Cluster Analysis

The UPGMA tree for the seven sampling sites produced from Nei genetic distance showed two clusters A and B (Figure 11) at 0.12 dissimilarity co-efficient. Cluster analysis or taxonomy analysis describes a natural population of the same species into distinctively related phylogenetic groups and subgroups (Oduro, 2009). The first main cluster (A) includes Elmina, Elmina Saiko, Half Assini, Jamestown and Tema. Cluster B contained Apam and Sekondi. Elmina Saiko and Half Assini formed a separate cluster among the sampling sites in the first cluster, while Elmina also formed a different cluster. However, the dendrogram (Figure 12) illustrates the association of the 195 samples studied based on the cluster analysis of their similarities.

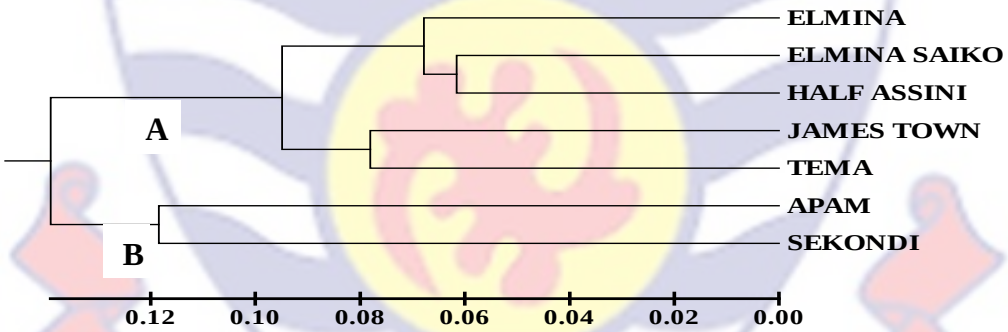

Figure 11: UPGMA trees of samples of *Scomber colias* from along the coast of Ghana generated using Nei's genetic distance matrix (values attached are branch length

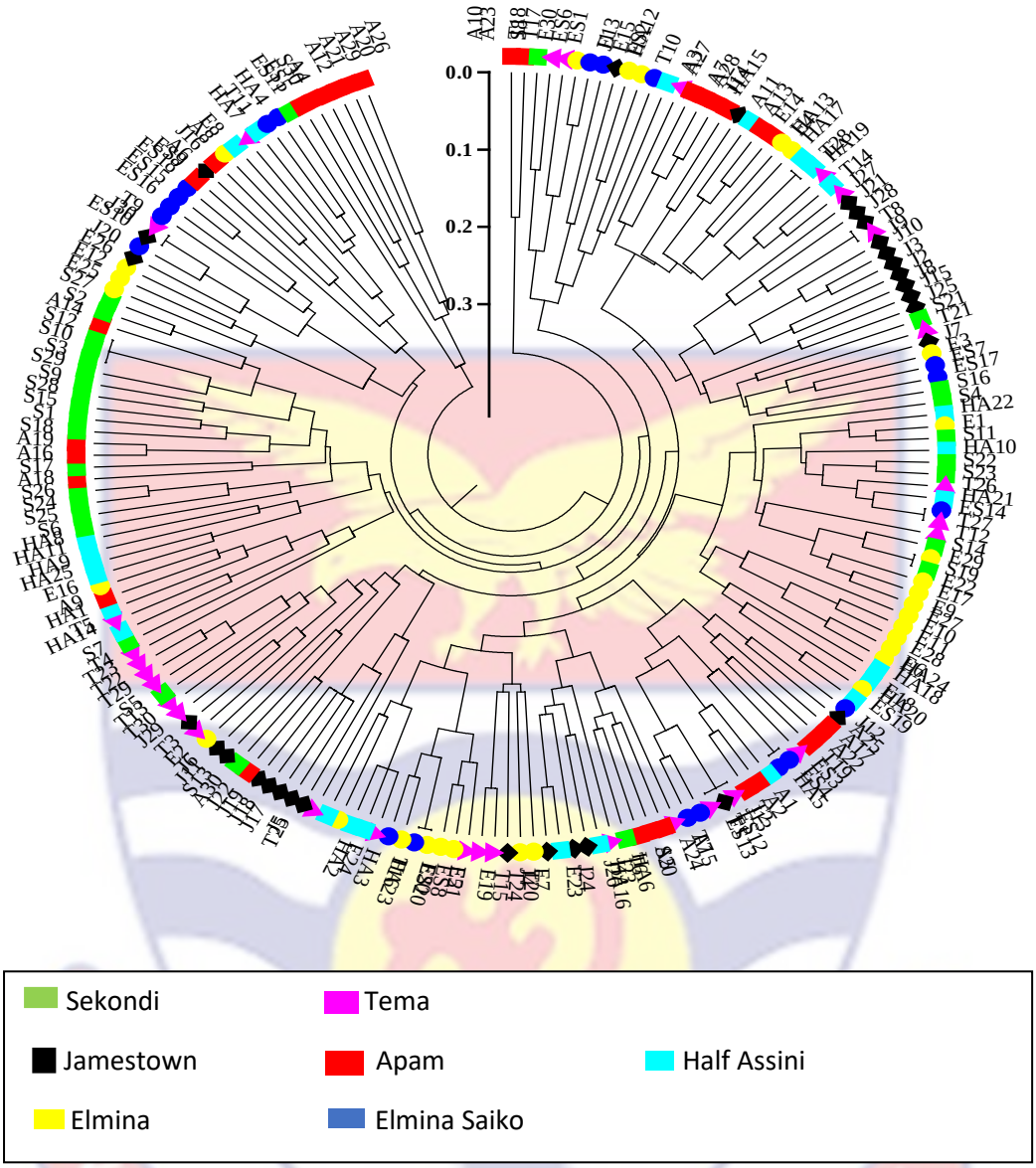

Figure 12: A dendrogram of 195 samples constructed from PowerMarker using eight microsatellite loci with UPGMA tree method

Sequence Editing, Alignments and Readings

FinchTV software version 1.4.0 was used to edit and view the sequences. Pre-alignment of the raw nucleotide sequences was examined manually for correct alignment. The sequences were first trimmed to remove low-quality regions and the reverse and forward sequences were aligned to obtain consensus nucleotide sequences. Sequences were copied and pasted in

online software, MUltiple Sequence Comparison by Log-Exptation (**MUSCLE**) for final alignment.

The sequence alignment yielded 529bp per taxon and was used for further studies. The 529bp acquired sequences were Blast searched (NCBI database) using BLASTN toolkit for confirmation. The mtDNA sequences of *Scomber japonicus* haplotype hap59 cytochrome *b* gene, partial cds; mitochondrial (Accession No. KY912249.1), *Scomber colias* haplotype 26 cytochrome *b* (cyt-*b*) gene, complete cds; mitochondrial (Accession No. KT230509.1), *Scomber colias* voucher BMVP/0913 cytochrome *b* gene, complete cds; mitochondrial (Accession No. EF439575.1) and *Scomber japonicus* haplotype hap10 cytochrome *b* gene, partial cds; mitochondrial (Accession No. KY912200.1), *Scomber scombrus* isolate ScoScoWM2 cytochrome *b* gene, partial cds; mitochondrial (Accession No. DQ080334.1) and *Oreochromis niloticus* haplotype HAP13 cytochrome *b* gene, partial cds; mitochondrial (MH041458.1) were retrieved from NCBI databases and use for comparative and phylogenetic analysis using MEGA X (Kumar et al. 2018). The similarity percentages for sequences were 99.62%, 100%, 99.43% and 97.72% respectively, with an E value of 0.0 for all. KT230509.1 was employed as an outgroup to root trees.

The sequences amplified with Sco-Cyt*B* forward primers formed good sequence reads for all the samples. Throughout the assessment of the chromatograms in the samples, the amplified region yielded a well-defined sequence peak indicating that no co-amplification of nuclear pseudogenes has occurred. Although poor reads (double peaks) were observed in few nucleotide sites for some of the samples (Figure 13 a and b).

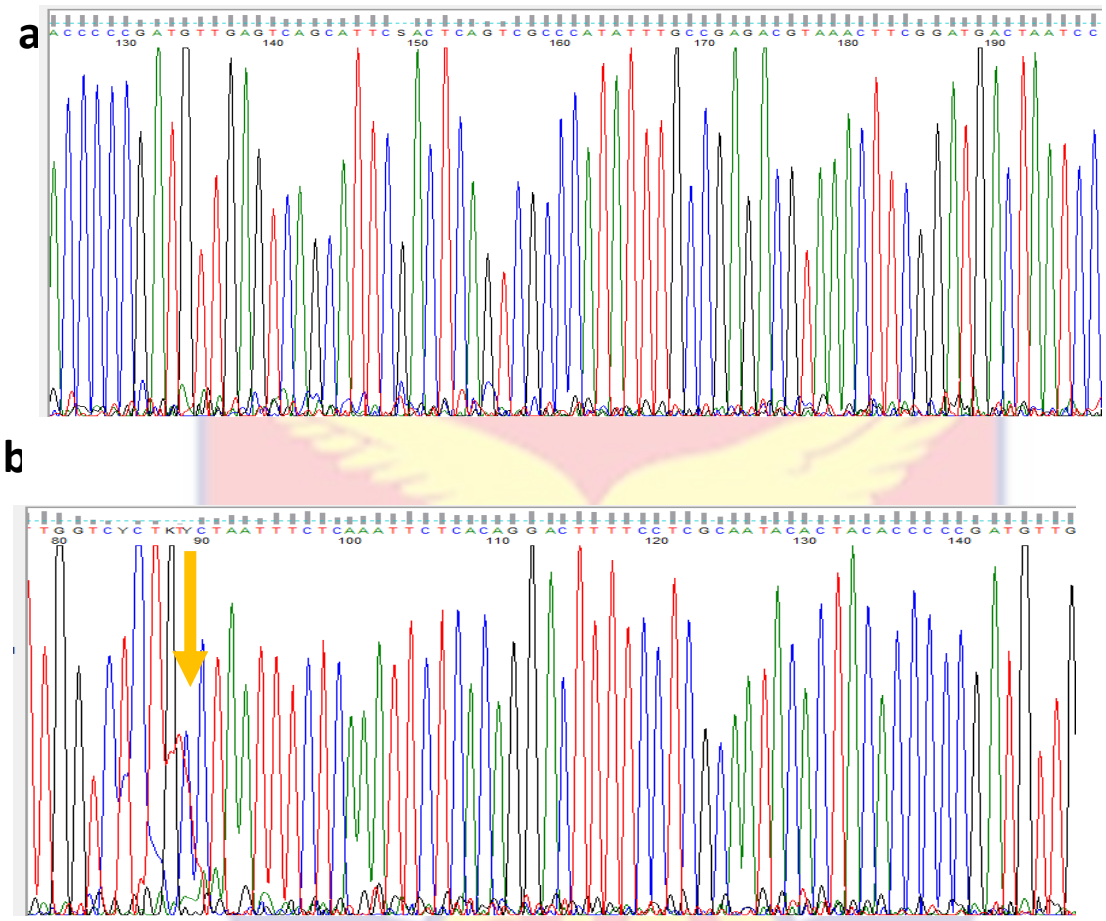

Figure 13: Sanger sequencing chromatographs of Apam6 (a) and Sekondi12 (b)
C G A T arrow showing double peaks

Sequence Analysis

The sequence analysis for all samples showed average nucleotide frequencies as 22.3% (A), 29.9% (T), 17.4% (G), 30.4% (C) and 47.8% (G~C content) (Appendix C). MH041458.1 had the highest nucleotide frequency as 24% (A) while DQ080334.1 had the least as 20.9% (A). Sekondi12 has the second highest nucleotide frequency as 22.9% (A), Apam13 and ElminaSaiko2 had frequencies as 22.7. EF439575.1 and KY912200.1 recorded 22.6% (A). Tema20, Elmina18, Elmina21, HalfAssini7 and HalfAssini11 recorded as 22.4% (A). Elmina4 had its frequency as 22.3% (A). 22.2% (A) was recorded for Apam6, Tema24, Elmina11, HalfAssini25 and KT230509.1. Jamestown8,

ElminaSaiko8, ElminaSaiko15, Sekondi15 and HalfAssini6 recorded 22.1% (A). 22.0% (A) was recorded for Sekondi7, Elmina6, HalfAssini9 and KY912249.1. The nucleotide frequencies for Thymine (T) were between 29.2 to 30.3% with KY912249.1 and MH041458.1 as highest (30.3%) and DQ080334.1 as lowest (29.2%). Apam13 had its frequency as 29.6%(T). 29.7% (T) was recorded for Sekondi12. Apam6, Jamestown8, Tema10, Tema20, Tema24, Elmina4, Elmina11, HalfAssini7 and KT230509.1 recorded 29.8% (T). KT230509.1, Elmina6, Elmina18, Elmina21, Sekondi15, HalfAssini6, KY912249.1 and EF439575.1 recorded 30.0% (T). ElminaSaiko8, HalfAssini9 and KY912200.1 recorded 31.0 and 32.0% (T) respectively. Percentage of Guanine (G) ranged between 14.9% (MH041458.1) to 19.2% (DQ080334.1). Sekondi12 and Apam13 had 16.8% (G) and 17.0% (G) respectively. 17.1% (G) was recorded for KY912249.1, EF439575.1 and KY912200.1. Elmina18 and Elmina21 had 17.3% (G). 17.4% (G) was recorded for HalfAssini7 and HalfAssini9. 17.5%% (G) was recorded for Apam6, Tema24, Tema10, ElminaSaiko8, Elmina11, HalfAssini25 and KT230509.1. Sekondi7, Tema20, Elmina4, Elmina6, Sekondi15, HalfAssini6, HALFASSINI11 and KY912249.1 had 17.6% (G). 17.7% (G) was recorded for Jamestown8 and KT230509.1. Cytosine percentage ranged from 29.9% (KY912249.1) to 30.7% (Apam13, Sekondi12, MH041458.1 and DQ080334.1). Sekondi7, Tema20, Elmina21, HALFASSINI11 and KY912200.1 recorded 30.2% (C). 30.3% (C) was recorded for Jamestown8, ElminaSaiko8, KT230509.1, Elmina4, Sekondi15 and HalfAssini6. Tema24, Tema10, Elmina6, Elmina11, Elmina18, HalfAssini7, HalfAssini9, KY912249.1 and EF439575.1 recorded 30.4% (C). 30.6% (C) was recorded for Apam6, HalfAssini25 and KT230509.1. Percentage

of G~C content ranged from 45.6% (MH041458.1) to 49.9% (DQ080334.1). 47% and 47.2% (G~C) were recorded for KY912249.1 and KY912200.1 respectively. Sekondi12, EF439575.1 and Elmina21 had 47.4 and 47.5% (G~C) respectively. 47.6% and 47.7% were recorded for Elmina18 and Apam13 respectively. 47.8% was recorded for Sekondi7, Tema20, ElminaSaiko8, HalfAssini7 and HALFASSINI11. Tema10, Tema24, Elmina4, Elmina11, Sekondi15, HalfAssini6 and HalfAssini9 had 47.9% (G~C). 48% was revealed for Apam6, KT230509.1, Elmina6, HalfAssini25, KY912249.1 and KT230509.1. 48.1% (G~C) was revealed for Jametown8.

Out of the 529bp, 365bp were conserved or constant sites and the remaining been polymorphic, singletons, parsimony informative sites and ambiguous sites or peaks (Appendix D). The polymorphisms included 81 transitions (25 A↔G and 56 C↔T) and transversions 45 (25 A↔C, 16 A↔T, 1 T↔G, and 3 C↔G). There were ambiguous sites at bases 31, 33, 34, 35, 37, 267, 273, 284, 342, 357, 398, 434, 478, 488, 504, 509 and 524 (Appendix E). There were 87 singletons and insertions (T) were found at bases 35 and 500.

Phylogenetic analyses

A neighbor-joining (NJ) phylogenetic tree was constructed to yield a graphical illustration of the pattern of divergences for all the samples. Different Scomber mitogenomes were included for comparative analyses. Based on the phylogeny and sequence similarity, the NJ tree displayed six major clusters (Figure 14). The nucleotide sequence of MH041458.1 formed cluster A, while that of DQ080334., KY912200.1 and Sekondi2 formed the basal clusters B, C and D. The remaining formed two distinct clusters, E and F, with E containing the nucleotide sequence of the cytochrome *b* gene of the following; HalfAssini7,

Tema10, Tema24, Elmina18 and ElminaSaiko2. F consist of sequences of ElminaSaiko8, Sekondi15, HalfAssini6, ElminaSaiko15, KY912249.1, Elmina6, Sekondi7, HalfAssini9, Elmina21, EF439575.1, Tema20, HALFASSINI11, Elmina4, Jametown8, Apam6, HalfAssini2, Elmina11, KT230509.1 and Apam13 (Figure 14).

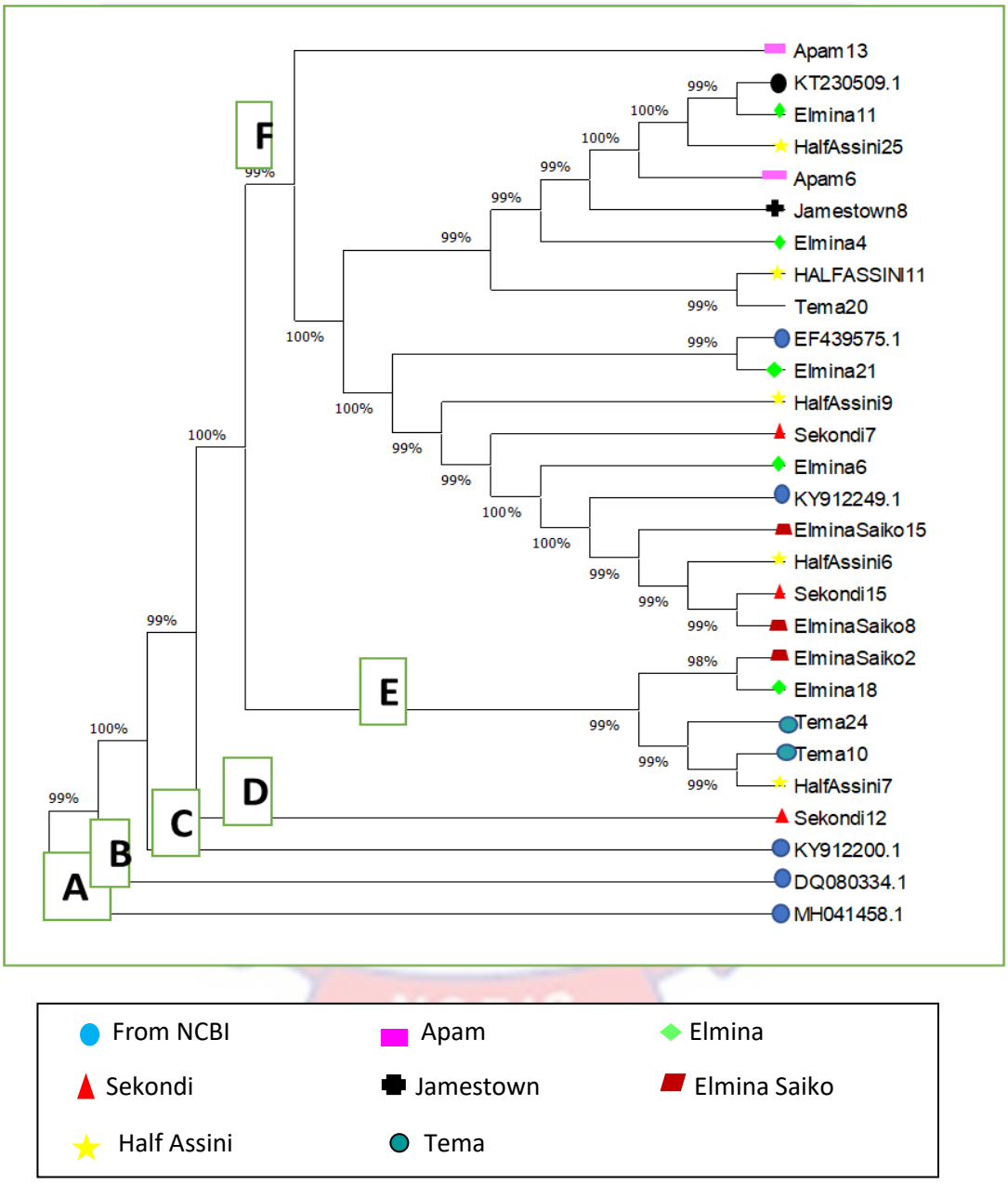

Figure 14: Phylogeny of selected samples across the population based on mtDNA cytochrome b sequences. Different mitogenesis from NCBI were added in the analysis.

Comparative analysis of genetic and phenotypic dendrograms

The tanglegram comparison revealed that both the positions and groupings of the populations were inconsistent across the genotypic and phenotypic dendrograms. Out of the seven populations, four of them maintained their position, that is, Apam, Sekondi, Jamestown and Elmina (Figure 15). However, the two dendrograms revealed two distinct clusters (A and B)**.** Apam and Sekondi appeared in cluster B for the genotypic and phenotypic dendrograms. Cluster A had two sub-clusters (I and II) for the two dendrograms. However, sub-cluster I of the genotypic dendrogram grouped Elmina, Elmina Saiko and Half Assini while that of the phenotypic dendrograms grouped Tema, Half Assini and Elmina. Subcluster II of the genotypic dendrogram Jamestown and Tema populations and Elmina Saiko and Jamestown populations appeared in subcluster II of the phenotypic dendrogram

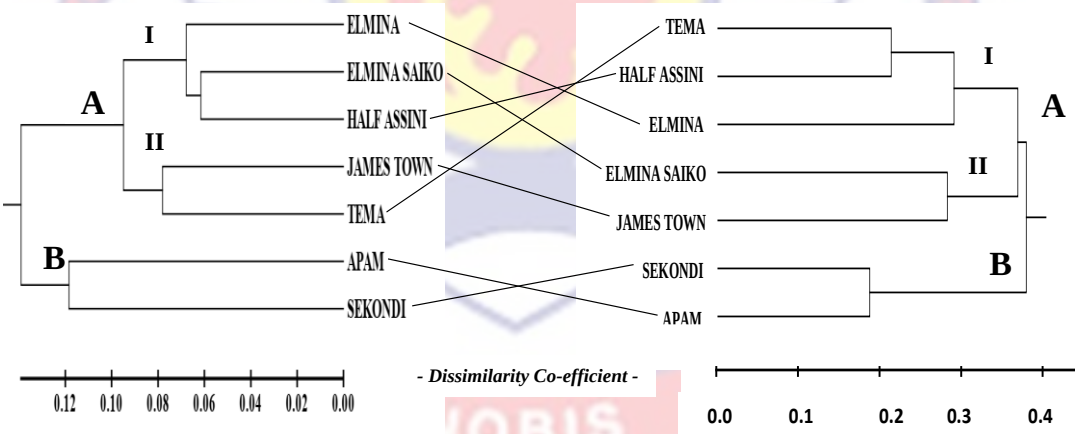

Figure 15: Tanglegram showing comparison of genotypic and phenotypic dendrograms

CHAPTER FIVE

DISCUSSIONS

This chapter discusses the results obtained from analysis of length-weight relationships, principal component analysis and genetic variation using microsatellite and mtDNA cytochrome *b* sequences

Length-weight relationship

The length–weight relationship is a vital biological parameter (Bagenal & Tech, 1978; Martin, 1949; Ricker, 1978). The maximum known total length for Atlantic chub mackerel from literature is 54 cm (Martins, 1996). The total length measurements taken for all specimens (N = 195) however, indicated the maximum total length as 41.0 cm.

The weight of *S. colias* obtained from the bycatch of commercial catches along the coast of Ghana ranged from 51.4 -337.4 g. All the length measurements taken had a modal length of 24 cm *TL*. According to Martins et al. (2013), two modal lengths (21.0 cm *TL* and 30.0 cm *TL*) were found for samples from the artisanal catches off Portugal, while purse seine and trawl samples were represented by a single mode in the 21 cm *TL* and 24- 26 cm *TL* classes, respectively. Keč & Zorica (2012) also recorded a single mode for the species caught in the Adriatic Sea. Earlier studies on the fish in Ghana (Amponsah, Ofori-Danson & Nunoo 2016; Kwei, 1971) did not report on the size range of the fish. Kwei's (1971) analysis of the length-weight relationship showed that *S. colias* had a maximum length (L_{max}) of 25.0 cm *FL* and weight (W_{max}) of ~300 g and out of this, Kassah (2020) was able to calculate the L_{max} to be 27.4cm *TL* using the equation determined for the fork length-relationship of *Scomber colias* in her studies. Comparing the L_{max} in the 1970s (27.4 cm *TL*) to the

present studies (41 cm *TL*), it can be deduced that, the landings in the early 70s contained fish smaller than the present samples. Similar variations in the size distribution of chub mackerel have been observed in Morocco and Mauritania-Senegal waters (Habashi & Wojciechowski, 1973; quoted in Hernández & Ortega, 2000), and off South Africa (Dietrich & Ritzhaupt, 1979; In Hernández & Ortega, 2000). Lorenzo & Pajuelo (1996) recorded L_{max} of 42.1 cm *TL* and Wmax of 746.6 g in waters off the Canary Islands, and Jurado-Ruzafa, Hernandez, & Santamaria (2017) reported L_{max} of 49.0 cm *TL* and W_{max} of 1,512 g in Mauritanian waters. An L_{max} of 70 cm TL were observed in catches off South Africa in 1984 (Ostapenko, 1988). In the Adriatic Sea, L_{max} of S. colias was reported (Keč & Zorica, 2013) to be 38 cm FL which is 41.7 cm TL (Kassah, 2020). It is therefore apparent that the maximum size of *S. colias* differs among locations and this may be as a result of differences in the genetic and environmental factors, fishing gears and fishing methods. Isometric growth of *S. colias* determined for all samples collected in this study confirmed results recorded in earlier studies (Sinovčić, Franičević, Zorica & Čikeš-Keč, 2004; Stergiou & Moutopoluos, 2001). This also confirmed Kwei"s (1971) analysis, which found that *S. colias* also exhibited isometric growth in Ghanaian waters. Several reports indicate considerable variations in the growth patterns of *S. colias* populations along the west coast of Africa. Isometric growth of the fish has been reported (Namibia, b= 3.1; South Africa, b= 3.3; Morocco to Senegal, b= 2.88-3.56) (Hernández & Ortega, 2000). Jurado-Ruzafa, Hernandez & Santamaria (2017) found positive allometric growth for the population in Mauritania. Daley and Leaf (2019) found negative allometric growth in the North-West Atlantic population. It is evident from these reports that isometric

or allometric growth in *S. colias* is not specific to geographical regions. Differences in the growth dynamics of *S. colias* noted among regions can be ascribed to diverse reasons, such as variations in sample sizes, diverse type of gear used to collect the fish and temporal variability (Okomoda, Koh, Hassan, Amomsakun and Shahreza, 2018).

Principal Component Analysis (PCA)

According to Hotelling (1933), PCA is an informative tool to determine unknown trends in a multidimensional set of data. At an Eigenvalue greater than 1, comprising 95.8% of the total variation, all the variables were found to be an important contributor to describing the shape of the *S. colias* (Table 6). The first two, PC1 and PC2 explained 98.6 % of the cumulative variation and this was mainly due to the high positive loading coefficient of the number total length, body weight, standard length, and fork length. The third cumulative of variation of PC1, PC2 and PC3 explained 99.8 % of the total variation and this was mainly due to the high positive loading of the total weight and body weight. These factors seemed to contribute to the isometric growth observed in the *S. colias* population. At PC4, total length, fork length and body weight were the major contributors at 100 %.

Cluster Analysis

From this study, the UPGMA cluster analysis showed groupings between samples from the various landing stations (Figure 10). The close relatedness of Apam and Sekondi in cluster B may imply that the similarity of specimens from these two landing sites is higher. In the same way, the grouping of Jamestown, Elmina Saiko, Elmina, Half Assini and Tema populations in cluster A, may also suggest that *Scomber colias* from these landing sites may

have close genetic relatedness. Fish, on the other hand, are known to exhibit a significant amount of environmentally driven morphological variation, which may indicate differing developmental or dietary contexts. Hence, environmental factors such as salinity, temperature, prolonged swimming, or food availability may determine the phenotypic differentiation in *Scomber colias* along the coast of Ghana (Allaya et al, 2016). In a study of the Atlantic bonito *Sarda sarda* which belongs to the *Scombridae* family by Franicevic et al. (2005), it was proposed that discrepancies in morphometric features between specimens from different geographical landing sites could be attributable to environmental variables or genetic structure. This, therefore, proposes that, the similarities and dissimilarities of the cluster analysis may be due to either genetic variations or environmental conditions, or even both.

Diversity within the *S. colias* population

Protecting fish resources and the prevention of severe future depletion in stocks depends on effective management, which also depends on information of gene flow levels, population structure and genetic variation within and between populations of commercially exploited species (Cha et al., 2010; Utter 1991). One of the basic aspects of fish population study is genetic variation. Identifying the genetic structure of a population is essential to evolutionary biology. It displays naturally existing genetic differences among members of the same species. Natural populations are separated into several populations, each of which has distinct genetic characteristics (Bezault et al., 2011). The mean total of Shannon diversity and heterozygosity of all the population was below 0.5 (Table 7) and suggests that there is moderate genetic variability of the *S. colias* population along the coast of Ghana. High Shannon

diversity index indicates high diversity, thus the closeness it is to 1, the more diversified a population is (Sarma & Das, 2004).

Heterozygosity on the other hand is a vital yardstick of population diversity at the genetic level (Xu, Primavera, De La Pena, Pettit & Belak 2001). Although heterozygosity varies between samples, the observed mean heterozygosity was lower than the expected mean heterozygosity for all the samples studied (Table 7). Expected heterozygosity is an estimate of the heterozygosity based on the assumption that the population is at Hardy-Weinberg equilibrium (HWE). Lower observed mean heterozygosity than expected mean heterozygosity shows that the *S. colias* population has deceased in size (Boopathi et al., 2019). Another reason might also be the inability to separate closely sized alleles and/or the presence of null alleles as a result of the presence of stutter bands in the electropherograms of the dinucleotide microsatellites used (Hassanien & Gilbey, 2005)

Average deviation Hardy-Weinberg within subdivisions (F_{IS}) and deviation from Hardy-Weinberg equilibrium in the total population (F_{IT}) according to study have been used to determine the levels of heterozygosities in the natural population in population genetics (Çiftci & Okumu, 2002). It has also been stated that there is an excess of heterozygosity especially when alleles are of low frequencies (Crow & Kimura, 1970). The negative values in this assessment (Table 9) show the presence of excess heterozygotes and that may be due to the presence of different genotypes that occurred in very low proportions. This may therefore suggest that sexual selection, mutation, migration, the allele frequencies, or the genotype frequencies are not consistent from generation to generation as expected under Hardy-Weinberg equilibrium

(HWE). This is most common in natural populations which usually contradict Wright's (1951) island model (Briñez, Caraballo & Salazar, 2011)

The results from the analysis of molecular variance showed a high genetic differentiation (F_{ST} = 0.240) among collected samples (Table 9). According to De Vicente, Lopez, and Fulton (2004) genetic differentiation is said to be large when the F_{ST} value falls from 0.15 to 0.25.

Also, percentage-wise, AMOVA revealed greater diversity among individuals within the *S. colias* population (66) compared to that within any given population, which is expected for any natural population when polymorphic loci are present in individuals of a population (Boopathi et al., 2019). The high genetic variation within the *S. colias* population proposes high genetic diversity within the *S. colias* population. The current results confirm those of Medina-Alcaraz, (2014).) who noted high genetic diversity in each population of *Scomber colias* collected from the Atlantic and Mediterranean Sea. High genetic variation in a species according to Allendorf and Phelps (1980) aids the ability of organisms to adjust to a dynamic environment and is needful for species survival.

The presence of private alleles (Table 7) among four sampling sites (Apam, Sekondi, Half Assini and Jamestown) did not affect the F_{ST}. According to Jost (2008), as heterozygosity becomes large, F_{ST} will naturally approach 0, indicating low differentiation even if all alleles at a locus are private.

Genetic differentiation between the *S. colias* population

The differentiation between populations is caused by migration, mutation, selection and drift (Gall, 1987; Whitlock, 2011; Holsinger & Weir, 2009). The total heterozygosity of the alleles was 0.1569 while the mean expected and observed heterozygosity were 0.244 and 0.152 respectively, an indication that the variability in the gene pool was maintained (Table 7). This variation can be presumed to be high, since gene diversity (0.64) and Shannon information index (0.406) were significantly greater than zero (P>0.05) (Table 7).

The low gene differentiation in Elmina and Elmina Saiko from this study as well as their location on the phylogenetic tree thus, their close relatedness shows that there may be increased level of inbreeding (Figure 11). There were two clusters within cluster A (Elmina, Elmina Saiko and Half Assini, Jamestown and Tema) and the second cluster B, also comprised of Apam and Sekondi. This shows that there is low genetic variation among the population. The second cluster contained the Apam and Sekondi population (Figure 11). However, AMOVA showed 14% of the variance that existed between the population. This implies a low genetic variation among the population (Appendix B).

Sequences variation

DNA sequencing is the leading technique selected in molecular-based research (Critiano, Cardoso & Fernandes-Salomão, 2014). Changes in an organism's mitochondrial DNA sequence are of four main groups: additions, deletions, sequence rearrangements and nucleotide substitutions (Gerbi & MacIntyre, 1997). However, emphasis has been made on nucleotide

substitutions in the derivation of phylogenetic relationships (Wilson et al., 1985). From the sequence data, amplified sequences formed good sequence reads although few double peaks were seen in few nucleotide sites for some samples. Each of these letter-specific combinations of signals and is defined in the IUPAC list of ambiguous nucleotides. Where R stands for either A or G, Y = C or T, S = G or C, W = A or T, K = G or T. The usefulness of mitochondrial DNA markers has been confirmed in most studies, however, a number of studies have revealed that its stability is subjected to compromise (Cristiano et al., 2012; Bertheau et al., 2011; Buhay, 2009). Numerous molecular processes undermine some general assumptions based on mtDNA unique features. One of the common issues is the incorporation of mitochondrial fragments into the nuclear genome referred to as nuclear mitochondrial pseudogenes (numts) (Lopez et al., 1994; Hazkani-Covo et al., 2010). Seeing double peaks in a chromatograph may imply that co-amplification of nuclear pseudogenes has occurred or the PCR product used for the sequencing was prepared from a diploid genomic DNA, where polymorphic regions will simultaneously show both nucleotides. Nuclear mitochondrial pseudogenes are non-transcribed and untranslated regions in the nuclear genome that occur as a result of the natural transfer of DNA from the mitochondrial to the nuclear genome during the evolutionary period (Hazkani-Covo & Zeller, 2010; Gaziev & Shaikhaev, 2010).

Based on lack of function in their nuclear genome and the presence of complex machinery to monitor and repair damaged DNA in the nucleus, numts evolved under molecular evolutionary processes that are different from the original mitochondrial copy. Numts have been observed in a number of eukaryote taxa from plants to higher vertebrates. This may bring about false

interpretations and misperceptions while conducting phylogeographic, phylogenetic and population genetic studies (Buhay, 2009; Hazkani-Covo & Zeller, 2010; Bensasson, Zhang, Hartl & Hewitt, 2001) therefore the key underlying assumption of homology between sequences is overlooked when both mitochondrial gene and nuclear copies are used in molecular analysis.

As defined for other teleosts, two main features were found. First, the most represented base in all cases was C; secondly, a bias against G was observed (Appendix C). The G~C content of the mitogenomes of all the sequenced samples (47% to 48%) and the average G~C content of all samples (47.8) fall within the limits observed in other Scombridae species (Catanese et al., 2008; Manchado, Catanese & Infante, 2004). However, these values were higher than in other teleosts (Hurst, Bartlett, Davidson & Bruce, 1999; Broughton et al., 2001; Inoue et al., 2001b; Oh et al., 2007; Wang et al., 2008; Zhu & Yue, 2008), the high G~C content seems to be related to higher temperatures in tropical waters thus, attaining thermostability is dependent on high G~C content for nucleic acids (Wang & Hickey, 2002; Galtier & Lobry, 1997; Nakashima, Fukuchi & Nishikawa, 2003). Therefore, the worldly distribution of Scomber species living in tropical and subtropical warm waters according to Collette and Nauen (1983) confirms such a hypothesis.

According to study, nearly all DNA sequences examined from any genome, transitions (A↔G, T↔C) are noted to occur at higher frequencies than transversions (T↔A, T↔G, C↔A, C↔G) (Brown et al. 1982; Curtis & Clegg 1984; Gojobori, Li & Graur, 1982; Wakeley 1994). Eighty-one transitions (25 A↔G and 56 C↔T) and transversions 45.

Phylogenetic assessment based on mtDNA cyt-*b* sequence

The phylogeny of scomber mackerel remains dependent on mtDNA sequence data (Infante et al., 2007). Although mtDNA sequences provide obvious benefits for phylogenetic studies, It is essential to know that they represent a single linkage group. Hence this disadvantage limits their application in determining the taxonomic relationships due to short internodes and for which lineage sorting causes non-congruence between species and gene trees (Hudson, 1992).

The pattern of the branching of the phylogenic tree of this study depicted that, the species evolved from a common ancestry (98% to 100% bootstrap consensus) (Figure 14). The phylogenetic relationships between species used in this study exhibited six different phylogroups (mainly A, B, C, D, E and F) (Figure 12). Although evolving from a common ancestry (lineage) there was heterogenous and homogenous distribution using mtDNA which is different from what was observed in some scombroid species. It has been reported that two divergent sets of linages with heterogenous distribution were observed among some scombroid species, including bigeye tuna, bluefin tuna, sailfish, blue marlin and swordfish (Chow et al. 2000; Alvarado Bremer, Naseri & Ely, 1998; Chow & Kishino 1995; Graves & McDowell 1995; Finnerty and Block 1992; Rosel & Block 1996).

Moreover, from the phylogenetic tree, the nucleotide sequence of cyt-*b* gene for Elmina11 formed a sister group with KT230509, revealing that they are closely related (Figure 14). Also, the nucleotide sequence of cyt-*b* for EF439575.1 and the of Elmina21 formed sister groups revealing their close relatedness. The nucleotide sequences of cyt-*b* gene for the following pairs;

HALFASSINI11 and Tema20, Sekondi15 and ElminaSaiko8 and Tema10 and HalfAssini7 formed sister groups and are therefore likely to be closely related to each other (Figure 14).

However, the nucleotide sequences of cyt-*b* gene for samples found within a population did not cluster or form clades, although the bootstrap consensus ranged from 98% to 100%. The observed patterns in these samples have showed the existence of variation among nucleotide sequences and even within a single population, this may therefore suggest that samples of *S. colias* within a particular population may possess a little degree of genetic divergence.

Genetic and phenotypic relatedness

A comparative analysis of the genotypic and phenotypic variations observed in a tanglegram revealed an inconsistent pattern of relatedness of *S. colias* at the different landing stations (Figure 15). This observed variation may be attributed to the difference in morphological and molecular characteristics. The random markers used in the genetic diversity study may have no link with genes that control traits or morphological parameters used in this study. According to Nkhata, et al. (2020), the inconsistency between genotypic and phenotypic clusters are attributed to environmental variance.

CHAPTER SIX

SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS

The significance of this study was to characterize *S. colias* species along the coast of Ghana using microsatellite and mitochondrial DNA cytochrome *b* markers to ascertain their genetic diversity and propose a road map for its sustainability, management and utilization in Ghana's fisheries industry.

The morphological parameters (length and weight) studied sought to determine the size distributions (based on length and weight) and also length-based estimates of growth to establish the current status of the species in the Ghanaian coastal waters. A combination of field-work involving the collection of samples landed from the artisanal fishery across the Central and Western coastlines of Ghana as well as laboratory methods were employed to obtain data which were used to accomplish the aim of the research. This study was the first of its kind as no genetic diversity study on *Scomber colias* in Ghanaian waters exist.

Summary of Key Findings

The results of the study indicate that one main cohort of fish with the modal length of 24 cm *TL* classes are the most exploited in Ghanaian waters. The overall length-weight relationship shows that adult fish in Ghanaian waters undergo isometric growth ($BW = 0.0114TL^{2.92}$). The weight of *S. colias* obtained from the bycatch of commercial trawlers along the coast of Ghana ranged from 51.4 -337.4 g. The Principal Component Analysis employed revealed four principal components which explained 100% of the total variation with a variation proportion of 95.8, 2.7, 1.2, and 0.2 % for total length (TL), body weight (BW), standard length (SL) and fork length (FL) respectively.

The findings of the analysis of molecular variance revealed a high level of genetic differentiation within the *S. colias* population (FST = 0.240). Also, AMOVA showed 14% of the variance that existed between the population.

The nucleotide sequences of cyt-*b* gene for samples demonstrated the existence of divergence among nucleotide sequences and even within a single sampling station.

The tanglegram generated showed similarities and divergences between the genotypic and phenotypic dendrograms.

Conclusions

Growth in all the samples exhibited isometric. The Principal Component Analysis revealed that all the morphological variables contributed to the shape of the *S. colias* specimens.

The mean observed heterozygosity (Ho) within the population was 0.152 whilst the mean expected heterozygosity (He) was 0.244. The mean gene diversity and PIC were 0.58 and 0.54 respectively.

It has been noted that molecular markers used in the genetic diversity studies were informative. However, it has been recommended that the limitations of markers can be better served if alternative markers are used concurrently.

The nucleotide sequences of cyt-*b* gene for samples demonstrated the existence of divergence within and among the nucleotide sequence of the *S.colias* population. However, the extent of divergence was known by the use of microsatellite loci markers. Results showed that there is high genetic divergence (F_{ST} = 0.240) in the *S. colias* population and relatively low divergence (14%) between the population.

The observed patterns in the nucleotide sequences of cyt-*b* gene for samples showed the existence of variation among nucleotide sequences for samples collected from the various sampling sites.

Recommendations

Small population size is associated with reduced genetic variation. The results of this studies point to the fact that there is low genetic diversity between the *S. colias* population, proof that the *S. colias* species are being overexploited and hence the decision to implement a seasonal closure will yield the best results if carried out during the major spawning period (July to August) to revive the species in Ghana.

The whole -genome sequence of *S.colias* should be carried out to give a highly detailed view of their genetic variation and gene structure.

Results of this study revealed isometric growth in all the specimens collected along the coast of Ghana therefore, carrying out a whole-genome sequence will also help in the identification of candidate genes responsible for performance traits.

Suggestions for Further Research

1. Sequencing across the whole genome of *S. colias*.
2. Study on the population dynamics and genetic diversity of *S. colias*.
3. Profiling the genetic diversity of large sample of *S. colias* across annual seasonal changes.

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APPENDICES

APPENDIX A: Descriptive statistics of total length (TL) and body weight (BW) for *Scomber colias* sampled from the selected sampling sites along the coast of Ghana

All fish	N	Mean TL ± s.e.(cm)	TL range (cm)	Mean BW± s.e.(g)	BW range
A	195	24.65 ± 0.25	13.5-41.0	144.18 ± 4.82	51.4 -337.4

APPENDIX B: Results of analysis of molecular variance (AMOVA) for analyses of all Scomber colias population

Source	d.f.	Sum of squares	MS	Est. Var.	%
Among Population	6	135.699	22.617	0.343	14%
Among Individuals within population	188	674.147	3.586	1.562	66%
Within Individuals	195	90.000	0.462	0.462	20%
Total	389	899.846		2.366	100%

APPENDIX C: A table showing counts and percentages of nucleotide sequences for all samples

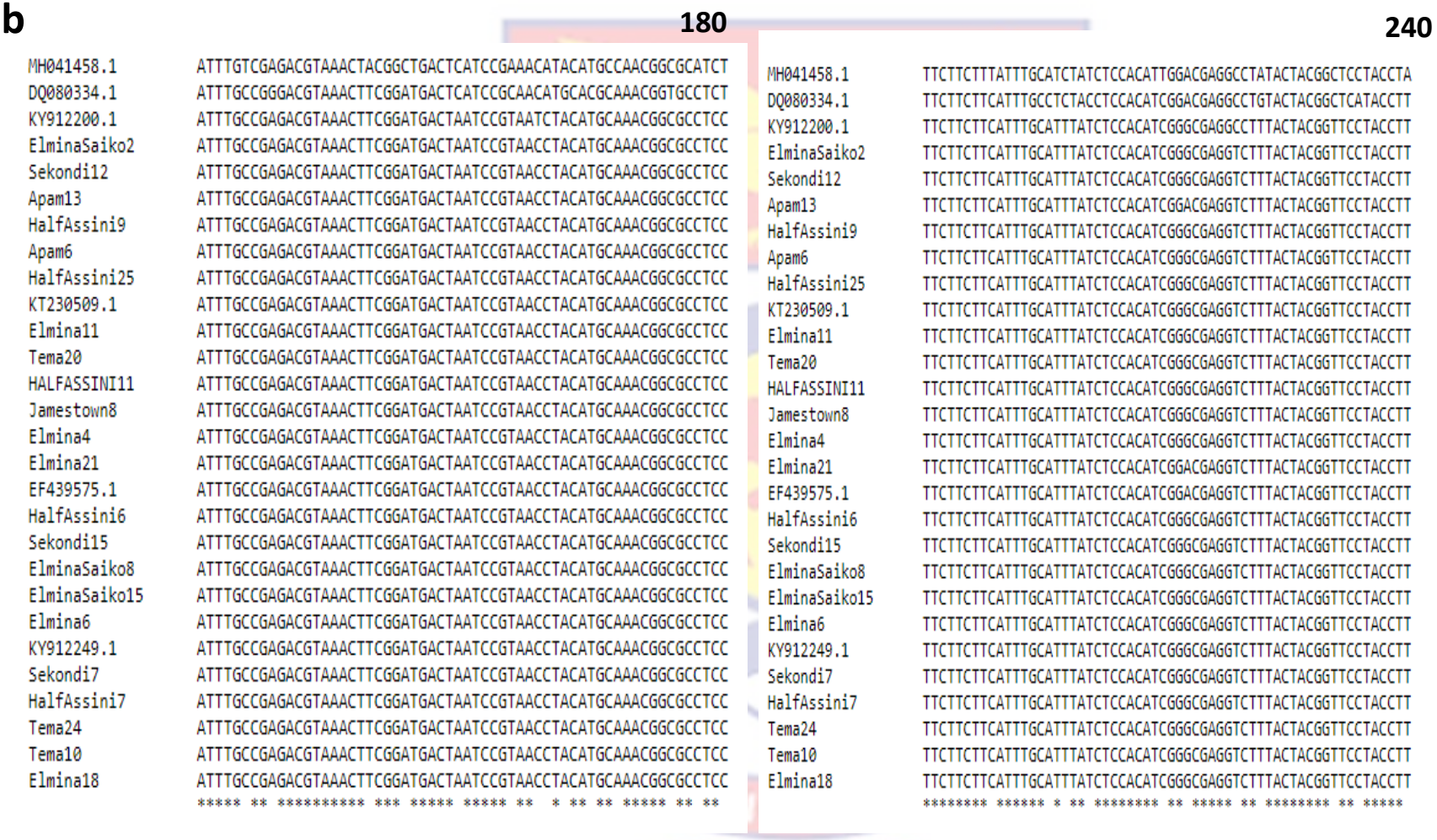
Samples	Adenine (A)		Thymine (T)		Guanine (G)		Cytosine (C)		
	Count	%	Count	%	Count	%	Count	%	
Apam6	117	22.2	157	29.8	92	17.5	161	30.6	48.0
Sekondi7	116	22.0	159	30.2	93	17.6	159	30.2	47.8
Apam13	119	22.7	155	29.6	89	17.0	161	30.7	47.7
Jamestown8	116	22.1	156	29.8	93	17.7	159	30.3	48.1
Tema24	117	22.2	157	29.8	92	17.5	160	30.4	47.9
Tema10	117	22.2	157	29.8	92	17.5	160	30.4	47.9
Tema20	117	22.4	156	29.8	92	17.6	158	30.2	47.8
KY912249.1	118	22.7	157	30.3	89	17.1	155	29.9	47
ElminaSaiko8	116	22.1	158	30.1	92	17.5	159	30.3	47.8
KT230509.1	116	22.1	157	30.0	93	17.7	159	30.3	48
Sekondi12	120	22.9	156	29.7	88	16.8	161	30.7	47.4
Elmina4	117	22.3	156	29.8	92	17.6	159	30.3	47.9
Elmina6	116	22.0	158	30.0	93	17.6	160	30.4	48
Elmina11	117	22.2	157	29.8	92	17.5	160	30.4	47.9
Elmina18	118	22.4	158	30.0	91	17.3	160	30.4	47.6
Elmina21	118	22.4	158	30.0	91	17.3	159	30.2	47.5
Sekondi15	116	22.1	157	30.0	92	17.6	159	30.3	47.9
HalfAssini6	116	22.1	157	30.0	92	17.6	159	30.3	47.9
HalfAssini7	117	22.4	156	29.8	91	17.4	159	30.4	47.8
HalfAssini9	115	22.0	157	30.1	91	17.4	159	30.4	47.9
HalfAssini25	117	22.2	157	29.8	92	17.5	161	30.6	48
HALFASSINI11	117	22.4	156	29.8	92	17.6	158	30.2	47.8
KY912249.1	116	22.0	158	30.0	93	17.6	160	30.4	48
KT230509.1	117	22.2	157	29.8	92	17.5	161	30.6	48
EF439575.1	119	22.6	158	30.0	90	17.1	160	30.4	47.4

KY912200.1	119	22.6	159	30.2	90	17.1	159	30.2	47.2
MH041458.1	126	24.0	159	30.3	78	14.9	161	30.7	45.6
DQ080334.1	110	20.9	154	29.2	101	19.2	162	30.7	49.9
Mean		22.3		29.9107143		17.4035714		30.3892857	47.775

APPENDIX D: List of all 529 sites (gaps excluded) found in Scomber 5S rDNA units. Numbers above sites indicate arbitrary positions with respect to the first sequence (Sco 1) in the 766 bp alignment. Dots indicate identity with reference sequence

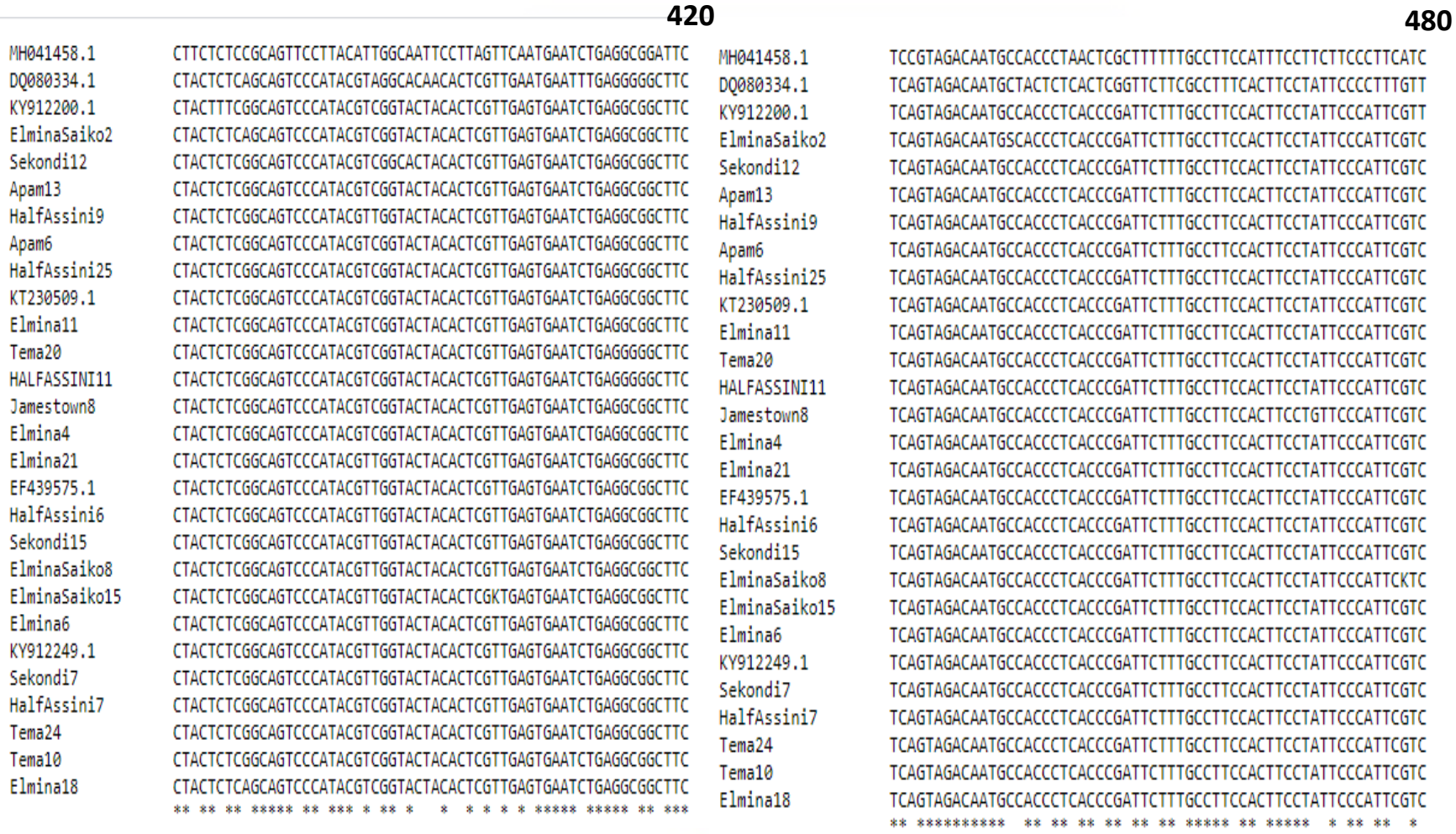
a

b



C

d



e

529

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MH041458.1          ATTGCAGCCGCAACAATAG-TTCACCTAATTTTCCTTCACGAAACT---
DQ080334.1          ATCTTAGCGGCAGCGGTTC-TCCACCTACTATTCCTTCATGAAACTGGG
KY912200.1          ATTCTAGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
ElminaSaiko2        ATCCTGGSAGCAACAATTC-TTCWCCTGYTATTCCTACATGAAACCGGG
Sekondi12           ATCCTAGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGA
Apam13              ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
HalfAssini9         ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Apam6               ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
HalfAssini25        ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
KT230509.1          ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Elmina11            ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Tema20              ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
HALFASSINI11        ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Jamestown8          ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Elmina4             ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Elmina21            ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
EF439575.1          ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
HalfAssini6         ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Sekondi15           ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
ElminaSaiko8        ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAAYCGGG
ElminaSaiko15       ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Elmina6             ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
KY912249.1          ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Sekondi7            ATCCTGGCAGCAACAATTCTTTCACCTGCTATTCCTACATGAAACCGGG
HalfAssini7         ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Tema24              ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Tema10              ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
Elmina18            ATCCTGGCAGCAACAATTC-TTCACCTGCTATTCCTACATGAAACCGGG
                    **     *  *** *  *   * * ***  * ***** ** ****
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APPENDIX E: A table showing ambiguous nucleotide bases from the Sanger sequencing data

Base pairs (bp)	Ambiguous nucleotides				
	K	R	S	W	Y
31					Sekondi12, Sekondi15
33					Apam13, Tema20, Jamestown8, Elmina4, HalfAssini6, HalfAssini7, HALFASSINI11
34	ElminaSaiko2, Sekondi12, Apam13, Sekondi15				Tema20, HALFASSINI11, HalfAssini6, HalfAssini7,
35					ElminaSaiko2, Tema20, HALFASSINI11, Jamestown8, Elmina4, Elmina21, HalfAssini6, Sekondi15, ElminaSaiko15, Sekondi7, HalfAssini7, Tema24, Tema10
37					ElminaSaiko2, Apam13, HalfAssini9, Elmina11, Tema20, HALFASSINI11, Jamestown8, Elmina4, HalfAssini7
267		HalfAssini9			
273					HalfAssini7
284				ElminaSaiko2	

342		HalfAssini9			
357		HalfAssini9			
398	ElminaSaiko15				
434			ElminaSaiko2		
478	ElminaSaiko8				
488			ElminaSaiko2		
504				ElminaSaiko2	
509					ElminaSaiko2
524					ElminaSaiko8