Thesis for the Degree of Master of Fisheries Science

Mitochondrial Genomes of *Cynoglossus senegalensis* and *Chrysichthys nigrodigitatus* from the coastal water of Cameroon

by

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Graduate School of Global Fisheries

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카메룬 연근해에 서식하는 *Cynoglossus senegalensis*와 *Chrysichthys nigrodigitatus*의 전장 미토콘드리아게놈의특성규명

Advisor: Prof. Park Wongyu

by

Fantong Zealous Gietbong

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Abstract

The complete mitogenomes of *Cynoglossus senegalensis* and *Chrysichthys nigrodigitatus* were determined by the combination of high-throughput sequencing and the traditional PCR-based cloning. The size of each complete mitochondrial genome was 16,519 bp for *C. senegalensis* and 16,514bp for *C. nigrodigitatus,* respectively. Translocation of putative control region (D-Loop) and a tRNA gene in the mitochondrial genome occurred and their location was identified between ND1 and tRNA-Gln genes. Phylogenetic analysis showed that *C. senegalensis* was most closely related to *Cynoglossus sinicus and Cynoglossus bilineatus,* which were geographically distant and further study should be conducted to understand the complicated evolution of fish in Cynoglossidae. Different from *C. senegalensis,* the mitogenome of *C. nigrodigitatus* showed a canonical mitochondrial genome organization in which 13 Protein-coding genes, 22 tRNA genes, 2 rRNA genes, and 2 noncoding regions including the light-strand replication origin (OL) and a putative

control region (CR) were conserved. All tRNA genes were predicted to fold into the typical cloverleaf secondary structures with the typical base-pairing except for tRNA-Ser (AGC). The phylogenetic analysis with currently known complete mitogenome sequences in Siluriformes showed that *C. nigrodigitatus* is most closely related to *Auchenoglanis occidentalis* forming a family Claroteidae cluster.

Keywords: Mitochondrial genome, *Cynoglossus senegalensis, Chrysichthys nigrodigitatus*, Cameroon,

List of Abbreviations

µL	Microliters
µM	Micromoles
COI	Cytochrome C oxidase subunit 1
CR	Control Region
dNTPs	Deoxyribonucleotide triphosphate
IRAD	Institute of Research and Agricultural Development
Kb	Kilobytes
OL	Light –Strand Replication Origin
Qv	Quality value
rRNA	Ribosomal Ribonucleic acid
tRNA	Transfer Ribonucleic acid

Introduction

Species identification is the first step for the scientific management of the fish resources. Traditionally, species identification is based on the external morphological features, including body shape, pattern of colors, and the measurements of other body parts (Strauss and Bond, 1990). Although morphological identification of fish species has been successfully used, it also requires considerable amount of time and efforts for the analysis. In some cases morphological identification is often difficult for those which have lost their characteristic features or which are in their early life stages (egg and larvae) (Strauss and Bond, 1990). Researchers have attempted to develop novel methods for identifying fish species without relying on morphological features. The genetic information is now alternatively used for the species identification for the fastness and accuracy. The most widely used genetic makers for the species identification are partial mitochondrial DNA sequences such as cytochrome C oxidase I (COI) or cytochrome B (Cyt B) (Palumbi, 1991; Vrijenhoek, 1994; Ward et al., 2005). However, full mitochondrial genome sequences would be also useful for the bio-geographical or evolutional information. Complete mitochondrial DNA information is also vital for the determination of the genetic structure, biology, and its aquaculture potential (Chauhan and Rajiv, 2010; Okumuş and Çiftci, 2003; Yáñez et al., 2014).

Moreover, because of the characteristics of coding content conservation, maternal inheritance, rapid evolution and low level intermolecular genetic recombination, mitogenomes have become increasingly effective and popular makers for molecular research, such as phylogenetic molecular evolution, population genetics, phylogenetics, and comparative and evolutionary genomics. In addition to comparing nucloetide and amino acid sequence applying to molecular evolution, the complete mitogenome of tRNA secondary strcture, gene arrangement and models of the control of replication and transcription have been used extensively for deep-level phelogenetic inference in taxonomy in the recent decades. As a result, more than 5,000 mitochondrial genomes have been deposited in the GenBank database (www.ncbi.nlm.nih.gov) from over 30,000 species identified based on the morphological characteristics (www.fishbase.org).

Cynoglossus is a genus in the family Cynoglossidae. They are in the Eastern Atlantic and widely found in the estuaries and in freshwater lagoons of West Africa. Some main characteristics which help distinguish the Cynoglossus from other genera of the Cynoglossidae include the flat and elongate body, the small eyes which were placed on left side of head, dorsal and anal fins lacking spinous rays, and absence or rudimentary pectoral fins (Paugy et al., 2003). According to Fish Base (http://www.fishbase.org/), the family Cynoglossidae has 3 genera and 67 species are currently recognized in the genus Cynoglossus. Many of them include the commercially important demersal species, and some species are nearly threatened in the IUCN red list (Desimone et al., 1990; List, 2015). Cynoglossus is a highly valued food fish and plays a vital role in the aquatic ecology and

fisheries in African water bodies. Four species of the genus Cynoglossus are currently known in Cameroon waters including *Cynoglossus browni* (Nigerian tongue soles), *Cynoglossus canarensis* (Canary tongue soles), *Cynoglossus monodi* (Guinea tongue soles), and *Cynoglossus senegalensis* (Senegalese tongue soles). The guinea tongue soles dominate the other *Cynoglossus* spp. and are mostly exploited by the commercial fishing. However, species identification in the genus *Cynoglossus* is often difficult due to its broad geographical range and overlapping distributions (Paugy et al., 2003). They can also show intra-specific variations and differences, and due to similarities in the early life stages (eggs and larvae), the young ones are more difficult to identify than adults(Strauss and Bond, 1990).

Chrysichthys is a genus in the family Bagridae (commonly called Bagrid catfish and known by the indigenous population as "yinda"). They are found near the shores and in the estuaries of all tropical and subtropical regions. Some species enter rivers and a few happens to live permanently in fresh water. The major characteristics which help distinguish the *Chrysichthys* from other genera of the Bagridae are the presence of its pointed snout, the rather small mouth, and the width of the premaxillary tooth plate (1/5-1/3.5, usually 1/4, of head length); vomerine dentition represented by a square to rectangular tooth plate which begins to develop at sizes between 60 and 70 mm standard length; palatine dentition developing at sizes over 100 mm standard length, initially as isolated teeth; second or third branched dorsal-fin ray always the longest; upper caudal-fin lobe much longer than lower lobe; gill rakers long and smooth; other characters, such as

length of dorsal fin, number of branched rays in anal fin, and number of gill rakers on first gill arch, are subject to intraspecific variations; sexually mature males and (some) ripe females show considerable morphological differences; such specimens have often been labelled as *Chrysichthys furcatus*; maturity occurs probably at a rather advanced stage (at over 200 mm SL) and leads to the inflation of head, broadening of mouth and premaxillary tooth plate, shortening of spines and overgrowth of fin-spines by thick skin, the fins becoming more rounded, and the caudal-fin lobes sometimes becoming sub equal; the body acquires an emaciated, thinned down appearance (Lévêque et al., 1990). According to (Lujan and Armbruster, 2012) some characters are only clear in mature males in reproduction: such as a short adipose fin, base contained 8-11 times in SL and measuring 28-64% of distance between dorsal fin and adipose fin; maxillary barbel not reaching beyond dorsal spine when extended; head swollen; skin mucous; spines thick and covered with skin. However, species identification in the genus *Chrysichthys* is always difficult for its broad geographical ranges and overlapping distributions (Benson et al., 2007). They can also show intra-specific variations and differences, and due to similarities in the early life stages (eggs and larvae), the young ones are more difficult to identify than adults(Strauss and Bond, 1990).

Molecular study on the complete mitochondial genomes of *Cynoglossus senegalensis* and *Chrysichthyes nigrodigitatus* will be performed to complete the genetic information for the future conservation and management of the fisheries resources.

According to Fish Base (http://www.fishbase.org/), there are currently 42 recognized species in the genus *Chrysichthys*. Species in *Chrysichthys* are commercially important with a high potentials for aquaculture (Duwal et al., 2016; Erondu, 1997; Ezenwa et al., 1986; Nwafili et al., 2015). The culture of the species of *Chrysichthys* is widely practiced in many countries of the West African sub region and constitute one of the largest fresh water cultivated fish (Ezenwa, 1981). *Chrysichthys* is highly valued food-fish and plays a vital role in the aquatic ecology and fisheries in African water bodies. It makes significant contribution to the artisanal fisheries of rivers and lagoons. Owing to its economic importance and sustainability for culture, considerable research has been develop to the study of several aspects of the species in Nigeria waters (Ekanem, 2000; Ezenwa and Ikusemiju, 1981; Ikusemiju and Olaniyan, 1977).Three species in the genus *Chrysichthys* are currently known in Cameroonian coastal waters including *Chrysichthys helicophagus*, *Chrysichthys nigrodigitatus* and *Chrysichthys auratus*.

It has been established that fish production of a country can be increased through the proper management of the fisheries resources and through fish culture in order to augment the natural yield. Although much information is available on the identification of fishes in different parts of the world, the identification of many fish species in the coastal waters of Cameroon have been little studied and as such vital information for their culture and sustainable management is scanty. (NGOANDE and Yongbi). The aim of this work is to characterize the mitochondrial genome of two commercially important fish species, *Cynoglossus senegalensis* and *Chrysichthys nigrodigitatus* in the coastal water of

Cameroon, in order to determine the genetic structure, understand the biology and aquaculture potentials and hence sustainable management.

Materials and Methods

2.1. Study Site: Geographical Location

Cameroon is located at the base of the Gulf of Guinea, in the Bight of Biafra. The coastal zone extends from Nigeria border ($4^0$40'N) to Equato-Guinean boarder ($2^0$20'N). From Campo at the Southern Border of River Nyong, the coastline is high and rocky, alternating with sandy beaches. From River Nyong to Tiko and Njangassa to Rio-del-Rey, the coastline is low and swampy, characterized by the presence of mangroves with alternating sandy beaches. Between Njangassa and Tiko, the coastline is covered with volcanic deposit from the active Mount Cameroon. The continental area (up to 200 m depth) is about 12,900 square kilometres. Human activities along the coastline include fishing, swimming, tourism, fish processing and business (Ssentogo and Njock, 1987).

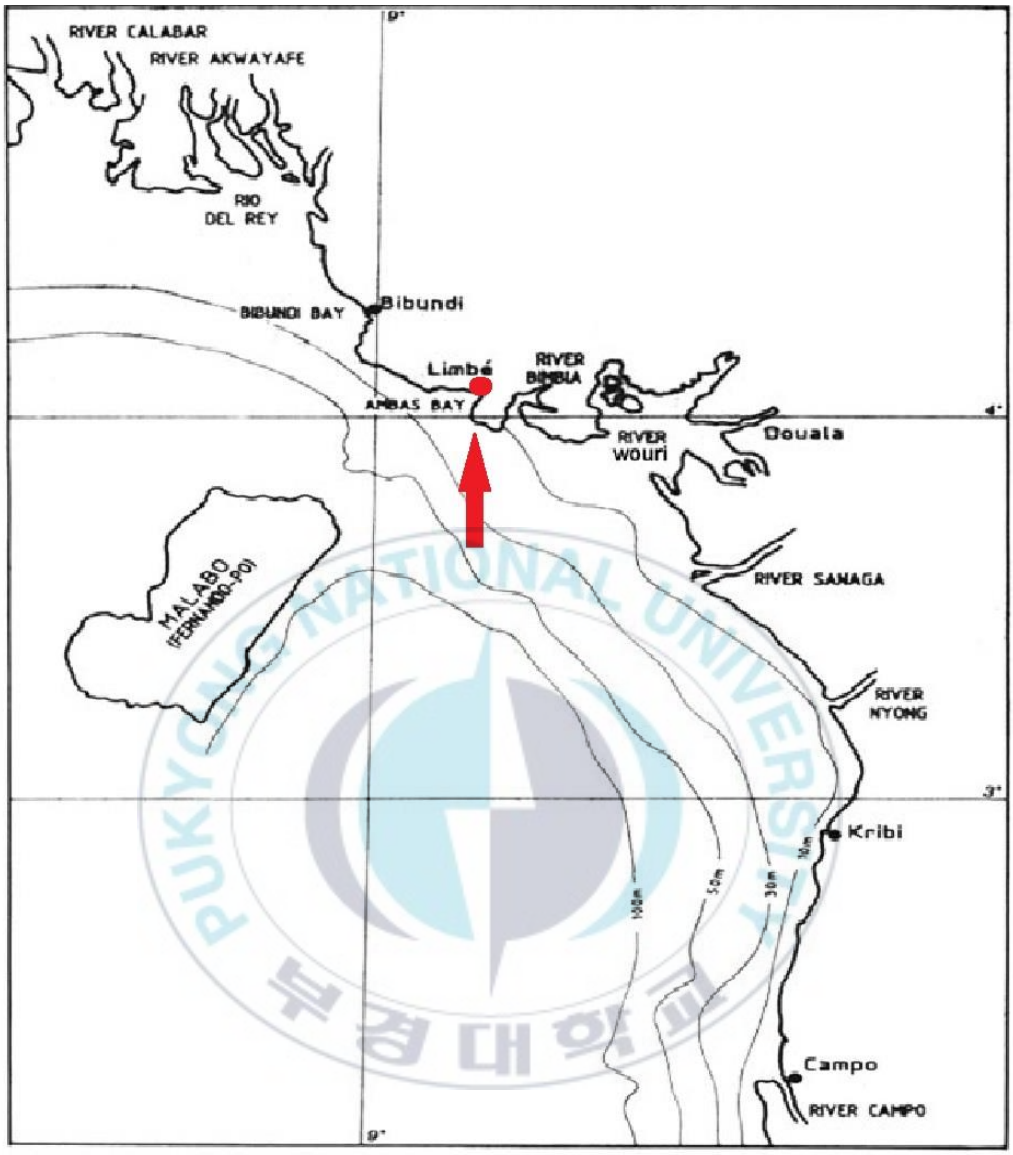

Figure 1. Map of Cameroon Coastline (Gabche and Hockey, 1995)

Collected fish were immediately stored in 96% ethanol and kept at -20ºC until further analysis (Knebelsberger and Stöger, 2012). Morphological identification was done by looking at body shape, type of scales, fin features, morphometric (i.e. Standard length, body width, and other relevant measurements), and meristic characteristics (Strauss and Bond, 1990).

2.1.1. Sample collection and Preservation

Fish samples were purchased from artisanal fishermen/fish market along the coastal region of Cameroon specifically at the Limbe Dockyard market. Fish samples were collected and stored in 96% ethanol and kept at -20ºC until further analysis. The type species were kept at the Fisheries and Oceanographic Research Station(IRAD Batoke) Cameroon.

2.2. Genomic DNA extraction

Genomic DNA was extracted using an Accuprep Genomic DNA Extraction Kit (Bioneer) following the manufacturer's instruction. A small portion of the muscle was cut, and was further homogenized by the use of Tissuelyser II (Qiagen). Purified genomic DNA was quantified with nano-drop (Thermo fisher Scientific D1000), aliquot, and stored at -70ºC for later use.

Two universal primer sets targeting cytochrome c oxidase (COI) region, BCL and BCH, (Ward et al., 2005), and targeting mitochondrial rRNA ITS- region, Fish_F2 and Fish_R1 (Palumbi, 1991), were used to obtain the partial sequences of each of the gene respectively.

Table 1. List of Species specific primers used during this study

Species	Primer ID	Sequence (5' to 3')	Amplicon Size
C. senegalensis	Cyno_CO1_ITS-F	CCG GAG CTG CAA CGG GAT GAA CTG	10.9 kb
	Cyno_CO1_ITS-R	CGA TTT GCA CGG ATG ACT TCT CAG TGT AAG GG	
	Cyno_ITS_CO1-F	CGG CTC TTA AGC GCG TAC ACA CCG	6.0 kb
	Cyno_ITS_CO1-R	GAG AGG CAG GGA TAG AAG TAA TAG GAC GGC	
C. nigrodigitatus	Chry_CO1_ITS-F	GGC AGG AAC AGG ATG AAC TGT TTA CCC	13.1 kb
	Chry_CO1_ITS-R	GGA TGT CTT CTC GGT GTA AGG GAG ATG C	
	Chry_ITS_CO1-F	GAA TTA GGC TCT GAG ACG CGC ACA CAC	5.0 kb
	Chry_ITS_CO1-R	GAT GGA GGA TAC ACC TGC AAG ATG GAG GG	

The quality of all the primers used in this experiment was analyzed by the Oligo Analyzer 3.1 (http://sg.idtdna.com/calc/analyzer) and commercially synthesized by Bioneer Co. (Korea).

2.2.1. PCR amplification and sequencing

Each PCR mixture (30μL) contain 19.8μL ultra-pure water, 2μL primer (1μM, forward and reverse), 0.3 μM Ex Taq DNA polymerase (TaKaRa, Japan), 3Um 10x Buffer, 3uL dNTPs (1 Um TaKaRa, Japan), and 100ng DNA as template. PCR was carried out under the following condition: initial denaturation step at 95ºC for 3 min, and followed by 35 cycles of denaturation at 95ºC for 30 s, annealing at 50ºC for 30 s, and extension at 72ºC for 45 s (COI target sequence) or 30 s (Mitochondrial rRNA-ITS). The process was completed with the final extension at 72ºC for 10 min. Two PCR products targeting the partial sequences of COI and Mitochondrial rRNA - ITS were then purified with Accuprep Gel purification kit (Bioneer, Korea) and ligated into a cloning vector (Promega, USA), sequenced in both directions.

In order to obtain two large PCR products (11kb), two pairs of sequence-specific primers sets for *Cynoglossus senegalensis* and *Chrysichthys nigrodigitatus* (Cyno_CO1_ITS-F and Cyno_CO1_ITS-R and Chry_CO1_ITS-F and Chry_CO1_ITS-R) were designed based on the obtained partial sequences of each region (Table 1). Each PCR reaction (30 μL) contained 19.8 μL ultrapure water, 2 μL of each primer (1 μM), 0.3 μL Ex Taq Hot Start Version DNA polymerase (TaKaRa, Japan), and 100 ng genomic DNA as template. PCR was carried out with two -step PCR protocol for long PCR under the following condition:

initial denaturation step at 94°C for 3 min, followed by 30 cycles denaturation at 98°C for 10s, and annealing and extension at 68°C for 10 min. The process was completed with a final extension at 72°C for 10 min. Two large PCR products were pooled together in equal concentration and fragmented to 350 bp in length by Covaris M220 (Covaris Inc.). TruSeq sample preparation kit V2 (Illumina, USA) was used for the construction of a library from fragmented sequence and the quality and quantity of the constructed library was measured using 2100 Bio analyzer (Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed by Illumina Miseq platform (2 × 300 bp pair ends) (Illumina, USA).

2.2.2. Assembly of mitochondrial genome by the bioinformatics analysis

Raw reads from MiSeq sequencer, with under Qv 20 and more than ambiguous nucleotides, were removed from raw read using CLC genomic Workbench v 7.5 (CLC BIO Aarhus, Denmark). Mothur software was used in pairing forward and reverse sequence with more than 7 bp overlapped and without mismatch. Paired sequence then assembled using Geneious R8 with minimum 20 bp of overlapping sequence and 100% overlap identity. Ambiguous sequence of the D-loop region was reconfirmed by the typical end point PCR and with sequence-specific primers and DNA sequencing of its PCR products by Sanger sequencing method.

Results and Discussion

3.1. Morphological and molecular Identification of *Cynoglossus senegalensis* and *Chrysichthys nigrodigitatus.*

We identified species of the collected species based on the morphological characteristics for *C. senegalensis* and *C. nigrodigitatus*, respectively, which was described in previously (Strauss and Bond, 1990). For the genus Cynoglossus, we identified the flat and elongated body, slightly tapering backward, snout broadly rounded, the rostral hooks rather short and extending to the anterior nostril; mouth hook-shape; eye small and placed on the left side of the head separated by wide interorbital space; preopercular margin not free, covered by skin and scales; dorsal and anal fins lacking spinous rays and confluent with caudal fin; pectoral fins absent or rudimentary; only the pelvic fin of eyed side present, united to anal fin; small ctenoid scales on eyed side and cycloid scales on blind side; median lateral line on blind side (Paugy et al., 2003). For the fish in the Chrysichthys, there is the presence of pointed snout, the rather small mouth, and the width of the premaxillary tooth plate (1/5-1/3.5, usually 1/4, of head length); vomerine dentition represented by a square to rectangular tooth plate which begins to develop at sizes between 60 and 70 mm standard length; palatine dentition developing at sizes over 100 mm standard length, initially as

isolated teeth; second or third branched dorsal-fin ray always the longest; upper caudal-fin lobe much longer than lower lobe; gill rakers long and smooth; other characters, such as length of dorsal fin, number of branched rays in anal fin, and number of gill rakers on first gill arch, are subject to intraspecific variations; sexually mature males and (some) ripe females show considerable morphological differences; such specimens have often been labelled as *Chrysichthys furcatus*; maturity occurs probably at a rather advanced stage (at over 200 mm SL) and leads to the inflation of head, broadening of mouth and premaxillary tooth plate, shortening of spines and overgrowth of fin-spines by thick skin, the fins becoming much more rounded, and the caudal-fin lobes sometimes becoming sub equal; the body acquires an emaciated, thinned down appearance (Lévêque et al., 1990). According to (Lujan and Armbruster, 2012), some characters are only clear in mature males in reproduction: such as a short adipose fin, base contained 8-11 times in SL and measuring 28-64% of distance between dorsal fin and adipose fin; maxillary barbel not reaching beyond dorsal spine when extended; head swollen; skin mucous; spines thick and covered with skin. These characteristics suggested that the collected samples were *Cynoglossus senegalensis* and *Chrysichthys nigrodigitatus* respectively.

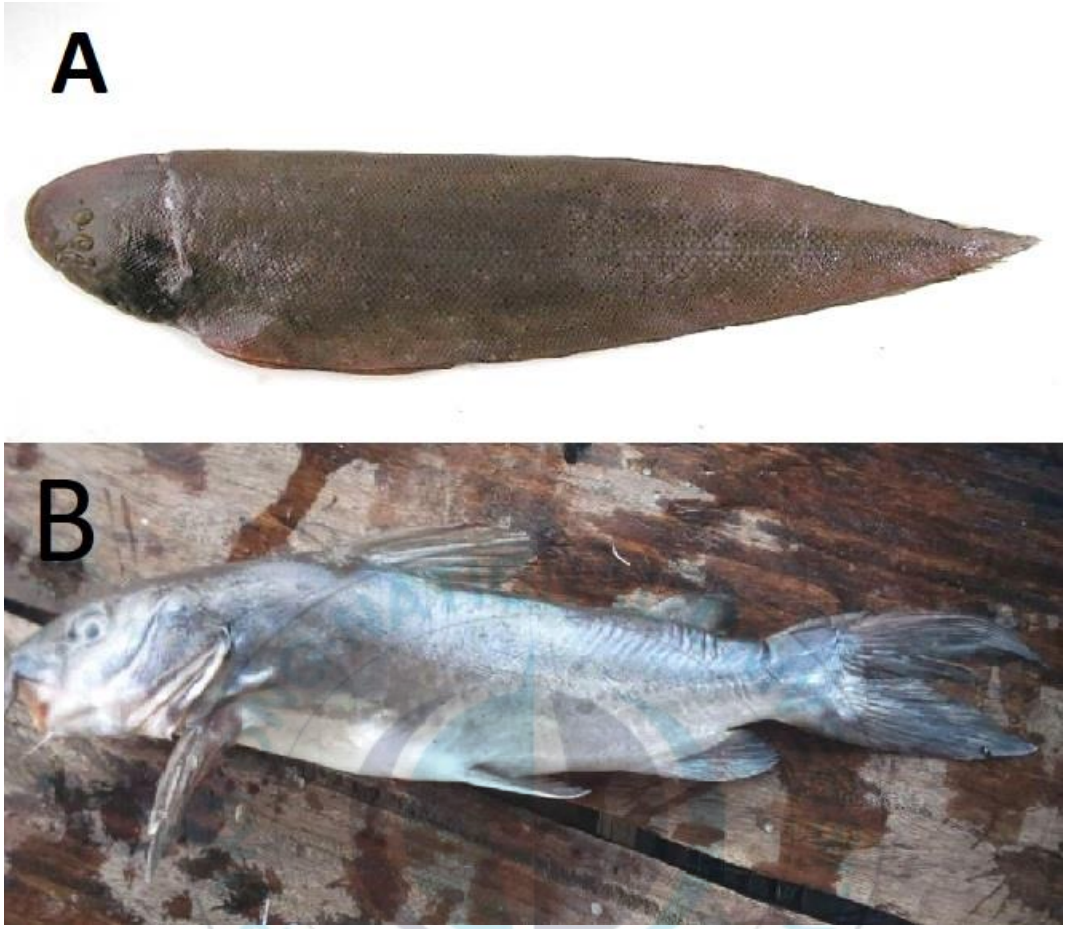

Figure 2. *Cynoglossus senegalensis* (A) and *Chrysichthys nigrodigtatus* (B) collected from the Coastal Waters of Cameroon**.**

According to Fish Base (http://www.fishbase.org/), the most closely related Cynoglossus species, *C. sinicus* and *C. bilineatus*, are distinguished from *C. senegalensis* with the eyed side brown with an irregular dark blotch on gill cover, blind side white. For the Chrysichthys species, the most closely related species *Auchenoglanis occidentalis* is distinguished from *C. nigrodigitatus* by the hind margin of adipose fin being rounded ; premaxillary dentition forming (in adults) two close-set oval plates (Lévêque et al., 1990). Maxillary barbel often blackish and only rarely reaching to anterior margin of opercula, usually not reaching beyond eye and much shorter than outer mandibular barbel. Molecular identification of the two samples *Cynoglossus senegalensis* and *Chrysichthys nigrodigitatus* confirmed the morphological identification. The COI region of the samples (707 -751 bp) exhibited 100% and 99% sequence identity to Cynoglossus sp. and Chrysichthys sp respectively (GenBank accession number: NC023224 and MH709123) collected from the coastal water of Cameroon.

3.2. Complete mitochondrial genome of *Cynoglossus senegalensis.*

In order to have additional information of *Cynoglossus senegalensis*, the complete mitochondrial genome sequence was determined by the NGS and bioinformatics sequence assembling. The mitochondrial genome was 16,519 bp in length (GenBank accession No. MH709122), which is longer in length than the closes sister *C. sinicus* with a length of 16,478 bp (Shi et al., 2015) and *C. bilineatus* with length 16,428 bp (Shi et al., 2016b).

The mitogenome of *C. senegalensis* consist of 13 protein-coding genes, 22 tRNA genes, 2 ribosomal RNA genes (12S and 16S), as well as a control region (D-Loop) and origin of light- strand replication (OL) (Fig. 3). Eleven protein was initiated with codon ATG except COX1 which uses GTG and ND3 uses ATT. There are six complete stop codon with TAA and TAG, while ND2, COX2, ND3, ND4, and ND5 uses T and COX3 uses TA. It can be observe that in this species, the H strands encode 29 genes while the L strands encodes for 8 genes. With the exception of ND6, all protein-coding genes were encoded by H strand (Fig. 3). Overlapping nucleotides were identified in three pairs of protein-coding genes (10 nucleotides for ATP8 and ATP6, 7 for ND4L and ND4, and 1 for ND5 and ND6). Among the 22 tRNA genes of the mitogenome of *Cynoglossus senegalensis*, which shows the difference in their sizes from 69 bp (tRNA-Phe) to 72 (tRNA-Gln), fifteen (15) tRNA genes encoded in H strand and seven (7) genes in the L strand (Fig 3).

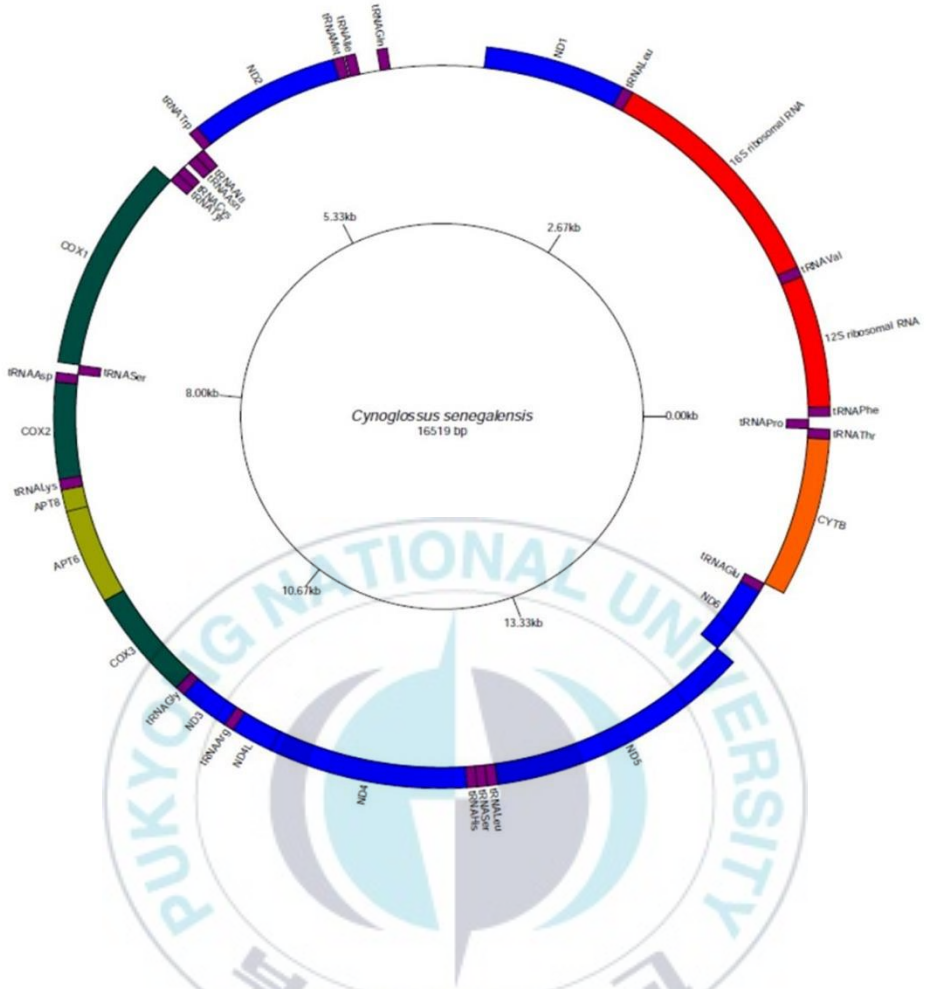

Figure 3. Mitochondrial genomic organization of *Cynoglossus senegalensis*

3.3. Organization of full length mitochondrial genome of *Cynoglossus senegalensis*

The gene order of *C. senegalensis* was identical to other flatfish in which a translocation of genes from the typical mitogenome (Kong et al., 2009). The *D-Loop* as the putative control region is typically located between tRNA-Pro and tRNA-Phe gene in Teleost mitochondrial genomic, however, only 22 bp intergenic spacer exist at this position. This area plays an important role in the initiation of transcription and replication as reported by (Taanman, 1999), which most variable mtDNA contained length of tandem repeats and variable number. The control region which is typically located between tRNA-Pro and tRNA-Phe, has been translocated to the position between ND1 and tRNA-Gln genes. A 675 bp non-coding region is present between ND1 and tRNA-Gln gene as the typical structure of control region in the flatfish (Mjelle et al., 2008). The 12S and 16S genes, 945 bp and 1691 bp in length, respectively, which is located between tRNA-Phe and tRNA-Leu $^{(UUA)}$ and tRNA-Val separates both of them. Twenty-two tRNA with length ranging from 69 bp to 77 bp (Table 2). Randomization of tRNA-Gln, tRNA-Ile and tRNA-Met genes was identified, in addition to the position of tRNA-Gln translocated from light-strand (L-strand) to heavy strand (H-strand) also found in other *Cynoglossus* genus groups (Kong et al., 2009; Mu et al., 2015; Shi et al., 2016a; Shi et al., 2015; Shi et al., 2016b; Wei et al., 2016). Among the protein-coding genes, three overlap nucleotides up to 10 bp, ATP8–ATP6,

ND4L–ND4, and ND5–ND6, were detected. The transfer RNA gene pairs (t RNA−Cys–tRNA−Tyr, tRNA-Gly-tRNA-Arg, and tRNA-His-tRNA-Ser), overlaps 1 bp as well. The base composition of *Cynoglossus senegalensis* was 5167 A (31.3%), 4727 T (28.6%), 4107 C (24.9 %), and 2518 G (15.2 %). The purines and pyrimidines are A+T content (59.9%), higher than G+C content (40.1%). The highest A+T content was observed in the putative control region (65.7%), which is similar to other previous studies. The putative control region of *C. senegalensis* (675 bp) was longest among two other *Cynoglossus* species including *C. sinicus* (659 bp) (GenBank accession No. NC023224) and *C. bilineatus* (655 bp) (GenBank accession No. 023226).

Table 2. Organization of the full-length mitochondrial genome of *Cynoglossus senegalensis*

Locus	Position		Length	anticodon	codon		space (+) overlap (-)
	Start	Stop			Start	Stop	
tRNA Phe	1	69	69	GAA			0
12S-rRNA	70	1014	945				0
tRNA Val	1015	1088	74	TAC			0
16S-rRNA	1089	2779	1691				0
tRNA Leu	2780	2855	76	TAA			-1
ND1	2855	3826	972		ATG	TAA	0
D-Loop	3827	4501	675				0
tRNA Gln	4502	4573	72	TTG			0
Noncoding Region	4574	4722	149				0
tRNA Ile	4723	4793	71	GAT			0
Noncoding Region	4794	4803	10				0
tRNA Met	4804	4877	74	CAT			0
ND2	4878	5922	1045		ATG	T--	0
tRNA Trp	5923	5991	69	TCA			2
tRNA Ala	5994	6064	71	TGC			2
tRNA Asn	6067	6139	73	GTT			0
OL	6140	6181	42				0
tRNA Cys	6182	6251	70	GCA			-1
tRNA Tyr	6251	6321	71	GTA			1
COX1	6323	7873	1551		GTG	TAA	1
tRNA Ser	7875	7943	69	TGA			3
tRNA Asp	7947	8015	69	GTC			2
COX2	8018	8708	691		ATG	T--	-1
tRNA Lys	8708	8784	77	TTT			0
ATP8	8785	8949	165		ATG	TAA	-10
ATP6	8940	9623	684		ATG	TAA	-1
COX3	9623	10407	785		ATG	TA-	0
tRNA Gly	10408	10478	71	TCC			0
ND3	10479	10827	349		ATT	T--	-1
tRNA Arg	10827	10897	71	TCG			-1
ND4L	10897	11193	297		ATG	TAA	-7
ND4	11187	12558	1372		ATG	T--	0
tRNA His	12559	12627	69	GTG			-1
tRNA Ser	12627	12696	70	GCT			1
tRNA Leu	12698	12772	75	TAG			-1
ND5	12772	14623	1852		ATG	T--	1
ND6	14625	15146	522		ATG	TAG	-1
tRNA Glu	15146	15216	71	TTC			1
Cyt B	15218	16357	1140		ATG	TAA	0
tRNA Thr	16358	16429	72	TGT			-2
tRNA Pro	16428	16497	70	TGG			-1
Noncoding Region	16497	16519	23				0

3.4. Structure of tRNA genes of *Cynoglossus senegalensis*

The 22 transfer RNA genes identified, showed a typical clover secondary structure, this was estimated by ARWEN (Laslett and Canbäck, 2007). With the exception of tRNA Ser with two arms, the other twenty-one tRNA structures were predicted to have typical three arms structure (Figure 4). The same result was also identified in other Cynoglossus species (Wei et al., 2016).

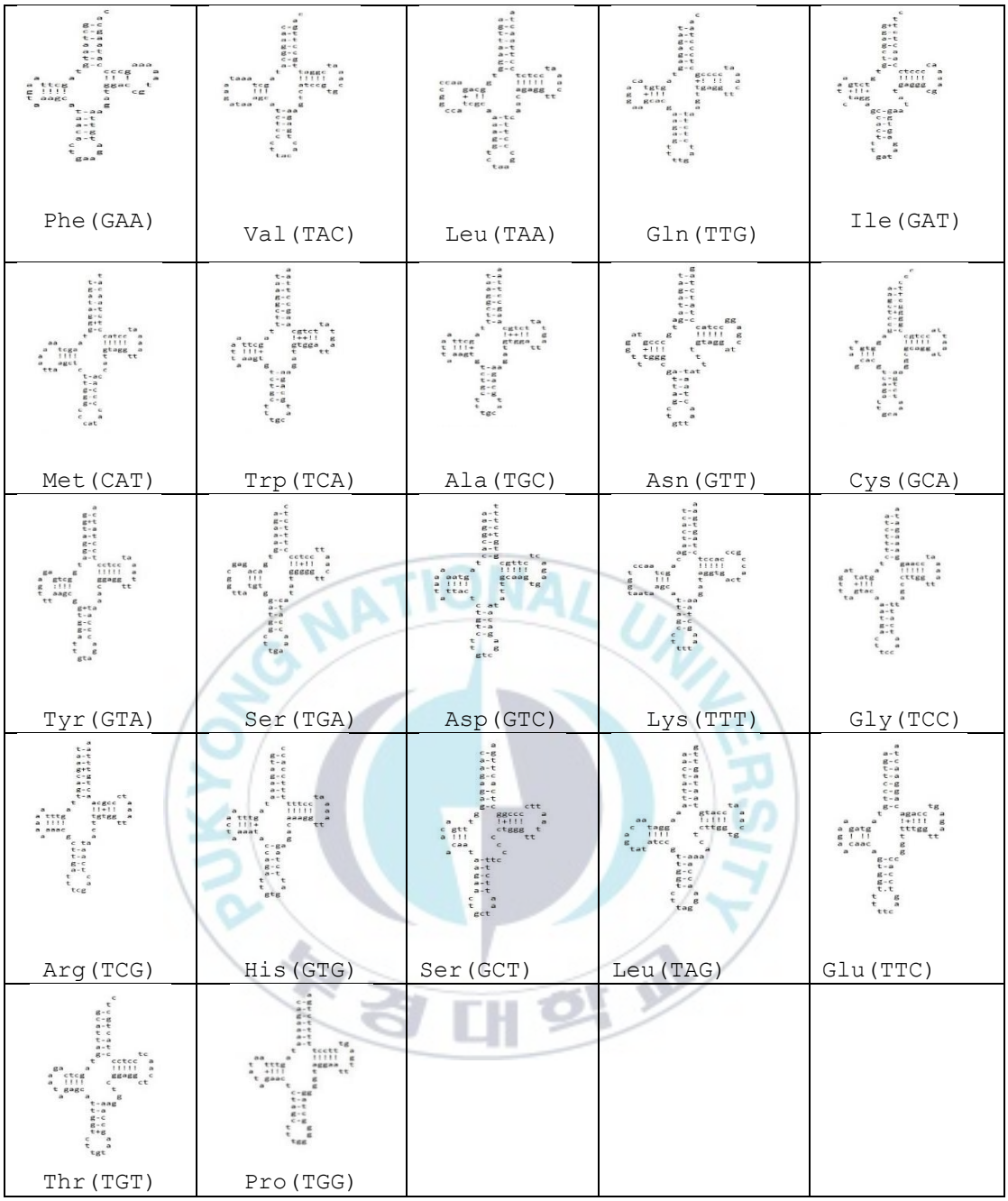

Figure 4. Structure of 22 tRNA genes in the mitochondrial genome of Cynoglossus *senegalensis*

3.5. Phylogenetic tree of *Cynoglosus senegalensis*

The Total mitochondrial DNA sequence of *C. senegalensis* showed (99-100%) identity with those of currently known two other *Cynoglossus* species among which *C. sinicus* is the most closely related to *C. senegalensis* (Fig 5). In order to know the better evolutionary relationship of *C. senegalensis*, its COI sequence was compared with those of other 11 Cynoglossus species. As shown in the analysis of the full mitochondrial genomes, *C. senegalensis* showed to be the most closely related to *C. sinicus* with 100% sequence identity. In fact, DNA sequence identity of two species *C. sinicus* and *C. bilineatus* was too high to be distinct with each other in the COI region (Fig 5). Although morphological keys can be used to discriminate the two proposed species, (Strauss and Bond, 1990), frequently it is misidentified as shown in the COI barcodes. For this reason, it is required to compare full-length mitochondrial sequences of the two species for a better and more reliable classification. As the lowest sequence identity to other Cynoglossus species, the control region of *C. senegalensis* mitochondrial genome would be the good candidate to discriminate them.

In this study, we identified that *C. senegalensis* inhabits the West-African coastline which stretch from Senegal to Congo (Obiekezie and Lick, 1994), and Cameroon, as well as three previously known Cynoglossus species, *C. browni*, *C. canarensis*, and *C. monodi* (Lévêque et al., 1990). Although *C. senegalensis* is originally distributed in the Eastern Atlantic Ocean, from Mauritania to Angola (Vreven et al., 2008). The result strongly Supported that

C. senegalensis is more widely distributed than we have thought and a large-scale survey should be made to know the spatiotemporal distribution of other Cynoglossus species in Cameroon.

In order to explore the phylogenetic position of *C. senegalensis* within the Cynoglossidae, a phylogenetic tree was constructed using Mega7 according to (Kumar et al., 2016), by applying the minimum evolutionary (ME) methods. The topological structure of the complete mitogenome of *C. senegalensis was* identified to be closely related to *C. sinicus and C. bilineatus* (Figure 5). Even though, *C. sinicus* (82%) and *C. bilineatus* (82%) inhabit the Indo-West Pacific (Shi et al., 2016b), they have similar molecular characteristic to their ancestor *C. zanzibarensis* (80%) which inhabit West Indian Ocean (Booth and Walmsley-Hart, 2000). This mitogenome information could further contribute to the study of *C. senegalensis* genetic population and gene reorganization.

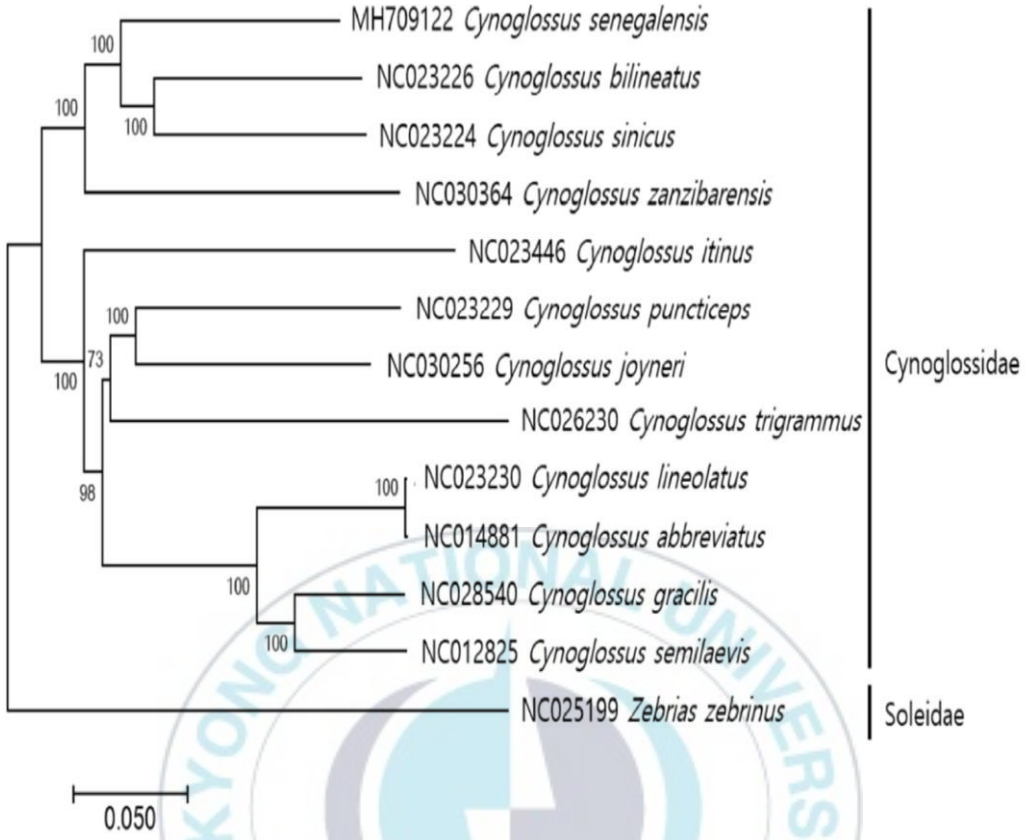

Figure 5. Phylogenetic tree of *Cynoglossus senegalensis*

3.6. The complete mitochondrial genome of *Chrysichthys nigrodigitatus*

We used the NGS and bioinformatics sequence assembling to obtain additional information for *Chrysichthys nigrodigitatus*. The complete mitochondrial genome size of *C. nigrodigitatus* (GenBank accession No: MH709123) was 16,514 bp in length, which was identical to the catfish *Arius arius* (Wang et al., 2016*)* and *Silurus asotus* (Wang et al., 2015). Its mitogenome consist of 13 protein-coding genes, 22 tRNAs, 2rRNA (12S and 16S), and 2 non-coding regions as typically seen in vertebrate mitogenome (Satoh et al., 2016). The protein-coding genes in the mitogenome of *C. nigrodigitatus* utilizes ATG as the start codon, except for COI which is initiated with GTG and ND6 with ATT (Fig. 5). The major noncoding region in the mitochondrial DNA is the control region which plays a vital role in replication and transcription as reported by (Saitoh et al., 2003; Satoh et al., 2016). The length of D-loop was 882 bp and OL 31bp, respectively. Among these tRNA genes, eight were encoded on L-strand, while the other sixteen were encoded on H-strand position. Fourteen (14) tRNA genes encodes in H strand and eight (8) genes encoded the L strand (Fig 5).

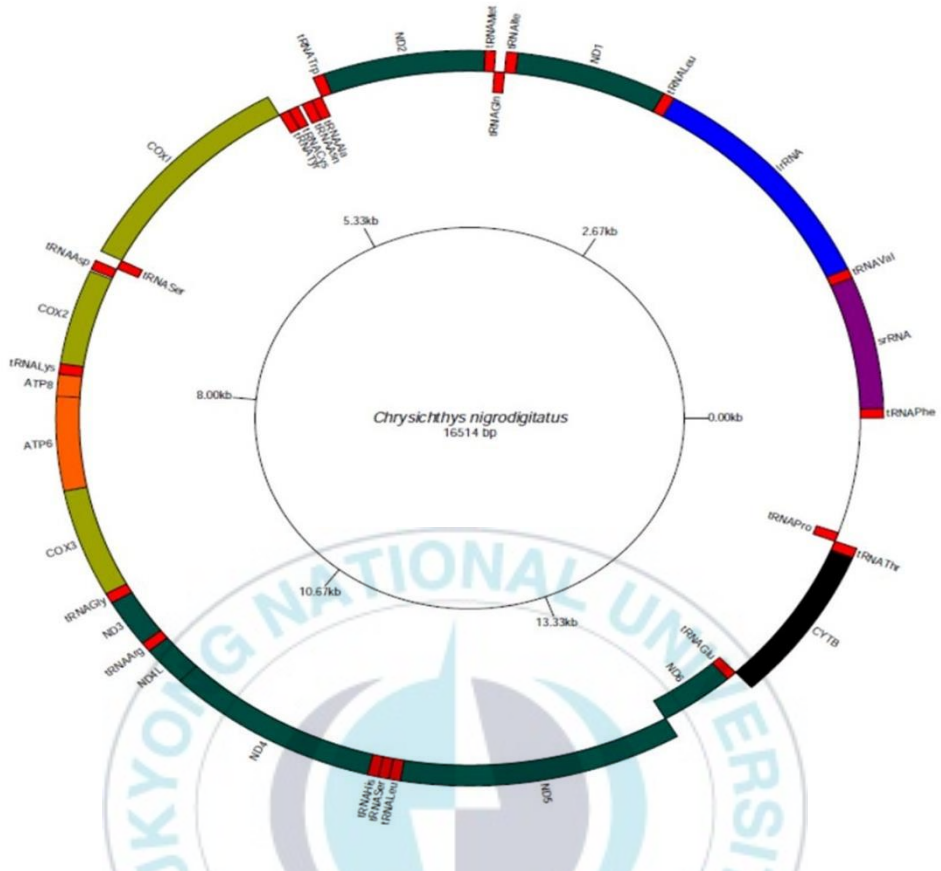

Figure 6. Mitochondrial genomic organization of *Chrysichthys nigrodigitatus*

3.7. Organization of full length mitochondrial genome of *Chrysichthys nigrodigitatus.*

In this species of Chrysichthys, we also observe that among the protein-coding genes, they were three overlap nucleotides of up to 10 bp between, ATP8–ATP6, ND4L–ND4, and ND5–ND6. The transfer tRNA gene pair, tRNA−Cys–tRNA−Tyr and tRNA-Gly-tRNA-Arg, overlap 1 bp. The base composition of *Chrysichthys nigrodigitatus* was 4,970 A (30.1%), 4,141 T (25.1%), 4,827 C (29.2%), and 2,576 G (15.6 %). The purine's and pyrimidine's are A+T content (55.2%) higher than G+C content (44.8%). The highest A+T content was observed in the putative control region (60.4%), this is similar to1 previous studies. With the exception of ND6, all protein-coding genes were encoded by H strand (Fig. 5). Among the thirteen genes, eleven of them began with typical start codon, ATG, with several stop codons including typical ones such as TAA (ND1, COX1, ATP8, ND4L and ND5), the stop codon TAG for ND6 (Fig. 5). The mitochondrial genome of *C. nigrodigitatus* contained 22 tRNA genes, which shows the difference in their sizes from 70 bp (tRNA-Phe) to 71 (tRNA-Gln). The 12S rRNA gene 958 bp of *C. nigrodigitatus* was located between the tRNA–Phe and tRNA–Val, whereas 830 bp of 16SrRNA was between tRNA–Val and tRNA–Leu. The putative control region of *C. nigrodigitatus* (882 bp) was longest among two other closely related species *Auchenoglanis occidentalis* (GenBank accession No. AP012005) and *Pangasionodon gigas* (GenBank accession No. AY76297).

Table 3. Organization of the mitochondrial genome of *Chrysichthys nigrodigitatus*

Locus	Position		length	anticodon	codon		space (+) overlap (-)
	Start	Stop			Start	Stop	
tRNA Phe	1	70	70	GAA			0
12S-rRNA	71	1028	958				0
tRNA Val	1029	1100	72	TAC			0
16S-rRNA	1101	270	-830				2500
tRNA Leu	2771	2845	75	TAA			0
ND1	2846	3820	975		ATG	TAA	0
tRNA Ile	3821	3894	74	TTG			-2
tRNA Gln	3893	3963	71	GAT			0
tRNA Met	3964	4031	68	CAT			1
ND2	4033	5077	1045				0
tRNA Trp	5078	5149	72	TCA			1
tRNA Ala	5151	5220	70	TGC			1
tRNA Asn	5222	5294	73	GTT	ATG	T--	0
OL	5295	5324	30				0
tRNA Cys	5325	5392	68	GCA			3
tRNA Tyr	5396	5465	70	GTA			1
COX1	5467	7017	1551		GTG	TAA	1
tRNA Ser	7019	7087	69	TGA			4
tRNA Asp	7092	7162	71	GTC			13
COX2	7176	7866	691		ATG	T--	-1
tRNA Lys	7866	7941	76	TTT			0
ATP8	7942	8109	168		ATG	TAA	-10
ATP6	8100	8782	683		ATG	TA-	0
COX3	8783	9566	784		ATG	T--	-1
tRNA Gly	9566	9640	75	TCC			-1
ND3	9640	9988	349		ATG	T--	0
tRNA Arg	9989	10058	70	TCG			0
ND4L	10059	10355	297		ATG	TAA	-7
ND4	10349	11729	1381		ATG	T--	0
tRNA His	11730	11799	70	GTG			0
tRNA Ser	11800	11867	68	GCT			3
tRNA Leu	11871	11943	73	TAG			0
ND5	11944	13767	1824		ATG	TAA	-4
ND6	13764	14282	519		ATT	TAG	-1
tRNA Glu	14282	14353	72	TTC			0
Cyt B	14354	15491	1138		ATG	T--	0
tRNA Thr	15492	15563	72	TGT			-3
tRNA Pro	15561	15631	71	TGG			0
D-Loop	15632	16514					0

3.8. Structure of tRNA genes of *Chrysichthys nigrodigitatus.*

All tRNA gene, were predicted to fold into typical cloverleaf secondary structure with the normal base pairing, except tRNA-Ser (Fig. 7). With the exception of tRNA Ser with two arms, the other twenty-one tRNA structures were predicted to have typical three arms structure.

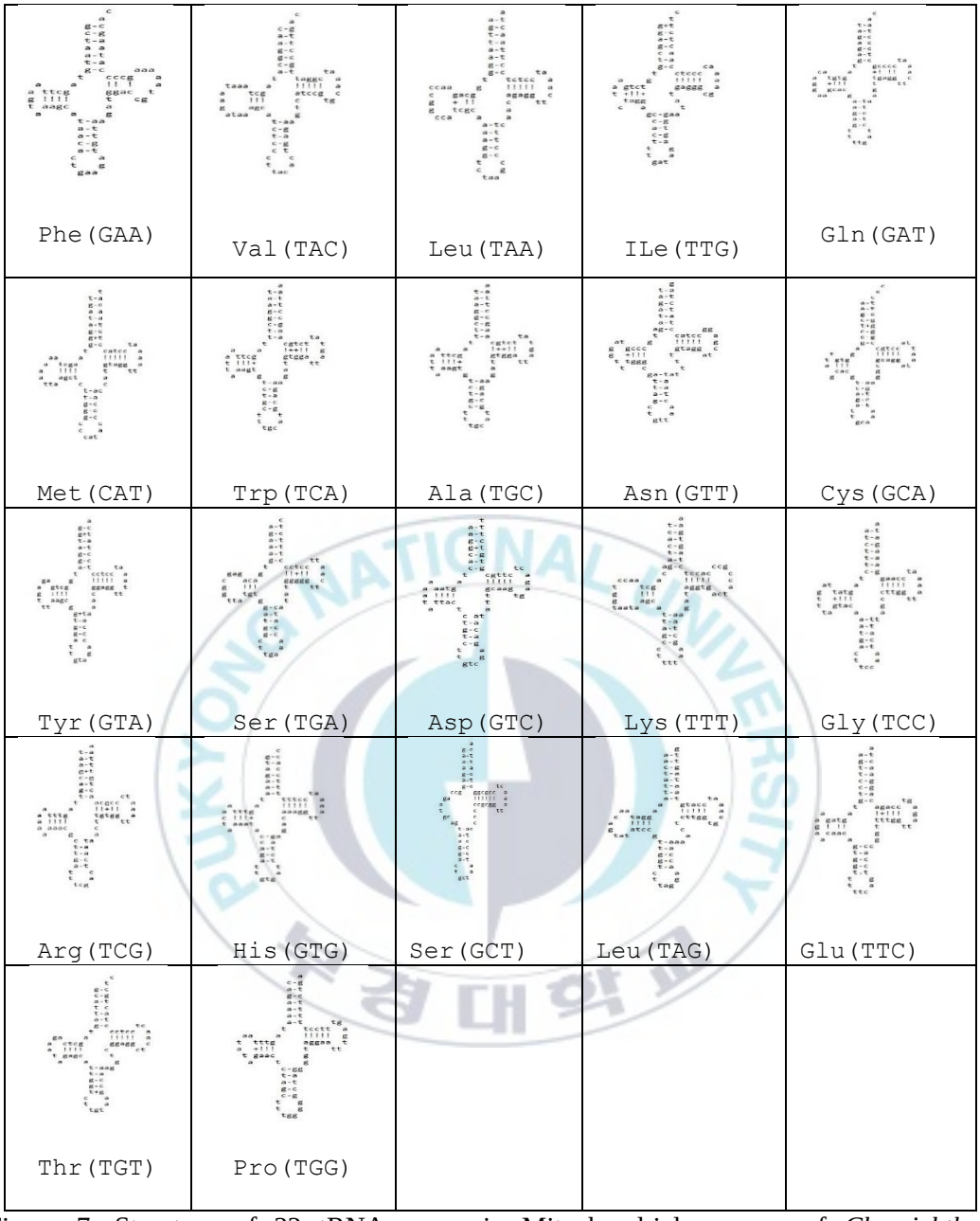

Figure 7. Structure of 22 tRNA genes in Mitochondrial genome of *Chrysichthys nigrodigitatus*

3.9. Phylogenetic tree of *Chrysichthys nigrodigitatus*

The total mitochondrial DNA sequence of *C. nigrodigitatus* showed 99% identity with other closely related species among which *Auchenoglanis occidentalis* is the most closely related to *C. nigrodigitatus* (Fig. 8). In order to know the better evolutional relationship of C. *nigrodigitatus*, its COI sequence was compared with those of other 12 species (Fig 8). As shown in the analysis of the full mitochondrial genomes, *C. nigrodigitatus* showed to be the most closely related to *Auchenoglanis occidentalis* with 99% sequence identity. In fact, DNA sequence identity of the two closely related species *A. occidentalis* and *Pangasianodon gigas* was too high to be distinguished with each other in the COI region (Fig. 8). Although morphological keys can be used to discriminate the two species, (Strauss and Bond, 1990), frequently it is misidentified as seen in the COI barcodes. For this reason, it is required to compare full-length mitochondrial sequences of two species.

In this study, we identified that *C. nigrodigitatus* is distributed in the Western-African inland waters including Cameroon (Adite et al., 2017), as well as two previously known *Chrysichthys* species, *C. helicophagus* and *C. auratus*. (Lévêque et al., 1990). Due to the fact that it is a widespread species in the West African inland waters, Cameroon inclusive, and has very good aquaculture potentials, its genetic diversity should be analyzed for a better management of the resources.

To understand the phylogenetic position of *C. nigrodigitatus* within Siluriformes order, we studied the evolutionary relationship by using MEGA7 with minimum evolutionary (ME)

algorithm (Kumar et al., 2016). As a result of the phylogenetic tree which was constructed with the full length mitogenome gene sequences, *C. nigrodigitatus* was most closely related to *A. occidentalis* which were those catfish clustered within the family Claroteidae. The mitogenome information could contribute to the study of the detail genetic diversity of Chrysichthys species.

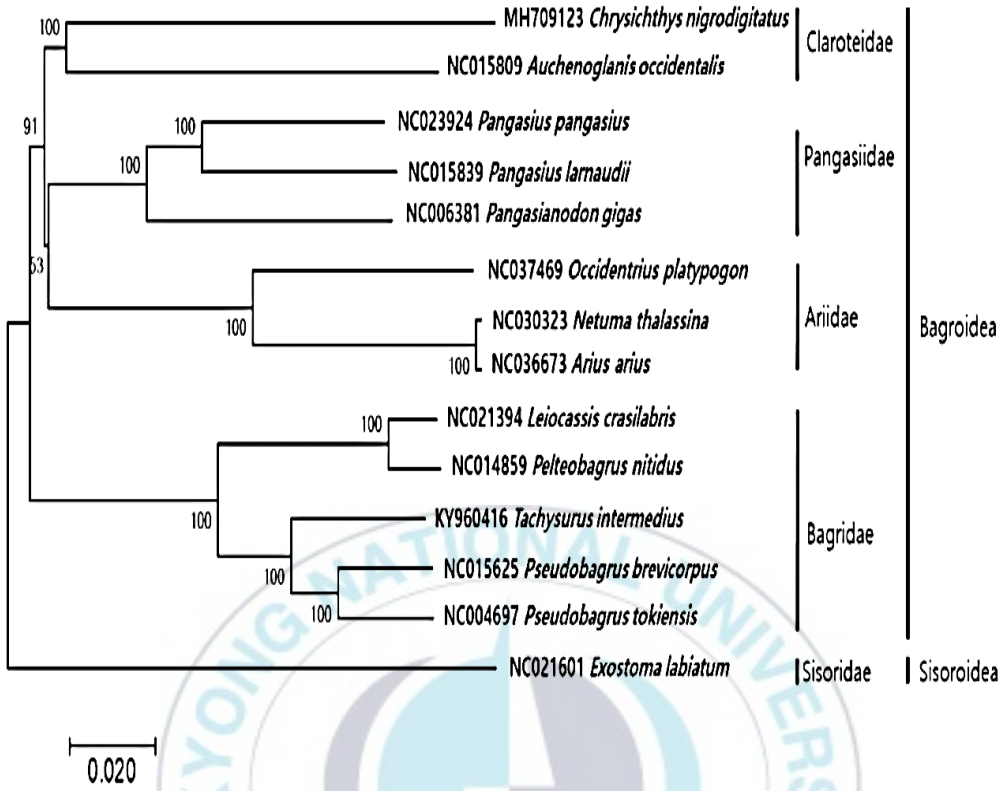

Figure 8. Phylogenetic tree of *Chrysichthys nigrodigitatus* within Order Siluriformes

Conclusion

We here reported the full-length mitochondrial genome sequences of two commercially important fish species, *Cynoglossus senegalensis* (MH706122*)* and *Chrysichthys nigrodigitatus* (MH709123), which were collected from Limbe dockyard fish market (4º00'09" N 09º14'40" E), Cameroon, Africa. The mitogenome size of *C. senegalensis* and *C. nigrodigitatus* were 16,519 bp and 16,514 bp, respectively. The mitochondrial genome structure of *C. senegalensis* was identical to those of other species in genus *Cynoglossus*, in which a translocation of the putative control region (D-loop) and tRNA-Gln occurred. As a result of the translocation, D-loop was located between ND1 and tRNA-Gln gene and tRNA-Gln was encoded by L-strand. For both species, all tRNA genes, were predicted to fold into a typical cloverleaf secondary structure with three arms except for tRNA-Ser which was predicted to form unusual two arms. The phylogenetic analysis using full mitochondrial genome showed that *C. senegalensis* is most closely related to *Cynoglossus sinicus* and *Cynoglossus bilineatus*. For *Chrysichthys nigrodigitatus*, it was observed to be closely related to *Auchenoglannis occidentalis* which were those catfish clustered within the family Claroteidae. The full mitochondrial genome information of *C. senegalensis* and *C. nigrodigitatus* would contribute to further studies for the scientific management of the resources.

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