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Sequence Length and Genetic Diversity in the *D-Loop* Region Amongst Some Catfishes Species from the River Nile in Egypt

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ABSTRACT

The present study aimed to assess the genetic diversity and genetics makeups of *D-loop* in some catfish species. The *mitochondrial (mt) D-loop* sequences varied in length, from 853 to 893bp. The *mt D-loop* region sequences' nucleotide were uploaded to GenBank with accession numbers (PQ589247-PQ589250). The A+T ratio of the *mt D-loop* is more concentrated than the C+G. The P-distances among the under studied catfishes, expanded from 0.0188 to 0.0823. The highest value (0.0823) was found between *Bagrus bajad* and *Chrysichthys auratus*. However, the lowest P-distance (0.0188) was found between *Clarias gariepinus* and *Bagrus bajad*. The results showed that the *mt D-loop* appear to be helpful in revealing the phylogenetic relationship of catfishes.

INTRODUCTION

Mitochondrial (mt) genomes in vertebrates are tiny, typically ranging from 16 to 17 kilobases in size, and the array of encoded genes is highly conserved. The mt genome is vital for the survival of nearly all eukaryotic organisms. The vertebrate mt genome generally encodes 13 proteins, two *ribosomal RNAs* (*rRNAs*), and 22 *transfer RNAs* (*tRNAs*), along with two noncoding regions: the control region (CR) and the origin of L-strand replication (OL). The mt genome tends to be conserved among vertebrates for 37 genes and two noncoding sections, ordered similarly from hagfish to eutherian mammals **(Anderson *et al*., 1981; Roe *et al*., 1985; Tzeng *et al*., 1992; Chang *et al*., 1994)**.

The evolutionary rates among genes within the mt genome exhibit significant variability, nonetheless, the noncoding segment of the mt genome, specifically the control region (CR), changes at a rate 2–5 times greater **(Meyer, 1993)**. Due to its unusually high mutation rate, the CR was rapidly deemed highly useful for exploring intraspecific evolutionary inquiries **(Brown *et al*., 1986; Palumbi, 1996)**. The control region (CR) is a vital noncoding element of the mt genome, responsible for the commencement of *mtDNA*

transcription and replication **(Clayton, 1991; Carrodeguas & Vallejo, 1997; Shadel & Clayton, 1997)**.

In numerous globally distributed taxonomic groups, accurately identifying fish and inferring evolutionary connections among species based on morphology is challenging. This is attributable to the morphological similarities among species resulting from convergent evolution, and the pattern of speciation is somewhat complex **(Rice & Westneat 2005; Duftner *et al*., 2007)**.

The *mtDNA* control region, referred to as the *displacement-loop (D-loop)* region, is located between *tRNAPro* and *tRNAPhe* in *mtDNA*. The control region is considered to be a non-coding portion of *mtDNA* and has proven to be an ideal marker for assessing the genetic structure of recently divergent or close-related species or populations **(Avise, 1994; Bremer *et al*., 1996; Iguchi *et al*., 1999; Rand, 2000; Tabata & Taniguchi, 2000; Ishikawa *et al*., 2001)**. The nucleotide sequence of the *D-loop* region is regarded as variable and does not influence transcription or replication. The *D-loop* is the most variable section of *mtDNA*. Significant genetic heterogeneity exists in the *D-loop* area, even among members of the same species **(Najjar Lashgari *et al*., 2017)**.

According to **Diogo (2004) and Nelson (2006)**, catfishes (Order Siluriformes) are a diverse group of ray-finned fishes that are distributed throughout the world and on all continents. Catfishes are predominantly freshwater species, except the two marine families: Ariidae and Plotosidae **(Kailola, 2004)**. More than 3,088 genuine species from 477 genera and 36 families are included in them **(Ferraris, 2007)**.

Because of their widespread, primarily freshwater distribution and diversity, catfishes are of significant interest to ecologists and evolutionary biologists. Siluriformes are the main component of biogeography on all scales, from local to global. In temperate regions, catfish are easy to raise, resulting in affordable and safe food at neighborhood supermarkets. Ordinarily, phylogenetic relationship between families, genera and species of Siluriformes are uncertain **(Lundberg *et al*., 2000; Meyer & Van de Peer, 2003; Punhal *et al*., 2018)**. The primary goal of this study was to examine the genetic diversity and genetics makeups of *D-loop* in some catfish species.

MATERIALS AND METHODS

Sample preparation and *DNA* extraction

Four catfish species (*Clarias gariepinus, Bagrus bajad, Schilbe mystus* and *Chrysichthys auratus*) were caught from the River Nile in Egypt and classified according to **Bishai and Khalil (1997)**. The caudal peduncle's muscular tissues were removed and kept at –20°C. The Biospin genomic *DNA* extraction kit was utilized to extract *DNA* from 15–25 mg of muscle tissue.

Polymerase chain reaction (PCR) amplification

The primers were utilized in accordance with **Cheng *et al*. (2012)** to amplify the *mt D-loop* region in the four catfish species. The PCR reactions comprise of a final reaction volume of 50µL, 1.0µL of genomic *DNA*, 1.0µL of each forward and reverse primers, and 25µL of PCR master mix. The PCR cycling parameters were: an initial denaturation of five minutes at 94°C; thirty cycles consisting of denaturation for one minute at 94°C, annealing for one minute at 54°C, and extension for one minute at 72°C, followed by a post-cycling extension of five minutes at 72°C. The PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide.

The sequencing of PCR products and phylogenetic tree construction

Macrogen (South Korea) performed the *DNA* sequencing. The *mt D-loop* sequences were uploaded to GenBank/NCBI in order to receive accession numbers. The MUSCLE method **(Edgar, 2004)** was used for sequence alignment with default settings. To conduct phylogenetic tree analyses utilizing two approaches—maximum likelihood and neighbor joining—MEGA version 11 was employed **(Tamura *et al.*, 2021)**. Bootstrap analysis was conducted using 1000 replicates **(Felsenstein, 1985)**. A graphical representation of the divergence was generated by computing the sequence divergences. By computing the sequence divergences, a graphical representation of the divergence amongst catfish species was created utilizing Kimura 2-parameter distances **(Kimura, 1980)**.

RESULTS AND DISCUSSION

The *mt D-loop* region sequences' nucleotide were uploaded to GenBank with accession numbers (PQ589247-PQ589250). The mt D-loop sequences varied in length, with *Schilbe mystus* having 893bp and *Bagrus bajad* having 853bp. The A+T ratio of the *mt D-loop* is more concentrated than the C+G. Additional information regarding the averages of the *mt D-loop* sequences in catfishes, as well as the nucleotide frequencies, A+T contents, and pyrimidine contents found in Table (1).

Table 1. Nucleotide frequencies, A+T contents, pyrimidines contents and their averages of *mt D-loop* sequence in catfish

No.	Species	Length	A	T	C	G	A+T	Pyrimidines C+T
1	*Clarias gariepinus*	864	32.29	30.44	22.57	14.70	62.73	53.01
2	*Bagrus bajad*	853	32.59	30.01	22.98	14.42	62.60	52.99
3	*Schilbe mystus*	893	33.26	29.68	23.29	13.77	62.94	52.97
4	*Chrysichthys auratus*	881	29.51	30.99	25.43	14.07	60.50	56.42
Avg.	-	-	31.91	30.28	23.57	14.24	62.19	53.85

The total length of the *MT D-Loop* varies greatly among species with *Schilbe mystus* having 893bp and *Bagrus bajad* having 853bp. **Lee *et al*. (1995)** reported that,

the length of control region segment is highly variable among even closely related species due to the presence of tandemly repeated sequences and large insertions. **Jamandre *et al*. (2014)** observed that the lengths of *Mugil cephalus* control region varied among sampling locations. In all understudied species, our analysis of the *mt D-loop* gene indicated a higher A+T composition than the C+G. The fish species *Leporinus elongatus* also presented a higher proportion of AT nucleotides in *the D-loop mtDNA* **(Martins *et al.*, 2003)**. The control region of *Setipinna taty* was rich in A+T (71.7%) **(Li *et al*., 2012)**. **Jamandre *et al*. (2014)** reported that *Mugil cephalus* CR is AT rich. **Satoh *et al*. (2016)** reported the CR was found to be AT rich, as reported in other vertebrates **(Brown *et al*., 1986; Saccone *et al*., 1987)**. **Nwafili and Gao (2016)** reported that the nucleotide composition of the control region fragment was A+T-rich (A, 28.5%; T, 34.3%). Additionally, a lower GC content was observed for the *D-loop* sequences in worldwide species of genus *Siganus* with an average of 30.6% **(Ali *et al*., 2021)**. The A+T content was rich (62.85%) in the nucleotide composition of the *D-loop* sequences in naked carp *Gymnocypris przewalskii* **(Fang *et al*., 2022)**.

The P-distances among the under studied catfishes, expanded from 0.0188 to 0.0823. The highest value (0.0823) was found between *Bagrus bajad* and *Chrysichthys auratus*. However, the lowest P-distance (0.0188) was found between *Clarias gariepinus* and *Bagrus bajad* (Table 2). The results revealed that *Bagrus bajad* found closely to species of family Clariidae as well as low P-distance between *Bagrus bajad* and *Clarias gariepinus* indicated a close relation between them. This is in line with the findings of **Kaleshkumar *et al*. (2015)**, who found that closely related species had low genetic distance values, but situations with significant genetic divergence are caused by the maximum genetic distance.

Catfishes sequencing was submitted for study together with 13 similar catfishes in addition, three outgroup species (order, Cypriniforms) from GenBank/NCBI to perform phylogenetic analysis utilizing the *mt D-loop* gene. The phylogenetic tree analysis showed that, the outgroup species formed a distinct cluster. Species within the family Clariidae constituted a distinct cluster. The species of rest families formed a separate cluster, except the understudied *Bagrus bajad*, which was found close to species of family Clariidae (Fig. 1a and b).

Several studies using many genetic markers occurred to illustrate the genetic variation and phylogenetic analysis in catfishes. **Uyoh *et al*. (2020)** studied the molecular characterization of two catfish species (*Chrysichthys nigrodigitatus* and *Chrysichthys auratus*) using *rRNA* and internal transcribed spacers. **Widayanti *et al*. (2021)** used the *12S rRNA* gene to investigate genetic variance and phylogeny of the baung fish. **Mahrous and Allam (2022)** studied the phylogenetic relationships among certain catfish species using the 12S rRNA and 16S rRNA genes.

Table 2. Pairwise distances by the mean of *mt D-loop* gene amongst catfishes with their linked species from the GenBank/NCBI.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	PQ589247.1_Clarias_gariepinus		0.0188	0.0657	0.0798	0.0685	0.0789	0.0012	0.0272	0.0245	0.0261	0.0262	0.0329	0.0552	0.0588	0.0615	0.0648	0.0679	0.0756	0.0779	0.0710
2	PQ589248.1_Bagrus_bajad	0.1831		0.0744	0.0823	0.0726	0.0853	0.0179	0.0386	0.0346	0.0377	0.0376	0.0501	0.0510	0.0543	0.0703	0.2020	0.0785	0.0811	0.0865	0.0809
3	PQ589249.1_Schilbe_mystus	0.7312	0.8235		0.0413	0.0734	0.0407	0.0655	0.0740	0.0700	0.0672	0.0631	0.0959	0.0585	0.0596	0.0706	0.2524	0.0196	0.0893	0.0830	0.0999
4	PQ589250.1_Chrysichthys_auratus	0.8199	0.8656	0.4659		0.0724	0.0123	0.0777	0.0837	0.0792	0.0703	0.0731	0.1081	0.0570	0.0563	0.0778	0.2858	0.0399	0.1182	0.1107	0.1250
5	AB919123.1_Eutropiichthys_vacha	0.7207	0.7412	0.7873	0.7587		0.0768	0.0672	0.0712	0.0700	0.0670	0.0729	0.1047	0.0488	0.0494	0.0480	0.2502	0.0656	0.0897	0.0925	0.0946
6	NC_042721.1_Chrysichthys_nigrodigitatus	0.8279	0.8871	0.4567	0.0953	0.7779		0.0776	0.0907	0.0775	0.0754	0.0711	0.1118	0.0600	0.0610	0.0787	0.3383	0.0361	0.1185	0.1064	0.1237
7	AF331461.1_Clarias_gariepinus	0.0012	0.1697	0.7205	0.7939	0.7044	0.8062		0.0279	0.0246	0.0261	0.0269	0.0342	0.0554	0.0590	0.0616	0.0697	0.0680	0.0759	0.0783	0.0712
8	NC_071774.1_Clarias_camerunensis	0.2867	0.4385	0.8126	0.8622	0.7737	0.9175	0.2922		0.0211	0.0197	0.0187	0.0202	0.0552	0.0566	0.0634	0.0189	0.0709	0.0732	0.0691	0.0704
9	NC_037193.1_Clarias_dussumieri	0.2478	0.3935	0.7605	0.8350	0.7348	0.8360	0.2473	0.2089		0.0229	0.0216	0.0255	0.0584	0.0641	0.0552	0.0331	0.0706	0.0756	0.0728	0.0780
10	KM363342.1_Clarias_macrocephalus	0.2595	0.4156	0.7345	0.7508	0.6998	0.7981	0.2588	0.2006	0.2346		0.0141	0.0164	0.0524	0.0549	0.0539	0.0416	0.0656	0.0728	0.0694	0.0714
11	KM259918.1_Clarias_batrachus	0.2669	0.4123	0.6980	0.7771	0.7269	0.7669	0.2722	0.1813	0.2152	0.1132		0.0026	0.0572	0.0568	0.0515	0.0356	0.0614	0.0847	0.0787	0.0768
12	MT376445.1_Clarias_magur	0.3035	0.4913	0.9206	1.0056	0.9041	1.0236	0.3113	0.1687	0.2317	0.1187	0.0044		0.0763	0.0736	0.0676	0.0350	0.0910	0.0870	0.0866	0.0834
13	NC_030188.1_Horabagrus_brachysoma	0.5959	0.5661	0.6656	0.6247	0.5115	0.6370	0.5877	0.5999	0.6251	0.5677	0.5975	0.7158		0.0084	0.0556	0.2159	0.0620	0.0821	0.0849	0.0897
14	MG986722.1_Horabagrus_nigricollaris	0.6155	0.5861	0.6738	0.6120	0.5183	0.6477	0.6074	0.5944	0.6592	0.5871	0.6008	0.7097	0.0523		0.0545	0.2064	0.0626	0.0824	0.0843	0.0905
15	EU560429.1_Hemibagrus_macropterus	0.6591	0.7411	0.7938	0.8229	0.4877	0.8324	0.6523	0.6965	0.6199	0.5982	0.5813	0.6828	0.5865	0.5784		0.1545	0.0651	0.0782	0.0795	0.0881
16	MT990828.1_Clarias_liocephalus	0.4396	1.1920	1.3904	1.4455	1.2431	1.5891	0.4597	0.0949	0.2110	0.2722	0.2251	0.2199	1.1805	1.1677	0.9657		0.2460	0.1194	0.1241	0.1246
17	NC_015837.1_Pareutropius_debauwi	0.7326	0.8393	0.1916	0.4469	0.6841	0.4026	0.7217	0.7780	0.7447	0.7040	0.6721	0.8638	0.6818	0.6743	0.7310	1.3771		0.0955	0.0880	0.0996
18	DQ105313.1_Cobitis_granoci	0.7838	0.8461	0.9393	1.1079	0.8938	1.1114	0.7859	0.7588	0.8016	0.7718	0.8402	0.8042	0.8611	0.8562	0.8360	0.7400	0.9478		0.0124	0.0119
19	EU670811.1_Cobitis_lutheri	0.8234	0.9160	0.8684	1.0467	0.9190	1.0442	0.8256	0.7421	0.7710	0.7551	0.8194	0.8145	0.8781	0.8698	0.8553	0.7406	0.8837	0.0885		0.0131
20	AY600880.1_Cobitis_sinensis	0.7548	0.8725	1.0247	1.1853	0.9378	1.1786	0.7567	0.7615	0.8336	0.7686	0.7985	0.7842	0.9441	0.9344	0.9249	0.7632	0.9938	0.0865	0.0987	

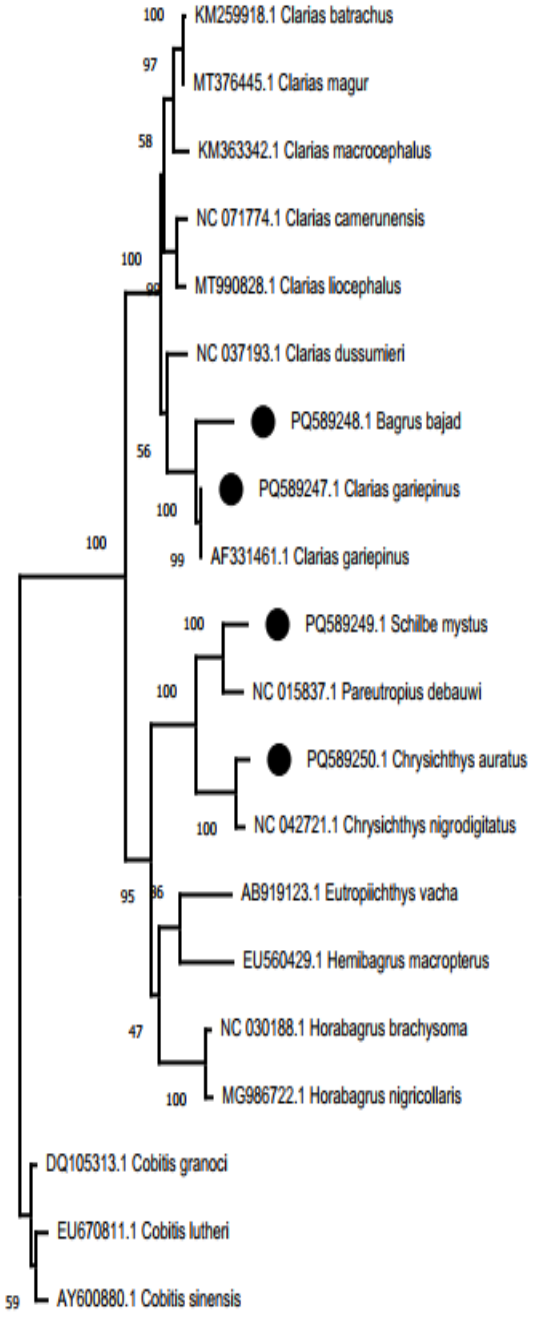

Fig. 1a. Maximum likelihood tree amongst catfishes with their linked species, in addition to the outgroup utilizing *mt D-loop*

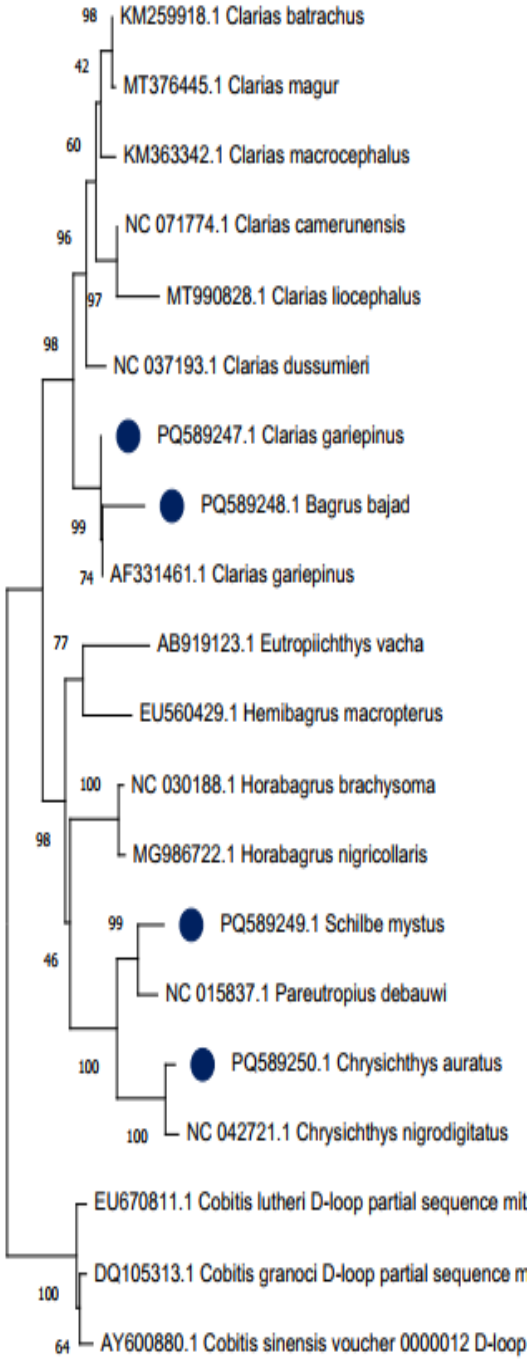

Fig. 1b. Neighbor joining tree amongst catfishes with their linked species, in addition to the outgroup utilizing *mt D-loop*

CONCLUSION

The purpose of this investigation was to evaluate the effectiveness of the mitochondrial D-loop region in studying the genetic makeup and genetic diversity of several catfish species. The results showed that the mt *D-loop* is useful in revealing the phylogenetic relationships among catfishes.

STATEMENT OF ETHICS

The Ethics of Animal Experiments Committee of the Faculty of Science of Port Said University accepted all animal experimentation practices (ERN: PSU. Sci. 74.).

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