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DNA Barcoding of some Species of Genus *Synodontis* (Family Mochokidae) from the River Nile in Egypt

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ABSTRACT

The current work aimed to study the phylogenetic lineages of some species of the genus *Synodontis* belonging to the family Mochokidae using the cytochrome c oxidase subunit I (*COI*) gene. The nucleotide sequences of *COI* were inserted into the GenBank with accession numbers (OP143814.1-OP143816.1). The length of the cytochrome c oxidase subunit I sequence stretched from 617 bp to 623 bp. The overall genetic distance was 0.09. The *COI* gene displayed A+T ratio bigger than the C+G ratio. The average nucleotide frequencies of adenine (A), thymine (T), cytosine (C) and guanine (G) were 27.30, 25.57, 29.93 and 17.20%, respectively. Among understudied *Synodontis* fish, the P-distances expanded from 0.0127 to 0.0161%. The highest value (0.0161) was found between *Synodontis* and *Synodontis schall*. While the lowest P-distance (0.0127) was found between *Synodontis batensoda* and *Synodontis clarias*. Furthermore, more investigations on the phylogenetics of the family Mochokidae are recommended.

INTRODUCTION

The Nile River is considered a superior natural resource in Egypt, offering large fertile deposits, fish and numerous aquatic animals. The River Nile is home to around 128 fish species from 27 families **(Witte *et al*., 2009; van Zwieten *et al*., 2011)**.

Family Mochokids is part of the 'Big Africa clade' **(Sullivan *et al*., 2006)**. This family has piqued the interest of biogeographical researchers because of their nearly pan-African spread. *Synodontis* is one of the biggest genera of African freshwater fishes, with 119 recognized species. The species-rich genus *Synodontis* is a specially good model for investigating the impact of geological events in fostering lineage diversification and shaping current biogeographic patterns on a continental scale (**Day *et al*., 2009; Pinton *et al*., 2013)**.

DNA markers, inclusive mitochondrial DNA (mtDNA), are widely applied in assessing the phylogenetic relationships between species **(FAO, 2013)**. Compared to the other mitochondrial genes, *Cox1* appears to have a stronger phylogenetic signal **(Trivedi *et al*., 2016)**. The *Cox1* gene has sufficiently and quickly evolved to distinguish between closely related species and investigate intraspecific diversity **(Strüder-Kypke & Lynn, 2010; Lin *et al*., 2015)**.

The current work aimed to study the phylogenetic lineages of some species of genus *Synodontis* belonging to family Mochokidae using cytochrome c oxidase subunit I (*COI*) gene.

MATERIALS AND METHODS

Samples collection

The Egyption River Nile is the study sampling region where the fish were compiled **(Bishai & Khalil, 1997)**. The muscles tissues of the samples were solitary and put at -20ºC to be used in DNA extraction.

DNA extraction and PCR amplification

The total genomic DNA was isolated by QIAamp DNA Mini kit (Qiagen, Germany) from the stored muscles tissues. To amplify cytochrome c oxidase subunit I (*COI*) gene in the *Synodontis* species, we used primers described in the study of **Ward *et al.* (2005)**. For PCR reactions, total volume of 50µL was inclusively used; 25µL master mix, 1µL from each of genomic DNA, forward and reverse primers. The thermal plan inclosed an initial step of 5 minutes at 95ºC, followed by 30 cycles of 60s at 94 ºC, 60s at 61°C, at 72°C for 60sec plus a final extension for 5 min. To see the PCR results, PCR products were electrophoresed by loading into 1.5% agarose gel stained with ethidium bromide.

The sequencing of PCR products and phylogenetic tree construction

To procure the accession numbers, the sequences of *COI* gene were exhibited into GenBank/NCBI. MUSCLE **(Edgar, 2004)** with default conditions was applied to accomplish the sequence alignment. Two phylogenetic methods, neighbour joining (NJ) and minimum evolution (ME), were used in MEGA version 11.0.11 **(Tamura *et al.*, 2021)**. The sequence divergences were performed by Kimura 2-parameter distances **(Kimura, 1980)**, and bootstrap analysis was implemented with 1000 replicates **(Felsenstein, 1985)**.

RESULTS

The sequences length of *COI* in three *Synodontis* fishes expanded from 617 bp. to 623 bp. The nucleotide sequences were inserted into the GenBank/NCBI with accession numbers (OP143814.1 - OP143816.1). Our results showed that the longest nucleotide sequence (623 bp.) was found in *Synodontis clarias*, while *Synodontis schall* showed the shortest sequence (617 bp.). The average frequencies of the nucleotides were 27.30, 25.57, 29.93 and 17.20% for adenine (A), thymine (T), cytosine (C) and guanine (G), respectively. The *COI* gene displayed A+T ratio bigger than the C+G ratio in all species (Table 1).

Table 1. Accession number, nucleotide frequencies, A+T contents and their averages of cytochrome c oxidase subunit I (*COI*) sequences in three *Synodontis* fishes

No.	Species	Accession number	Base pair length	Nucleotide Number %				A+T Content (%)
				A%	T%	C %	G%	
1	*Synodontis batensoda*	OP143814.1	621	27.70	26.08	29.31	16.91	53.78
2	*Synodontis clarias*	OP143815.1	623	27.61	25.68	29.53	17.17	53.29
3	*Synodontis schall*	OP143816.1	617	26.58	24.96	30.96	17.50	51.54
	Average %	-	620.3	27.30	25.57	29.93	17.20	52.87

The final alignments included 626 bp, among which 552 conserved sites, and 69 variable sites are presented in Fig. (1). The sequences obtained from three *Synodontis* species from the River Nile in Egypt, as well as 41 related sequences, and the out group from GenBank were applied in this study for a more compound phylogenetic analysis (Table 2).

Fig. 1. Alignment of (*COI*) sequences in three *Synodontis* fishes. Dots show similar nucleotides while A, C, G, and T display the variance nucleotides

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Table 2. The understudied *Synodontis* species with their linkage *Synodontis* species, and the out-group species from the GenBank/NCBI by employing the cytochrome c oxidase subunit I (*COI*) gene.

No.	Accession number	Species	No.	Accession number	Species
1	OP143814.1	*Synodontis batensoda*	25	HF565872.1	*Synodontis congica*
2	OP143815.1	*Synodontis clarias*	26	HF565891.1	*Synodontis gobroni*
3	OP143816.1	*Synodontis schall*	27	HF565905.1	*Synodontis macrostiga*
4	HF565870.1	*Synodontis clarias*	28	HF565864.1	*Synodontis brichardi*
5	HF565960.1	*Synodontis sorex*	29	HF565860.1	*Synodontis annectens*
6	HF565876.1	*Synodontis euptera*	30	HF565981.1	*Synodontis vanderwaali*
7	HF565879.1	*Synodontis irsacae*	31	HF565990.1	*Synodontis woosnami*
8	KU569038.1	*Synodontis eupterus*	32	HF565902.1	*Synodontis leopardina*
9	HF565941.1	*Synodontis polli*	33	HF565910.1	*Synodontis multipunctata*
10	HF565975.1	*Synodontis aff.*	34	HF565911.1	*Synodontis nebulosa*
11	MF595261.1	*Synodontis petricola*	35	HF565875.1	*Synodontis decora*
12	HM882972.1	*Synodontis resupinata*	36	HF565958.1	*Synodontis smiti*
13	HF565985.1	*Synodontis violacea*	37	KT193138.1	*Synodontis schoutedeni*
14	MK074667.1	*Synodontis flavitaeniatus*	38	MK074653.1	*Synodontis batesii*
15	HF565909.1	*Synodontis membranacea*	39	HF565904.1	*Synodontis lucipinnis*
16	HF565992.1	*Synodontis zambezensis*	40	KT193108.1	*Synodontis congicus*
17	KT193195.1	*Synodontis notatus*	41	MK074650.1	*Synodontis batesii*
18	HM882974.1	*Synodontis batensoda*	42	HF565959.1	*Synodontis soloni*
19	HF565857.1	*Synodontis angelica*	43	HF565865.1	*Synodontis caudovittata*
20	HF565912.1	*Synodontis njassae*	44	LC487135.1	*Synodontis schall*
21	HF565986.1	*Synodontis rukwaensis*	Out-group	MZ636448.1	*Hemibagrus macropterus*
22	KT193194.1	*Synodontis angelicus*		KJ909362.1	*Hemibagrus microphthalmus*
23	HF565855.1	*Synodontis albolineatus*		EU490862.1	*Hemibagrus wyckioides*
24	HF565866.1	*Synodontis centralis*			

Overall, the distance value among all fish species was 0.09%. Among all *Synodontis* species, the P-distances extended from 0.0024 to 0.0171%. The highest value (0.0171) was found between *Synodontis congica* and the understudied *Synodontis schall*. While, the lowest P-distance (0.0024) was found between *Synodontis aff* and *Synodontis petricola*. Among the *Synodontis* fish under study, the P-distances expanded from 0.0127 to 0.0161%. The highest value (0.0161) was found between *Synodontis batensoda* and *Synodontis schall*. While, the lowest P-distance (0.0127) was found between *Synodontis batensoda* and *Synodontis clarias* (Table 3).

To implement the phylogenetic tree analysis based on (*COI*) sequences, three *Synodontis* species were analyzed together with the 41 linked *Synodontis* species and the outgroup species from GenBank/NCBI as shown in Table (2). For widely interpretative phylogenetic relationships based on (*COI*) gene, two phylogenetic methods, neighbour joining and minimum evolution were employed (Figs. 2, 3).

Table 3: Pairwise distances by employing the *COI* gene in three *Synodontis* fishes and their linked *Synodontis* species with the outgroup.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
1	*Synodontis_batensoda*		0.0127	0.0161	0.0127	0.0128	0.0091	0.0117	0.0098	0.0121	0.0119	0.0113	0.0059	0.0126	0.0127	0.0029	0.0134	0.0124	0.0000	0.0134	0.0137	0.0143	0.0134	0.0124	0.0142	0.0126	0.0148	0.0132	0.0134	0.0118	0.0134	0.0134	0.0136	0.0144	0.0136	0.0141	0.0134	0.0133	0.0135	0.0115	0.0138	0.0136	0.0144	0.0159	0.0159	0.0242	0.0266	0.0263
2	*Synodontis_clarias*	0.0728		0.0146	0.0000	0.0097	0.0107	0.0110	0.0111	0.0114	0.0115	0.0113	0.0120	0.0123	0.0120	0.0120	0.0124	0.0119	0.0127	0.0129	0.0125	0.0127	0.0129	0.0129	0.0121	0.0124	0.0129	0.0127	0.0129	0.0126	0.0130	0.0130	0.0131	0.0129	0.0132	0.0134	0.0135	0.0132	0.0136	0.0116	0.0133	0.0139	0.0139	0.0146	0.0144	0.0245	0.0253	0.0271
3	*Synodontis_schall*	0.1034	0.0879		0.0149	0.0154	0.0154	0.0150	0.0158	0.0153	0.0145	0.0150	0.0155	0.0106	0.0169	0.0153	0.0143	0.0163	0.0163	0.0163	0.0144	0.0155	0.0163	0.0136	0.0152	0.0171	0.0134	0.0158	0.0161	0.0161	0.0161	0.0160	0.0162	0.0161	0.0163	0.0178	0.0160	0.0161	0.0142	0.0155	0.0165	0.0144	0.0167	0.0000	0.0000	0.0259	0.0301	0.0292
4	*Synodontis_clarias*	0.0728	0.0000	0.0896		0.0097	0.0107	0.0110	0.0111	0.0113	0.0114	0.0113	0.0119	0.0123	0.0123	0.0119	0.0124	0.0122	0.0126	0.0128	0.0124	0.0126	0.0128	0.0128	0.0121	0.0126	0.0128	0.0127	0.0129	0.0125	0.0129	0.0129	0.0130	0.0129	0.0131	0.0137	0.0134	0.0131	0.0135	0.0116	0.0135	0.0138	0.0139	0.0148	0.0146	0.0243	0.0251	0.0271
5	*Synodontis_sorex*	0.0767	0.0487	0.1005	0.0484		0.0114	0.0107	0.0113	0.0105	0.0109	0.0108	0.0125	0.0117	0.0124	0.0119	0.0126	0.0133	0.0127	0.0132	0.0126	0.0122	0.0132	0.0120	0.0136	0.0137	0.0130	0.0130	0.0133	0.0138	0.0136	0.0133	0.0133	0.0116	0.0136	0.0136	0.0125	0.0121	0.0139	0.0104	0.0150	0.0141	0.0132	0.0153	0.0151	0.0243	0.0265	0.0280
6	*Synodontis_euptera*	0.0433	0.0561	0.0955	0.0558	0.0635		0.0111	0.0028	0.0115	0.0114	0.0108	0.0076	0.0119	0.0125	0.0088	0.0119	0.0120	0.0090	0.0129	0.0128	0.0141	0.0129	0.0116	0.0138	0.0126	0.0129	0.0133	0.0124	0.0074	0.0136	0.0136	0.0137	0.0137	0.0137	0.0130	0.0123	0.0132	0.0128	0.0112	0.0129	0.0130	0.0132	0.0151	0.0152	0.0247	0.0262	0.0273
7	*Synodontis_irsacae*	0.0662	0.0582	0.0879	0.0579	0.0600	0.0594		0.0115	0.0048	0.0051	0.0051	0.0114	0.0116	0.0122	0.0110	0.0087	0.0119	0.0116	0.0125	0.0080	0.0088	0.0125	0.0119	0.0125	0.0123	0.0109	0.0123	0.0120	0.0124	0.0122	0.0126	0.0123	0.0082	0.0123	0.0124	0.0120	0.0125	0.0123	0.0048	0.0131	0.0125	0.0130	0.0148	0.0147	0.0258	0.0273	0.0290
8	*Synodontis_eupterus*	0.0488	0.0599	0.0997	0.0596	0.0637	0.0048	0.0632		0.0119	0.0115	0.0110	0.0084	0.0123	0.0128	0.0095	0.0122	0.0124	0.0097	0.0132	0.0131	0.0145	0.0132	0.0122	0.0142	0.0129	0.0133	0.0133	0.0127	0.0071	0.0135	0.0135	0.0137	0.0139	0.0136	0.0129	0.0127	0.0135	0.0134	0.0113	0.0132	0.0136	0.0136	0.0155	0.0155	0.0243	0.0267	0.0278
9	*Synodontis_polli*	0.0703	0.0603	0.0903	0.0600	0.0582	0.0636	0.0131	0.0674		0.0060	0.0060	0.0119	0.0111	0.0115	0.0114	0.0091	0.0115	0.0120	0.0124	0.0089	0.0088	0.0124	0.0120	0.0123	0.0120	0.0106	0.0126	0.0125	0.0132	0.0132	0.0128	0.0132	0.0096	0.0130	0.0126	0.0128	0.0124	0.0126	0.0058	0.0135	0.0128	0.0139	0.0152	0.0151	0.0262	0.0277	0.0283
10	*Synodontis_aff.*	0.0662	0.0618	0.0857	0.0615	0.0617	0.0594	0.0147	0.0613	0.0200		0.0024	0.0116	0.0117	0.0132	0.0112	0.0073	0.0126	0.0118	0.0134	0.0078	0.0090	0.0134	0.0126	0.0135	0.0131	0.0099	0.0129	0.0129	0.0129	0.0128	0.0131	0.0129	0.0076	0.0129	0.0132	0.0127	0.0128	0.0130	0.0030	0.0139	0.0132	0.0137	0.0144	0.0143	0.0264	0.0283	0.0298
11	*Synodontis_petricola*	0.0622	0.0618	0.0901	0.0615	0.0617	0.0556	0.0147	0.0574	0.0200	0.0032		0.0110	0.0115	0.0128	0.0111	0.0078	0.0122	0.0112	0.0129	0.0077	0.0090	0.0129	0.0125	0.0134	0.0126	0.0105	0.0128	0.0129	0.0128	0.0128	0.0130	0.0128	0.0082	0.0128	0.0131	0.0126	0.0124	0.0129	0.0030	0.0134	0.0131	0.0137	0.0149	0.0147	0.0261	0.0286	0.0295
12	*Synodontis_resupinata*	0.0184	0.0664	0.1005	0.0660	0.0740	0.0337	0.0637	0.0391	0.0679	0.0637	0.0597		0.0120	0.0124	0.0054	0.0124	0.0121	0.0058	0.0134	0.0130	0.0128	0.0134	0.0126	0.0146	0.0126	0.0139	0.0135	0.0135	0.0101	0.0138	0.0138	0.0140	0.0135	0.0139	0.0139	0.0136	0.0140	0.0135	0.0111	0.0140	0.0137	0.0147	0.0152	0.0152	0.0234	0.0250	0.0242
13	*Synodontis_violacea*	0.0741	0.0658	0.0567	0.0655	0.0673	0.0673	0.0638	0.0712	0.0583	0.0638	0.0638	0.0714		0.0130	0.0122	0.0115	0.0121	0.0124	0.0122	0.0111	0.0120	0.0122	0.0112	0.0141	0.0126	0.0123	0.0121	0.0122	0.0142	0.0124	0.0123	0.0124	0.0136	0.0125	0.0132	0.0120	0.0141	0.0125	0.0113	0.0135	0.0127	0.0129	0.0104	0.0105	0.0231	0.0271	0.0270
14	*ynodontis_flavitaeniatus*	0.0780	0.0674	0.1074	0.0690	0.0712	0.0732	0.0714	0.0772	0.0679	0.0800	0.0758	0.0773	0.0755		0.0131	0.0140	0.0069	0.0128	0.0082	0.0140	0.0155	0.0082	0.0112	0.0111	0.0076	0.0144	0.0085	0.0092	0.0155	0.0090	0.0087	0.0087	0.0156	0.0089	0.0094	0.0098	0.0095	0.0122	0.0124	0.0091	0.0124	0.0109	0.0166	0.0166	0.0249	0.0272	0.0281
15	*Synodontis_membranacea*	0.0049	0.0667	0.0965	0.0664	0.0702	0.0412	0.0600	0.0468	0.0642	0.0600	0.0600	0.0166	0.0717	0.0820		0.0125	0.0127	0.0029	0.0135	0.0128	0.0134	0.0135	0.0122	0.0139	0.0129	0.0139	0.0128	0.0130	0.0110	0.0128	0.0131	0.0132	0.0135	0.0132	0.0141	0.0130	0.0135	0.0131	0.0108	0.0141	0.0132	0.0140	0.0150	0.0151	0.0239	0.0257	0.0262
16	*Synodontis_zambezensis*	0.0803	0.0699	0.0879	0.0695	0.0738	0.0670	0.0394	0.0709	0.0435	0.0285	0.0321	0.0734	0.0638	0.0881	0.0738		0.0124	0.0132	0.0124	0.0059	0.0108	0.0124	0.0128	0.0141	0.0128	0.0105	0.0124	0.0126	0.0139	0.0124	0.0127	0.0125	0.0102	0.0125	0.0130	0.0127	0.0141	0.0135	0.0078	0.0133	0.0138	0.0137	0.0140	0.0140	0.0245	0.0271	0.0291
17	*Synodontis_notatus*	0.0736	0.0691	0.1028	0.0708	0.0832	0.0689	0.0690	0.0728	0.0675	0.0755	0.0714	0.0729	0.0690	0.0268	0.0774	0.0792		0.0125	0.0080	0.0131	0.0147	0.0080	0.0117	0.0112	0.0033	0.0143	0.0073	0.0075	0.0147	0.0079	0.0075	0.0080	0.0155	0.0081	0.0078	0.0077	0.0107	0.0120	0.0116	0.0072	0.0122	0.0090	0.0160	0.0160	0.0252	0.0253	0.0251
18	*Synodontis_batensoda*	0.0000	0.0725	0.1051	0.0721	0.0761	0.0429	0.0656	0.0484	0.0699	0.0656	0.0617	0.0182	0.0734	0.0794	0.0049	0.0796	0.0749		0.0133	0.0136	0.0141	0.0133	0.0123	0.0140	0.0127	0.0146	0.0130	0.0132	0.0117	0.0132	0.0133	0.0134	0.0142	0.0135	0.0142	0.0133	0.0132	0.0134	0.0115	0.0139	0.0135	0.0142	0.0160	0.0160	0.0239	0.0262	0.0262
19	*Synodontis_angelica*	0.0821	0.0713	0.1025	0.0709	0.0792	0.0751	0.0732	0.0791	0.0717	0.0798	0.0756	0.0834	0.0672	0.0340	0.0839	0.0753	0.0338	0.0813		0.0131	0.0154	0.0000	0.0115	0.0110	0.0079	0.0141	0.0077	0.0084	0.0151	0.0082	0.0080	0.0084	0.0152	0.0083	0.0100	0.0087	0.0107	0.0115	0.0121	0.0105	0.0117	0.0095	0.0159	0.0160	0.0239	0.0265	0.0266
20	*Synodontis_njassae*	0.0848	0.0721	0.0881	0.0717	0.0781	0.0753	0.0358	0.0792	0.0436	0.0322	0.0322	0.0819	0.0600	0.0883	0.0781	0.0199	0.0836	0.0840	0.0796		0.0100	0.0131	0.0126	0.0138	0.0131	0.0102	0.0120	0.0134	0.0143	0.0120	0.0123	0.0120	0.0097	0.0120	0.0139	0.0133	0.0142	0.0132	0.0076	0.0139	0.0135	0.0144	0.0141	0.0141	0.0257	0.0272	0.0293
21	*Synodontis_rukwaensis*	0.0868	0.0719	0.0968	0.0715	0.0697	0.0834	0.0394	0.0875	0.0397	0.0394	0.0394	0.0755	0.0678	0.0991	0.0800	0.0527	0.0942	0.0860	0.0988	0.0490		0.0154	0.0138	0.0145	0.0152	0.0111	0.0153	0.0155	0.0154	0.0160	0.0156	0.0160	0.0091	0.0160	0.0153	0.0158	0.0147	0.0149	0.0086	0.0171	0.0151	0.0162	0.0153	0.0152	0.0260	0.0280	0.0274
22	*Synodontis_angelicus*	0.0821	0.0713	0.1025	0.0709	0.0792	0.0751	0.0732	0.0791	0.0717	0.0798	0.0756	0.0834	0.0672	0.0340	0.0839	0.0753	0.0338	0.0813	0.0000	0.0796	0.0988		0.0115	0.0110	0.0079	0.0141	0.0077	0.0084	0.0151	0.0082	0.0080	0.0084	0.0152	0.0083	0.0100	0.0087	0.0107	0.0115	0.0121	0.0105	0.0117	0.0095	0.0159	0.0160	0.0239	0.0265	0.0266
23	*Synodontis_albolineatus*	0.0741	0.0736	0.0809	0.0732	0.0714	0.0631	0.0675	0.0689	0.0680	0.0719	0.0719	0.0755	0.0613	0.0584	0.0717	0.0777	0.0620	0.0734	0.0622	0.0779	0.0862	0.0622		0.0119	0.0118	0.0125	0.0116	0.0105	0.0135	0.0120	0.0118	0.0121	0.0140	0.0121	0.0137	0.0112	0.0105	0.0060	0.0121	0.0128	0.0063	0.0123	0.0133	0.0133	0.0252	0.0281	0.0288
24	*Synodontis_centralis*	0.0924	0.0733	0.0975	0.0729	0.0873	0.0851	0.0749	0.0892	0.0735	0.0815	0.0815	0.0937	0.0871	0.0600	0.0899	0.0875	0.0637	0.0915	0.0599	0.0855	0.0939	0.0599	0.0658		0.0116	0.0148	0.0110	0.0103	0.0153	0.0114	0.0111	0.0116	0.0146	0.0117	0.0116	0.0128	0.0092	0.0118	0.0132	0.0127	0.0120	0.0134	0.0150	0.0150	0.0237	0.0266	0.0265
25	*Synodontis_congica*	0.0777	0.0732	0.1115	0.0748	0.0875	0.0749	0.0731	0.0789	0.0716	0.0796	0.0755	0.0791	0.0731	0.0303	0.0816	0.0834	0.0064	0.0791	0.0338	0.0836	0.0986	0.0338	0.0623	0.0677		0.0148	0.0073	0.0079	0.0154	0.0079	0.0075	0.0080	0.0160	0.0081	0.0083	0.0080	0.0106	0.0124	0.0118	0.0076	0.0125	0.0088	0.0167	0.0168	0.0259	0.0256	0.0259
26	*Synodontis_gobroni*	0.0936	0.0742	0.0816	0.0738	0.0761	0.0773	0.0564	0.0813	0.0529	0.0487	0.0525	0.0862	0.0701	0.0883	0.0867	0.0544	0.0922	0.0927	0.0881	0.0507	0.0584	0.0881	0.0717	0.0942	0.0966		0.0141	0.0144	0.0150	0.0146	0.0143	0.0146	0.0108	0.0144	0.0156	0.0153	0.0135	0.0129	0.0113	0.0149	0.0132	0.0166	0.0134	0.0132	0.0255	0.0285	0.0314
27	*Synodontis_macrostiga*	0.0865	0.0735	0.1028	0.0731	0.0815	0.0836	0.0734	0.0834	0.0761	0.0779	0.0779	0.0879	0.0693	0.0397	0.0841	0.0755	0.0303	0.0857	0.0322	0.0715	0.0991	0.0322	0.0644	0.0580	0.0303	0.0840		0.0086	0.0147	0.0023	0.0017	0.0028	0.0140	0.0028	0.0090	0.0090	0.0106	0.0120	0.0119	0.0091	0.0122	0.0102	0.0155	0.0155	0.0247	0.0266	0.0272
28	*Synodontis_brichardi*	0.0822	0.0734	0.1004	0.0730	0.0794	0.0712	0.0693	0.0751	0.0719	0.0758	0.0758	0.0836	0.0673	0.0415	0.0798	0.0796	0.0303	0.0815	0.0376	0.0840	0.0968	0.0376	0.0525	0.0563	0.0339	0.0883	0.0359		0.0146	0.0085	0.0088	0.0087	0.0148	0.0086	0.0094	0.0085	0.0107	0.0105	0.0120	0.0093	0.0108	0.0096	0.0157	0.0158	0.0247	0.0255	0.0264
29	*Synodontis_annectens*	0.0666	0.0765	0.1025	0.0761	0.0864	0.0320	0.0715	0.0302	0.0800	0.0736	0.0736	0.0522	0.0879	0.1008	0.0603	0.0857	0.0959	0.0660	0.0983	0.0903	0.0966	0.0983	0.0794	0.1005	0.1025	0.0946	0.0942	0.0919		0.0150	0.0150	0.0150	0.0143	0.0151	0.0156	0.0145	0.0155	0.0138	0.0126	0.0154	0.0141	0.0149	0.0157	0.0158	0.0246	0.0272	0.0288
30	*Synodontis_vanderwaali*	0.0887	0.0755	0.1051	0.0751	0.0857	0.0857	0.0734	0.0855	0.0803	0.0779	0.0779	0.0900	0.0714	0.0434	0.0841	0.0755	0.0339	0.0879	0.0358	0.0715	0.1036	0.0358	0.0684	0.0620	0.0339	0.0883	0.0032	0.0359	0.0963		0.0029	0.0027	0.0140	0.0028	0.0093	0.0090	0.0110	0.0121	0.0119	0.0090	0.0123	0.0101	0.0158	0.0158	0.0254	0.0267	0.0282
31	*Synodontis_woosnami*	0.0887	0.0755	0.1051	0.0751	0.0836	0.0857	0.0755	0.0855	0.0781	0.0800	0.0800	0.0900	0.0714	0.0415	0.0862	0.0775	0.0321	0.0879	0.0340	0.0736	0.1013	0.0340	0.0664	0.0600	0.0321	0.0862	0.0016	0.0377	0.0963	0.0048		0.0033	0.0142	0.0033	0.0093	0.0091	0.0108	0.0123	0.0122	0.0093	0.0125	0.0104	0.0157	0.0158	0.0251	0.0270	0.0276
32	*Synodontis_leopardina*	0.0906	0.0774	0.1071	0.0770	0.0834	0.0877	0.0753	0.0875	0.0822	0.0798	0.0798	0.0920	0.0732	0.0414	0.0881	0.0773	0.0356	0.0898	0.0376	0.0734	0.1056	0.0376	0.0703	0.0638	0.0356	0.0903	0.0048	0.0376	0.0983	0.0048	0.0065		0.0141	0.0031	0.0094	0.0090	0.0111	0.0122	0.0119	0.0092	0.0124	0.0102	0.0159	0.0159	0.0256	0.0267	0.0284
33	*Synodontis_multipunctata*	0.0894	0.0781	0.1014	0.0777	0.0697	0.0817	0.0357	0.0836	0.0454	0.0305	0.0341	0.0821	0.0845	0.1042	0.0826	0.0507	0.1036	0.0886	0.0994	0.0470	0.0431	0.0994	0.0867	0.0966	0.1082	0.0604	0.0930	0.0951	0.0862	0.0930	0.0952	0.0949		0.0141	0.0154	0.0152	0.0151	0.0147	0.0081	0.0163	0.0150	0.0159	0.0158	0.0158	0.0267	0.0285	0.0297
34	*Synodontis_nebulosa*	0.0909	0.0776	0.1074	0.0772	0.0879	0.0879	0.0755	0.0877	0.0781	0.0800	0.0800	0.0922	0.0734	0.0415	0.0884	0.0775	0.0357	0.0900	0.0377	0.0736	0.1059	0.0377	0.0705	0.0640	0.0357	0.0862	0.0048	0.0377	0.0986	0.0048	0.0065	0.0065	0.0952		0.0095	0.0090	0.0111	0.0123	0.0120	0.0092	0.0125	0.0102	0.0160	0.0160	0.0256	0.0271	0.0277
35	*Synodontis_decora*	0.0887	0.0797	0.1148	0.0813	0.0853	0.0794	0.0731	0.0792	0.0736	0.0796	0.0796	0.0879	0.0775	0.0469	0.0884	0.0792	0.0355	0.0900	0.0506	0.0879	0.0963	0.0506	0.0805	0.0637	0.0392	0.0988	0.0413	0.0450	0.1030	0.0431	0.0431	0.0449	0.1036	0.0450		0.0102	0.0117	0.0129	0.0118	0.0088	0.0132	0.0114	0.0176	0.0176	0.0267	0.0254	0.0272
36	*Synodontis_smiti*	0.0859	0.0792	0.1020	0.0787	0.0768	0.0748	0.0689	0.0787	0.0755	0.0753	0.0753	0.0873	0.0669	0.0468	0.0835	0.0791	0.0319	0.0851	0.0394	0.0834	0.1028	0.0394	0.0599	0.0736	0.0355	0.0963	0.0375	0.0356	0.0935	0.0375	0.0393	0.0392	0.0988	0.0393	0.0503		0.0125	0.0123	0.0123	0.0095	0.0126	0.0044	0.0157	0.0158	0.0249	0.0251	0.0262
37	*Synodontis_schoutedeni*	0.0823	0.0800	0.1028	0.0796	0.0753	0.0791	0.0734	0.0830	0.0740	0.0738	0.0697	0.0879	0.0877	0.0488	0.0841	0.0881	0.0582	0.0815	0.0564	0.0905	0.0924	0.0564	0.0544	0.0449	0.0582	0.0819	0.0585	0.0605	0.1008	0.0625	0.0605	0.0644	0.0974	0.0646	0.0682	0.0742		0.0107	0.0126	0.0115	0.0108	0.0127	0.0158	0.0158	0.0247	0.0279	0.0282
38	*Synodontis_batesii*	0.0827	0.0800	0.0876	0.0796	0.0862	0.0753	0.0697	0.0813	0.0723	0.0763	0.0763	0.0840	0.0714	0.0666	0.0803	0.0821	0.0662	0.0819	0.0623	0.0824	0.0952	0.0623	0.0199	0.0660	0.0703	0.0761	0.0686	0.0526	0.0881	0.0686	0.0707	0.0705	0.0913	0.0707	0.0744	0.0680	0.0585		0.0124	0.0124	0.0016	0.0135	0.0139	0.0139	0.0278	0.0276	0.0290
39	*Synodontis_lucipinnis*	0.0639	0.0616	0.0902	0.0613	0.0552	0.0570	0.0120	0.0589	0.0174	0.0051	0.0051	0.0593	0.0595	0.0699	0.0576	0.0303	0.0633	0.0635	0.0676	0.0304	0.0360	0.0676	0.0681	0.0758	0.0654	0.0558	0.0677	0.0656	0.0697	0.0677	0.0699	0.0697	0.0324	0.0699	0.0654	0.0693	0.0699	0.0703		0.0134	0.0126	0.0131	0.0155	0.0155	0.0271	0.0290	0.0306
40	*Synodontis_congicus*	0.0883	0.0814	0.1091	0.0830	0.0983	0.0773	0.0813	0.0813	0.0841	0.0881	0.0838	0.0896	0.0834	0.0431	0.0923	0.0834	0.0302	0.0896	0.0544	0.0879	0.1145	0.0544	0.0763	0.0738	0.0337	0.0944	0.0431	0.0468	0.1008	0.0431	0.0450	0.0449	0.1105	0.0450	0.0429	0.0484	0.0622	0.0723	0.0826		0.0126	0.0108	0.0162	0.0162	0.0252	0.0259	0.0275
41	*Synodontis_batesii*	0.0848	0.0822	0.0898	0.0817	0.0883	0.0773	0.0717	0.0834	0.0744	0.0784	0.0784	0.0862	0.0734	0.0686	0.0824	0.0843	0.0682	0.0840	0.0644	0.0845	0.0974	0.0644	0.0216	0.0680	0.0723	0.0781	0.0707	0.0545	0.0903	0.0707	0.0728	0.0725	0.0935	0.0728	0.0765	0.0701	0.0605	0.0016	0.0724	0.0744		0.0137	0.0141	0.0141	0.0276	0.0273	0.0292
42	*Synodontis_soloni*	0.0968	0.0854	0.1089	0.0850	0.0873	0.0851	0.0791	0.0892	0.0860	0.0857	0.0857	0.0981	0.0770	0.0564	0.0942	0.0896	0.0409	0.0959	0.0450	0.0942	0.1096	0.0450	0.0699	0.0798	0.0409	0.1076	0.0468	0.0448	0.1001	0.0468	0.0487	0.0485	0.1056	0.0487	0.0600	0.0114	0.0763	0.0784	0.0780	0.0580	0.0805		0.0164	0.0164	0.0259	0.0250	0.0274
43	*Synodontis_caudovittata*	0.1020	0.0886	0.0000	0.0903	0.1011	0.0939	0.0886	0.0981	0.0915	0.0864	0.0908	0.0988	0.0558	0.1056	0.0949	0.0864	0.1011	0.1033	0.1008	0.0867	0.0974	0.1008	0.0796	0.0959	0.1096	0.0824	0.1011	0.0987	0.1008	0.1033	0.1033	0.1053	0.1019	0.1056	0.1148	0.1003	0.1011	0.0862	0.0902	0.1073	0.0883	0.1070		0.0000	0.0253	0.0293	0.0285
44	*Synodontis_schall*	0.1022	0.0866	0.0000	0.0883	0.0990	0.0941	0.0866	0.0983	0.0895	0.0844	0.0888	0.0990	0.0559	0.1058	0.0951	0.0866	0.1012	0.1035	0.1010	0.0868	0.0954	0.1010	0.0798	0.0961	0.1098	0.0804	0.1012	0.0989	0.1010	0.1035	0.1035	0.1055	0.0998	0.1058	0.1151	0.1005	0.1012	0.0863	0.0902	0.1075	0.0885	0.1072	0.0000		0.0253	0.0294	0.0286
45	*Hemibagrus_macropterus*	0.1910	0.1961	0.1997	0.1949	0.1981	0.1966	0.2042	0.1936	0.2088	0.2100	0.2071	0.1863	0.1866	0.1960	0.1902	0.1908	0.2038	0.1890	0.1873	0.1989	0.2042	0.1873	0.2042	0.1901	0.2095	0.2017	0.1989	0.1960	0.1998	0.2046	0.2017	0.2071	0.2129	0.2075	0.2154	0.2013	0.1968	0.2286	0.2135	0.1989	0.2255	0.2129	0.1960	0.1964		0.0239	0.0230
46	*Hemibagrus_microphthalmus*	0.2109	0.2003	0.2300	0.1991	0.2087	0.2051	0.2171	0.2104	0.2219	0.2240	0.2270	0.1973	0.2192	0.2134	0.2042	0.2091	0.1989	0.2087	0.2071	0.2145	0.2230	0.2071	0.2159	0.2062	0.2017	0.2327	0.2075	0.1989	0.2141	0.2075	0.2104	0.2100	0.2270	0.2104	0.2009	0.1936	0.2164	0.2164	0.2252	0.2046	0.2134	0.1936	0.2256	0.2261	0.1784		0.0145
47	*Hemibagrus_wyckioides*	0.2057	0.2095	0.2265	0.2095	0.2199	0.2103	0.2288	0.2159	0.2245	0.2331	0.2299	0.1904	0.2154	0.2158	0.2061	0.2267	0.1940	0.2048	0.2042	0.2325	0.2164	0.2042	0.2215	0.2027	0.2027	0.2523	0.2097	0.2007	0.2230	0.2158	0.2127	0.2184	0.2363	0.2127	0.2117	0.1962	0.2158	0.2221	0.2348	0.2097	0.2252	0.2082	0.2220	0.2225	0.1631	0.0912	

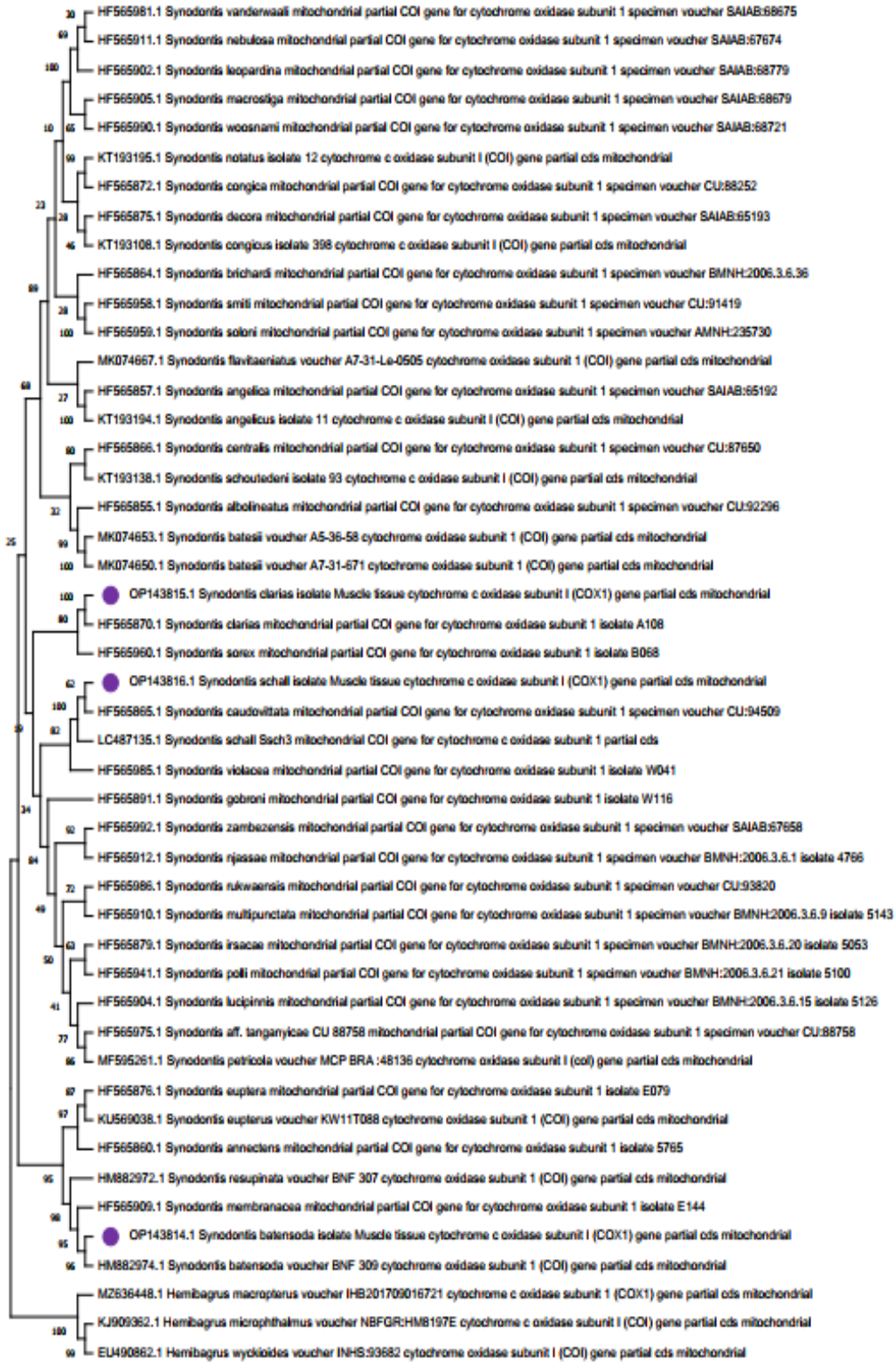

Fig. 2. Neighbour joining phylogenetic tree in three *Synodontis* fishes and their linked *Synodontis* species with the outgroup by employing the *COI* gene.

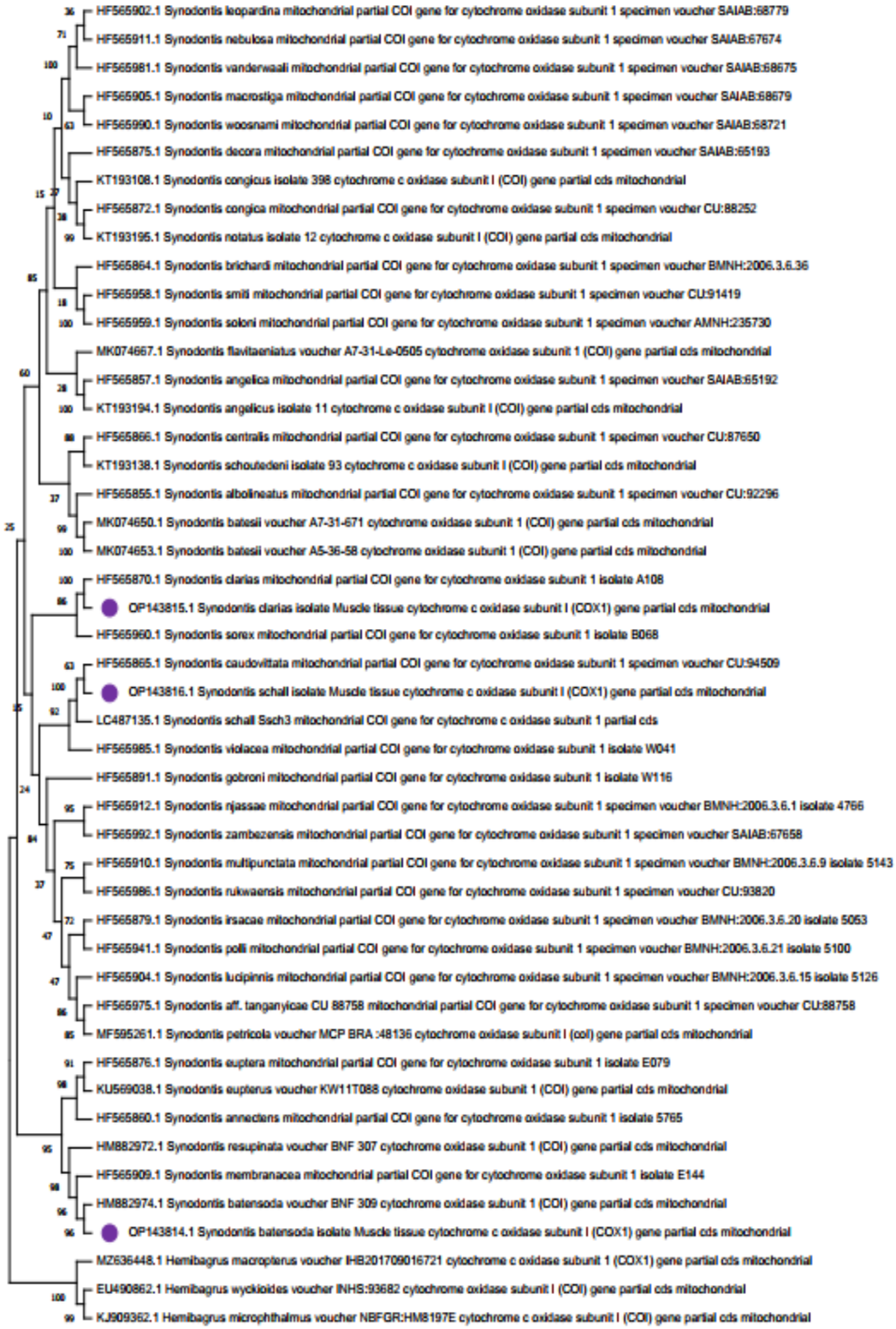

Fig. 3. Minimum evolution phylogenetic tree in three *Synodontis* fishes and their linked *Synodontis* species with the outgroup by employing the *COI* gene.

With some differences in support values, the two phylogenetic methods revealed nearly the same relations and displayed five main lineaments. (1) The outgroup species were found in a separate cluster. (2) All species of genus *Synodontis* were separated into two main clades; the first contains *Synodontis batensoda, S. membranacea, S. resupinata, S. annectens, S. eupterus* and *S. euptera*, while the second clade contains the rest species. (3) Understudied *Synodontis batensoda* was closely related to *Synodontis batensoda* (GenBank), *S. membranacea* and *S. resupinata*. (4) Understudied *Synodontis clarias* was closely related to *Synodontis clarias* (GenBank) and *S. sorex*. (5) The fifth main lineament was that the *Synodontis schall* under study was closely related to *Synodontis* schall (GenBank), *S. caudovittata* and *S. violacea*.

DISCUSSION

Taxonomy, as well as understanding the processes that lead to diversification and the origin of population structure, depend on the identification of independent lineages and species **(Hebert *et al*., 2004; Jörger & Schrödl, 2013; Gill *et al*., 2016)**. Unfortunately, African fishes DNA barcoding remains in the minority stage. Thus, a digital DNA barcode atlas for all African fishes is necessary for African countries. This would help recognize the fish species, observe the biogeographic distributions of the population and monitor the quickly extinction of threatened species by the help of a barcode reader **(Elsaied *et al*., 2021)**.

The average length of nucleotide sequences using *COI* in three *Synodontis* fishes was about 620 bp., which was in the expected length according to several studies **(Lakra *et al*., 2011; Bingpeng *et al*., 2018; Ali *et al*., 2020)**. The final alignments consisted of 626 bp. The highest sites were conserved sites (552); this result parallels recent findings of **Ferrari *et al*. (2022)** who applied *COI* barcoding to some fish samples of Amphiliidae and Cichlidae and found that the final consensus length was 684 bp., among which 426 (62.28%) were conserved sites.

The *COI* gene displayed A+T ratio bigger than the C+G ratio in all species, and the average proportion of A+T was 52.87; these data were in agreement with many previous studies **(Ward *et al*., 2005; Hubert *et al*., 2008; Lara *et al*., 2010; Bingpeng *et al*., 2018; Ferrari *et al*., 2022)**.

Sachithanandam *et al*. (2015) reported that through sequence analysis of *COI* gene, the intrageneric genetic distances are highly clearly distinguished. This coordinated with our result where the highest genetic distance in understudied species (0.0161) was found between *Synodontis batensoda* and *Synodontis schall*.

In the phylogenetic trees, the understudied species and their related conspecies from GenBank were clustered under the same nodes which revealed identical phylogenetic relationship among the species, this agree with **(Lakra *et al*., 2011)**.

The data of neighbour joining and minimum evolution methods using cytochrome c oxidase subunit I sequences showed that species identification and phylogenetic linkages, based on morphological and molecular methods, are widely coordinated. This finding are consistent with those of **Mat Jaafar *et al*. (2012)**. This revealed that DNA barcoding has great competence in species identification **(Bingpeng *et al*., 2018)**.

The current work illustrated the efficiency of *COI* in species identification which is in agreement with many similar studies. **Lakra *et al*. (2011)** used mtDNA (*COI*) to study the DNA barcoding of several fishes from the Indian Ocean and reported that, morphological characters mightily authenticated the ability of *COI* in the identification of fish species with designated DNA barcodes that make DNA barcoding approach successful. The work of **Sachithanandam *et al*. (2015)** once again affirmed the usefulness and efficiency of *COI* sequences in identifying marine fish species. In addition, the study of **Turan *et al*. (2017)** robustly authenticated the effectiveness of *COI* in the identification of the pufferfish species. Recently, the review of **Elsaied *et al*. (2021)** illustrated how DNA barcoding approaches have developed in African fisheries to resolve the fish identification problems, evolutions, differentiations of population and biogeographic distributions.

CONCLUSION

The current work was accomplished to assess the phylogenetic relationships of some species of genus *Synodontis* belonging to family Mochokidae using cytochrome c oxidase subunit I (*COI*) sequences. The results revealed the advantage of *COI* gene in exposing phylogenetic linkages among *Synodontis* species. Remarkaly, the phylogenetics of the family Mochokidae require further examination.

ETHICS STATEMENT

All procedures of animal experiments were achieved according to the Ethics of Animal Experiments Committee of South Valley University, Faculty of Science (Permit No.: 007/03/2023).

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