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Assessments of genetic variations of freshwater fishes *Malopterus electricus, Synodontis shall, Labeo niloticus* and *Lates niloticus*

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ABSTRACT
Freshwater fishes varied morphologically, but genetic assessments need some clarification. The present study is carried out to illustrate genetic variations between the freshwater fishes ; *Malapterurus electricus* (Gmelin, 1789), *Synodontis schall* (Bloch & Schneider, 1801), *Labeo niloticus* (Forsskål, 1775), and *Lates niloticus* as well as the brain isoenzymes electrophoresis of lactic dehydrogenase and glucose-6-phosphate dehydrogenase. The fishes were collected from River Nile were investigated for brain isoenzyme electrophoresis of lactic dehydrogenase and glucose-6-phosphate dehydrogenase and genetic variation using ten primers. The result of the study showed variations in the expression of the assayed isoenzyme electrophoresis. Also, application of the RAPD PCR using 10 primers revealed marked variations between the studied fishes. The primer OPB-3 expressed the production of nine unique bands between the study fishes, Nevertheless, OPA-2 and OPB-7 led to the production of eight unique bands, while the primers of OPA-5, OPA-7, OPA-9 and OPB-8 exhibited seven unique bands. Finally, the authors concluded that fish species varied in genetic expression and isoenzymes activities especially lactic dehydrogenase and glucose-6-phosphate dehydrogenase of the studied freshwater fishes.

INTRODUCTION

Fishes are the most abundant group of vertebrates with over 33,000 organisms living in aquatic environments and performing a wide range of biological functions in ecosystems (**Helfman *et al*., 2009; Martinez *et al*., 2018**). Heterozygosity (i.e., the proportion of diploid individuals with 2 separate alleles at a single locus) and the total number of alleles present in a population are also significant (**Frankham *et al*., 2014**). Following screened blunt snout bream with 88 SRAP primer combinations, of which 13 primer pairs produced stable and reproducible amplification patterns, **Ji *et al*. (2014)** reported significant genetic differentiation in the studied five species. *Oreochromis niloticus* collected from freshwater rivers, streams and canals were investigated using RAPD-PCR against Five random decamer primers and showed varying gene polymorphism (**Mahboob *et al*., 2019**).

Lactic dehydrogenase and glucose 6-phosphate dehydrogenase are important in the biological function of fishes. LDH is a key enzyme in managing of energy metabolism, catalyzing the transfer of pyruvate to lactate and regulating the levels of these metabolites related to oxygen supply. It can be found at the intersection of glycolysis, the metabolic pathway that convert glucose into pyruvate, and the tricarboxylic acid cycle, a series of enzyme-catalyzed chemical reactions that produce lactic acid. The distribution of LDH-A and LDH-B supports the concept of metabolic compartmentalization indicating that LDH-B enrichment in neurons and LDH-enrichment in astrocytes. The expression of RNA message for the A and B subunits have been determined (**Bittar *et al.*, 1996; Laughton *et al.*, 2000; Pellerin, 2003**).

LDH is formed by two different genes, LDH-A and LDH-B (Bittar et al., 1996), and they are handled differently (**O,Brien *et al.*, 2007**). For examples, because of their intrinsic bioenergetics properties associated dissemination over longer distances, larger neurons in the neocortex of primates with larger brains are likely to require complex responses to oxygen supply (**Sherwood et al., 2006**).

Glucose-6-phosphate dehydrogenase is involved in the first step in the pentose phosphate pathway, via conversion of glucose (a type of sugar found in most carbohydrates) to another sugar, ribose-5-phosphate, the main component of nucleotide (**Ge *et al.*, 2020**).

Following determination of glucose-6-phosphate dehydrogenase isoenzyme in fishes; *Albula neoguinaica* and *A. glossodonta* (Albulidae, Anquilliformes) (Shaklee and Tamaru,81), and butterflyfish, *Chaetodon miliaris* and *C. aurega* (Chaetodontidae); a goatfish, *Upeneus arge* (Mullidae); a goby, *Bathygobius fuscus* (Gobiidae); and a snapper, *Pristipomoides filamentosus* (Lutjanidae),**Kidder (1983)** identified two groups of isozymes of G6PD (specific for glucose-6-phosphate (G6P) and NADP+) and H6PD (capable of utilizing galactose-6-phosphate. Both of the isozymes detected can catalyze the oxidation of fructose-6-phosphate at a rate equivalent to that for G6P, suggesting that glucose-6-phosphate dehydrogenase can substitute glucosephosphate isomerase in monosaccharide metabolism. **Tian *et al.* (1999)** and **Hu et al. (2013)** mentioned that the intracellular reductant NADPH is mainly produced by the pentose phosphate pathway by Glucose-6-phosphate dehydrogenase (G6PDH). Data of studies showed that G6PDH inhibitors potentiated H 2 O 2 -produced cell death and overexpression of G6PDH enhanced resistance to H 2 O 2 -induced cell death. Also, G6PDH plays a critical role in cell death by affecting the redox potential.

The present study is carried out to illustrate genetic variations between the freshwater fishes ; *Malapterurus electricus* (Gmelin, 1789), *Synodontis schall* (Bloch & Schneider, 1801), *Labeo niloticus* (Forsskål, 1775), and *Lates niloticus* as well as the brain isoenzymes electrophoresis of lactic dehydrogenase and glucose-6-phosphate dehydrogenase.

MATERIALS AND METHODS

Eight adult teleost fishes were collected from the River Nile in Dakahlea Governorate, Damietta Branch, Egypt. The investigated fishes are illustrated (Fig.1):

1- *Malapterurus electricus* (Gmelin, 1789); it is belonged to Order: Siluriformes and Family: Malapteruridae.

2- Synodontis schall (Bloch & Schneider, 1801); it is to Order: Siluriformes and Family: Mochokidae.

3- *Labeo niloticus* (Forsskål, 1775), commonly named: Nile carp. It is belonged to Order: Cypriniformes and Family: Cyprinidae.

4- *Lates niloticus* (Linnaeus, 1758), commonly named: Nile perch. It is belonged to to Order: Perciforms and Family: Centropomidae.

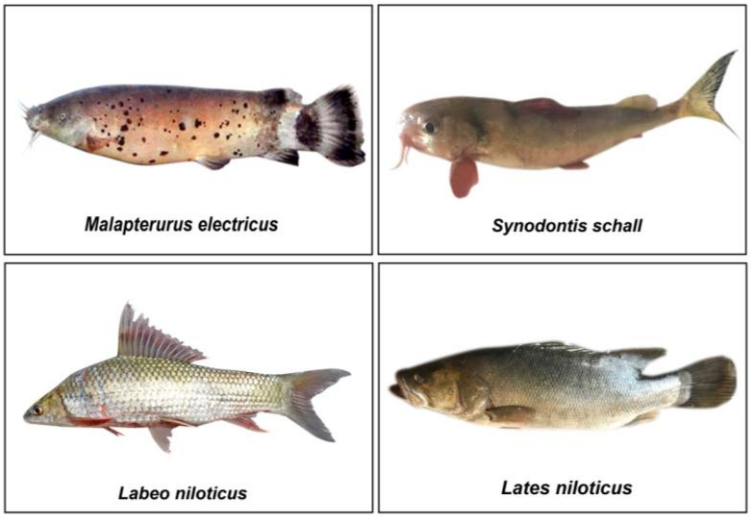

Fig. 1. Lateral view macrograph of the studied fishes.

The studied fishes were captured and their brains were dissected and their cerebellum were separated and subjected for investigations as follows:

1. DNA Extraction:

Knowing weight of fresh brain specimens of the studied fishes were lysed with proteinase K in 1.5-ml microcentrifuge tube and microcentrifugation was carried out. For DNA extraction using QIAamp DNA Mini Kit (Cat. No. 51304, Qiagen, Germany). Purification of DNA in the elution buffer was carried out and stored at 4 ^{0}C.

2. Random amplified polymorphic DNA (RAPD)-PCR:

Twenty RAPD primers were used with all of the species to assess the genetic diversity (OPA1to OPA10 and OPB1 to OPB10). All primers were obtained from Metabion International AG, Germany. Table (1) shows a list of primers and their sequences. The extracted DNA is considered as a template of DNA. The PCR mixture is composed of Emerald Amp GT PCR master mix (2X Premix) 25µl, template DNA (< 500 ng), forward primer (0.2 µM), reverse primer (0.2 µM) and 50 µL distilled water. The PCR amplifications were carried out at 94°C for 5 min (initial denaturation), then 40 cycles of denaturation at 94°C for 30 sec, followed by 30 second annealing at 35°C (for OPA3-OPA5,OPA7,OPA8,OPA10,OPB1,OPB2,OPB4) or at 37°C (for OPA1,OPA2,OPA6, OPA9,OPB3,OPB5-OPB10) after that extension was made at 72°C for 30 sec. Lastly, final extension at 72°C for 7 min (Table 1).

Table 1. Sequence of assayed primers.

Primer	Sequence
0PA-1	CAGGCCCTTC
0PA-2	TGCCGAGCTG
0PA-3	AGTCAGCCAC
0PA-4	AATCGGGCTG
0PA-5	AGGGGTCTTG
0PA-6	GGTCCCTGAC
0PA-7	GAAACGGGTG
0PA-8	GTGACGTAGG
0PA-9	GGGTAACGCC
0PA-10	GTGATCGCAG
OPB-1	GTTTCGCTCC
OPB-2	TGATCCCTGG
OPB-3	CATCCCCCTG
OPB-4	GGACTGGAGT
OPB-5	TGCGCCCTTC
OPB-6	TGCTCTGCCC
OPB-7	GGTGACGCAG
OPB-8	GTCCACACGG
OPB-9	TGGGGGACTC
OPB-10	CTGCTGGGAC

3. Isoenzyme electrophoresis:

The cerebellum samples were homogenized in 0.2 M Tris-Hcl (pH 7.5) containing 20% sucrose electrophoresis according to **Laemmli (1970).** The protein bands were stained with coomassie blue R-250 (60mg/L) in an acidic medium (**Andrews, 1986**). For visualization of the tested enzymes, electrophoresis was carried out in the selected incubated medium for each kind of the enzyme.

Lactic dehydrogenase: LDH isoenzyme was determined according to **Sarkar *et al.* (1978)**. After electrophoresis, the gel was incubated with H2O 18.4 ml, Tris 1M, tetrazolium-blue 1 mg/ml, phenazine methosulphate 1.6 mg/ml, Na-lactate 10Mm and NAD 10 Mm to develop color reaction for 20 min. In the color reaction, NAD and lactate serve as substrates, phenazine-methosulphate is the primary electron acceptor and tetrazolium-blue is the final electron acceptor.

Glucose 6 phosphate dehydrogenase: It was determined with gel electrophoresis at 4C in polyacrylamide slabs using a method based on **Gaal *et al.* (1980)**.The stacking gel (2.8%acrylamide) was prepared in 50 mm Tris-Pi(PH 6.3)and 20 % sucrose and the separating gel (5% acrylamide)in 0.75 M Tris Pi (PH 8).The electrophoretic buffer contained 5 mM Tris,80 mm aspartate ,and 20µM NADP+ at PH 7.4.Gels were stained for G6PD activity at 30 C in a solution in a volume of 20 ml containing 1.2mmol Tris-Pi (PH 8.5),25%(v/v)glycerol,30µmol glucose6-P,4gmol NADP+,6mg p-nitroblue tetrazoloum, and 0.5mg phenazine methosulfate.

4. **Statistical Analysis**

The data were presented as means ± standard deviation by using one way ANOVA test post hoc analysis (version 16 for Windows) and considered statistically significant at $P < 0.05$. For RAPD-PCR, the base pair values were used to perform agglomerative hierarchical clustering (ACH) of the different studied species using cluster analysis using XLSTAT software (2015). Bands are considered either polymorphic (present only in some species and missing in another) or unique (if present in one and missing in another) or monomorphic (present in all).

RESULTS

1. Random Amplified Polymorphic DNA (RAPD)-PCR:

A total of 20 random RAPD primers were used, with 17 of them yielding apparent DNA bands, resulting in 109 polymorphic bands including 89 unique bands with an average 5.45 per primer. None of the primers generated monomorphic bands in the studied species, (100 % polymorphism). The length of bands produced varied between112 and 1315 bp (base pair). The Primers OPA-2, OPA-9 and OPB-3 yield the most bands (10) while the primer OPB-2 produces the fewest (1). The DNA of the analyzed fish samples was tested against the primers shown.

With the used primers, *Lates niloticus* possesses the most total bands (41 DNA bands), followed by *Malapterurus electricus* (39 DNA bands) then *Labeo niloticus* (28 DNA bands). The smallest number of bands *Synodontis schall* (26 DNA bands). Following the use of the primers OPA-4, OPA-6 and OPB-4, there is no expressed bands (Tables 2-3).

Table 2. Numerical data based on the RAPD-PCR technique among the studied species.

Primer	Sequence	*Malapterurus electricus*		*Synodontis schall*		*Labeo niloticus*		*Lates niloticus*	
		PB	UB	PB	UB	PB	UB	PB	UB
0PA-1	CAGGCCCTTC	0	0	1	1	2	2	0	0
0PA-2	TGCCGAGCTG	6	4	1	0	4	3	1	1
0PA-3	AGTCAGCCAC	0	0	1	0	2	1	3	2
0PA-4	AATCGGGCTG	0	0	0	0	0	0	0	0
0PA-5	AGGGGTCTTG	0	0	7	7	1	0	1	0
0PA-6	GGTCCCTGAC	0	0	0	0	0	0	0	0
0PA-7	GAAACGGGTG	4	3	1	1	2	1	2	2
0PA-8	GTGACGTAGG	1	1	2	1	3	3	1	0
0PA-9	GGGTAACGCC	3	1	2	1	3	2	6	3
0PA-10	GTGATCGCAG	4	2	4	3	2	1	0	0
OPB-1	GTTTCGCTCC	2	2	1	1	2	1	2	1
OPB-2	TGATCCCTGG	1	1	0	0	0	0	0	0
OPB-3	CATCCCCCTG	1	0	0	0	0	0	10	9
OPB-4	GGACTGGAGT	0	0	0	0	0	0	0	0
OPB-5	TGCGCCCTTC	4	2	0	0	3	1	4	1
OPB-6	TGCTCTGCCC	2	1	1	0	1	1	2	2
OPB-7	GGTGACGCAG	4	3	2	1	1	1	4	3
OPB-8	GTCCACACGG	4	3	1	1	1	1	3	2
OPB-9	TGGGGGACTC	1	0	2	1	0	0	0	0
OPB-10	CTGCTGGGAC	2	1	0	0	1	0	2	2
Total		39	24	26	18	28	18	41	28

Abbreviations; PB: Polymorphic bands, UB: Unique bands

Table 3. Numerical data based on the RAPD-PCR technique among the studies species.

Primer	Sequence	TB	MB	PB	UB	%PB
0PA-1	CAGGCCCTTC	3	0	3	3	100
0PA-2	TGCCGAGCTG	10	0	10	8	100
0PA-3	AGTCAGCCAC	4	0	4	3	100
0PA-4	AATCGGGCTG	0	0	0	0	0
0PA-5	AGGGGTCTTG	8	0	8	7	100
0PA-6	GGTCCCTGAC	0	0	0	0	0
0PA-7	GAAACGGGTG	8	0	8	7	100
0PA-8	GTGACGTAGG	6	0	6	5	100
0PA-9	GGGTAACGCC	10	0	10	7	100
0PA-10	GTGATCGCAG	8	0	8	6	100
OPB-1	GTTTCGCTCC	6	0	6	5	100
OPB-2	TGATCCCTGG	1	0	1	1	100
OPB-3	CATCCCCCTG	10	0	10	9	100
OPB-4	GGACTGGAGT	0	0	0	0	0
OPB-5	TGCGCCCTTC	7	0	7	4	100
OPB-6	TGCTCTGCCC	5	0	5	4	100
OPB-7	GGTGACGCAG	9	0	9	8	100
OPB-8	GTCCACACGG	8	0	8	7	100
OPB-9	TGGGGGACTC	2	0	2	1	100
OPB-10	CTGCTGGGAC	4	0	4	3	100
TOTAL		109		109	88	

Abbreviations; TB: Total bands number, MB: Monomorphic bands, PB: Polymorphic bands, UB: Unique bands, %PB: Percent of polymorphic bands

Table (4) and Figure (2) illustrate the genetic relationship between the studied species. The highest similarity was between *Synodontis schall* and *Malapterurus electricus* as they have the least genetic distance (1.017) while the genetic distance between *Lates niloticus and Labeo niloticus was* 1.185.

Table 4. Cluster joining of studied fishes

Clusters Joining		At Distance	No. of Members
Synodontis schall	***Malapterurus electricus***	1.017	2
Lates niloticus	***Labeo niloticus***	1.185	2
Synodontis schall, Malapteruruss electricus	***Lates niloticus, Labeo niloticus***	1.208	4

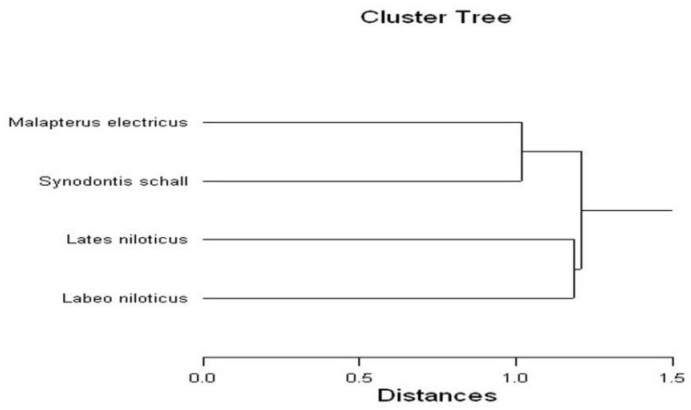

Fig.2. Cluster analysis of the four studied species depending on their RAPD-PCR data generated by Pearson distance and complete linkage method. Bar refers to the genetic distance. Note; the least genetic distance was between *Synodontis schall* and *Malapterurus electricus (*1.017) followed by *Lates niloticus and Labeo niloticus (*1.185).

Numerical analysis of the bands were illustrated. For the unique bands; *Synodontis schall*. had a single unique band at the base pair 385 , while Labeo niloticus had two unique bands at the base pair 579 and 1034. Primer OPA-2 showed four unique bands in *Malapterurus electricus*, three unique bands in *Labeo niloticus* and one in *Lates niloticus*. Primer OPA-3 revealed a unique band for *Labeo niloticus* at 791 base pair and two unique bands for Lates niloticus at 707 and 965 base pairs. Primer OPA-5 shows eight polymorphic bands, 7 band of which are unique to *Synodontis schall* , while primer OPA-7 generates seven distinct bands; three bands for *Malopterus electricus* at base pair 430, 519 and 1315, one band for *Synodontis shall* at 954 bp, one band for *Labeo niloticus* at 1062 bp and two bands for *Lates niloticus* at 479 and 868 bp. Primer OPA-8 developed five unique bands; one band for *Malapterurus electricus* at 325 bp, one for *Synodontis shall* at 625bp and 3 for *Labeo niloticus* at 729, 848, 921 bp. Primer OPA-9 produced seven unique DNA bands; one for *Malapterurus electricus* at 509 bp, another for *Synodontis schall* at 678 bp, 2 for *Labeo niloticus* at 612, 545 bp and three for *Lates niloticus* at 306, 744, 1024 bp.

Also, Primer OPA10 yielded 6 unique bands; 2 for Malapterurus electricus at 678, 1213 bp, three for Synodontis schall at 296, 391, 928 bp and one for Labeo niloticus at 1084 bp, compared to primer OPB-1 which revealed five unique bands; 2 for Malapterurus electricus at 277, 391 bp, one for Synodontis schall at 638bp, one for Labeo niloticus at 958 bp and one for Lates niloticus at 727 bp. Primer OPB-2 yielded a unique band specific for *Malapterurus electricus* at 519 bp. Primer OPB-3 possessed nine unique bands specific for Lates niloticus. Primer OPB-5 exhibited four unique bands; two for *Malapterurus electricus* at 613, 1230 bp, one band for *Labeo niloticus* at 1024 and one band for *Lates niloticus* at 791bp. Primer OPB-6 showed four unique bands; one for *Malapterurus electricus* at 112 bp, one for *Labeo niloticus* at 422 bp and two bands for *Lates niloticus* at 285, 664 bp. Primer OPB-7 produced eight unique bands; three for

Malapterurus electricus at 221, 304, 585 bp, one for Synodontis schall at 664 bp, one for *Labeo niloticus* at 531 bp and three for *Lates niloticus* at 178, 266, 466 bp. Primer OPB-8 produced. Primer OPB-8 produced seven unique bands; three for *Malapterurus electricus* at 378, 578, 979 bp, one for *Synodontis schall* at 411bp, one for *Labeo niloticus* at 821 bp and 2 for *Lates niloticus* at 221, 493 bp. Primer OPB-9 produced only a unique band specific for *Synodontis schall* at 242 bp. Primer OPB-10 produced three unique bands; one for *Malapterurus electricus* at 772 bp and two for lates at 149 and 277 bp. The other three used primers generated no band markers (Figs. 3-6).

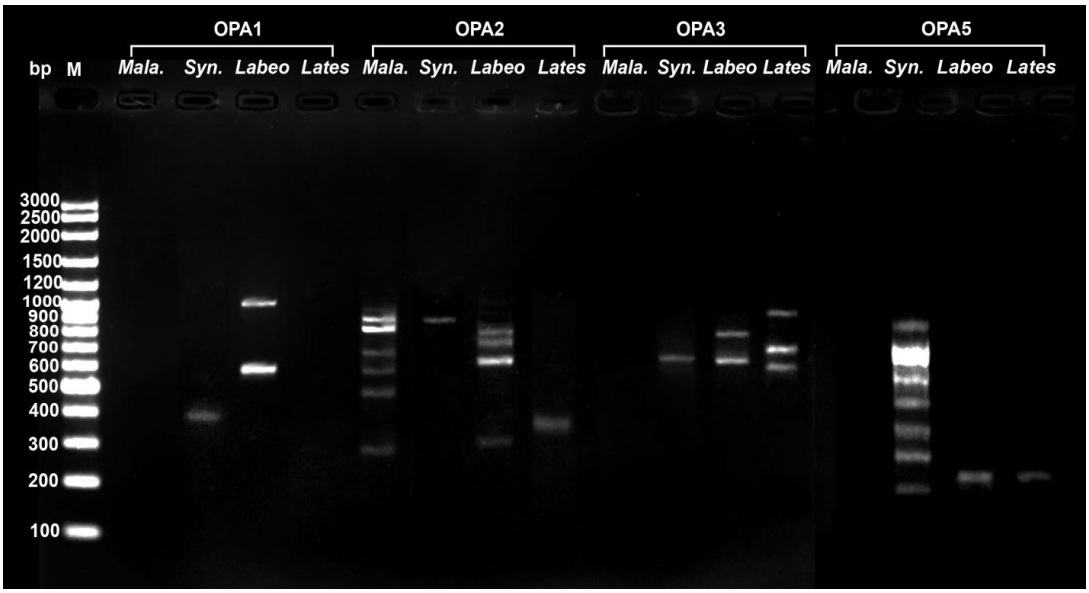

Fig. 3. Agarose gel electrophoresis showing expression of OPA1 in *Labeo niloticus*. OPA2 is expressed in *Malapterurus electricus* and *Labeo niloticus*. OPA3 is expressed in *Labeo* –and *Lates niloticus*. OPA5 is expressed in *Synodontis shall* and *Labeo niloticus*

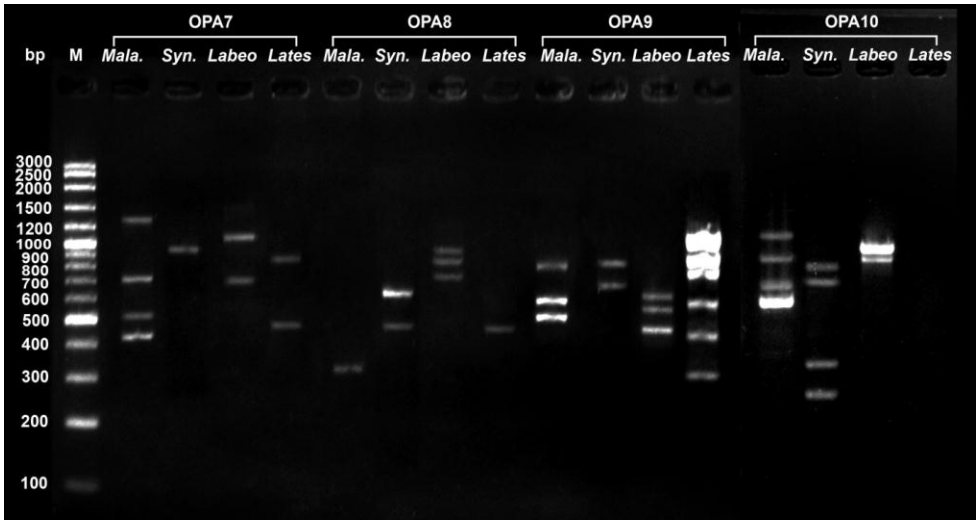

Fig.4. Agarose gel electrophoresis showing expression of OPA7 of 4 bands in *Malopterus electricus*, one band in *Synodontis shall*, two bands in Labeo niloticus and two varying bands in *Lates niloticus*. OPA8 expressed only one band in *Malopterus electricus*, 2 bands in *Synodontis shall*,3 bands in *Labeo niloticus* and one band in *Lates niloticus*. OPA9 expressed 3 bands in *Malapterurus electricus*, two bands in *Synodontis shall*, 3 bands in *Labeo niloticus* and 6 bands in *lates niloticus*. OPA 10 expressed 4 bands in *Malapterurus electricus*, 4 bands in *Synodontis shall*, 2 bands in *Labeo. niloticus*.

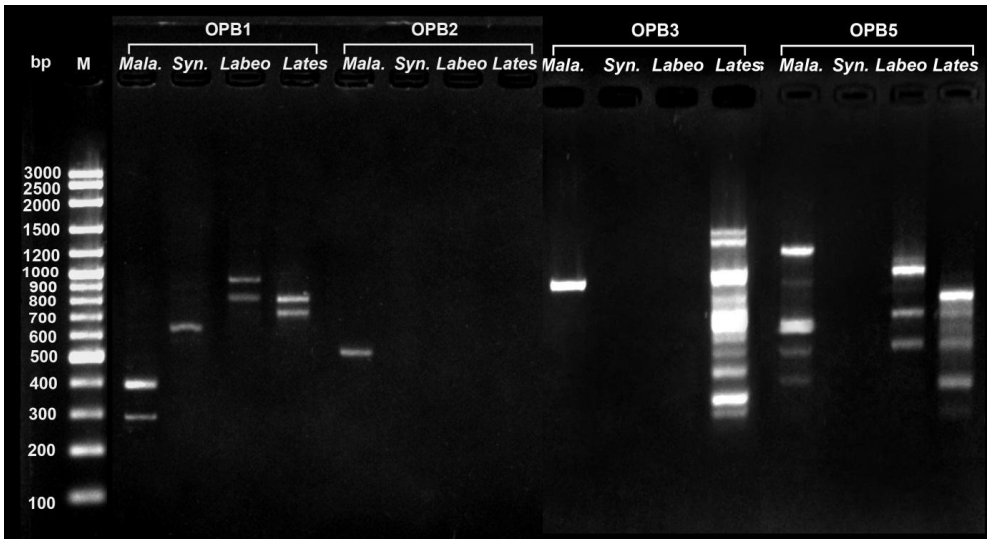

Fig. 5. Agarose gel electrophoresis of brain DNA of studied fishes with OPB1,2,3 and OPB5 primers. OPB1 expressed only two bands in *Malapterurus.electricus*, 1 band in *Synodontis schall*,2 bands in *Labeo niloticus* and 2 bands in *Lates niloticus*. OP B2 expressed only one band in *Malapterurus electricus.* OPB3 is expressed in *Malapterurus electricus* and *lates niloticus*. OPB5 is expressed in *Malapterurus electricus*, *Labeo niloticus* and *Lates niloticus*.

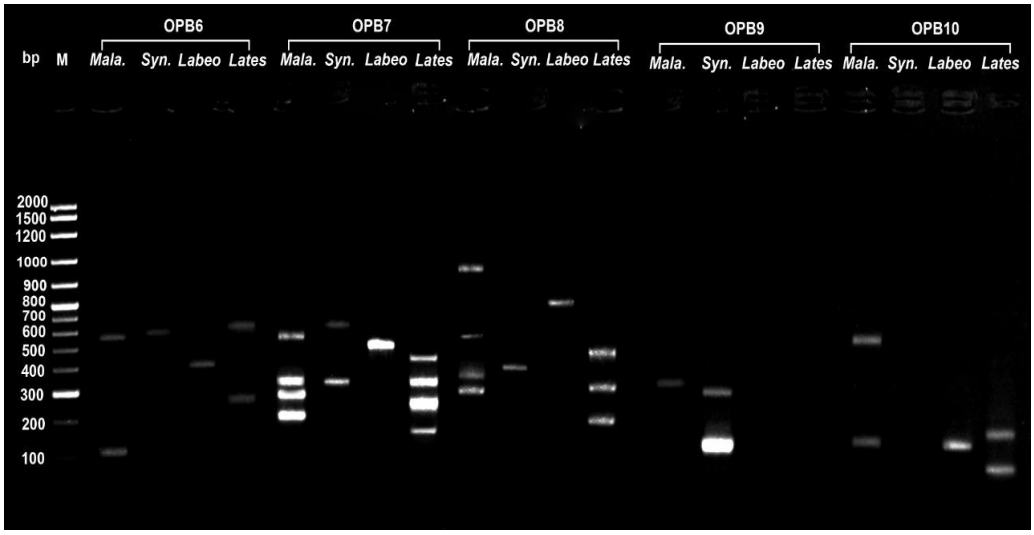

Fig. 6. Agarose gel electrophoresis of brain DNA of studied fishes with OPB 6,7,8,9 and OPB 10 primers. Note varying expressed bands in different types of fishes.

2. Isoenzyne electrophoresis of lactic dehydrogenase and glucose 6-phosphate dehydrogenase:

Figs. (7 & 8) illustrated the brain isoenzyme fractions of lactic dehydrogenase and glucose-6-phosphate dehydrogenase. Brain lactic dehydrogenase expressed five isoenzyme fractions with almost equal intensity in the studied fishes. However, glucose-6-phosphate dehydrogenase expressed four bands. In spawning *Malapterurus electricus*, the first band is duplicated. The rate of mobility is highly expressed in spawning *Synodontis schal* in comparison with the other studied fishes.

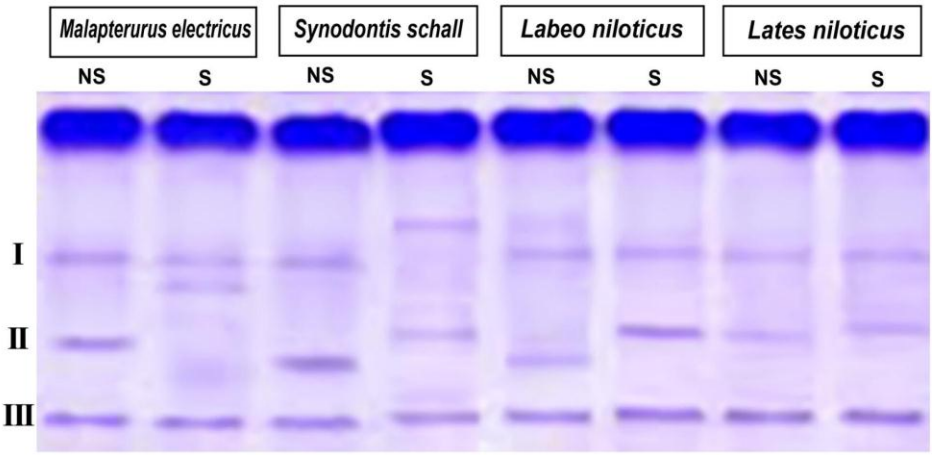

Fig.7. SDS-PAGE isoenzyme electrophoresis of glucose-6-phosphate dehydrogenase. The enzyme expressed three isoenzyme fractions. Isoenzyme faction I is duplicated in *Malapterurus electricus*. Isoenzyme fraction II is missing in spawning season of *Malapterurus electricus*. Isoenzyme fraction III is similar in all studied fishes during both seasons.

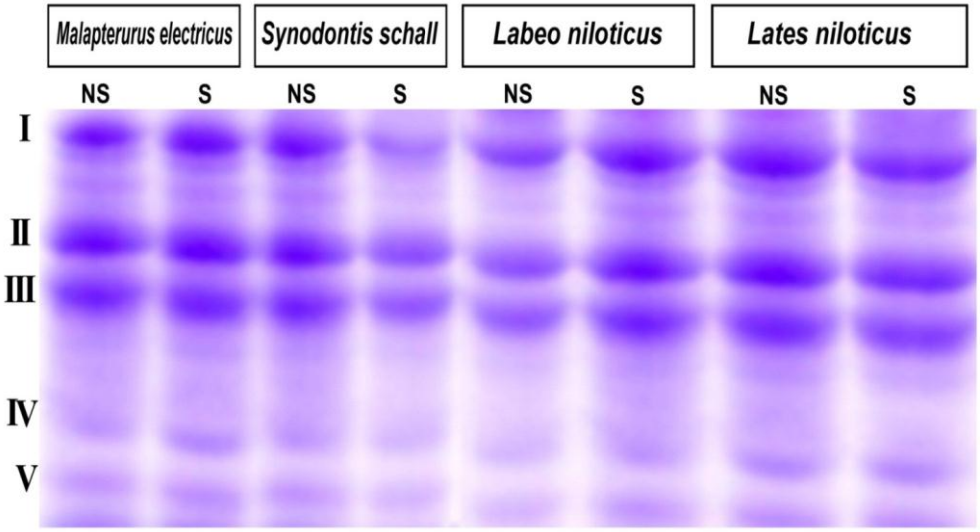

Fig. 8. SDS-PAGE isoenzyme electrophoresis of lactic dehydrogenase. The enzyme expressed five isoenzyme fractions and similar in the studied fishes. The studied fishes exhibited similar isoenzyme fraction except faint isoenzyme fractions IV and V in spawning *Synodontis schall* and non-spawning *Labeo niloticus*.

DISCUSSION

According to the current results, brain lactic dehydrogenase in the tested fishes expressed five isoenzyme fractions of approximately similar strength. Glucose-6-phosphate dehydrogenase, on the other hand, showed four bands. In spawning *Malapterurus electricus*, the first band is duplicated. The rate of mobility is highly expressed in spawning *Synodontis schall* in comparison with the other studied fishes.

Lactate dehydrogenase A (LDHA) is a cytosolic enzyme, involved in bothb anaerobic and aerobic glycolysis. It catalyzes the conversion of lactate to pyruvate with the reduction of NAD+ to NADH and vice versa (**Schumann *et al.*, 2002**). It is transferred from astrocytes to neurons for energetic requirement and maintain modulating neuronal functions, such as plasticity, excitability and memory consolidation (**Magistretti and Allama, 2018**). Lactate is formed from glucose or glycogen in astrocytes (**Bittner *et al.*, 2011; Choi *et al.*, 2012; Lerchundi *et al.*, 2015**). It servea as an energy substrate for neurons. Stimulation of the climbing fiber of cerebellar Purkinje cells increased the extracellular lactate by 30% within 30 s of stimulation (**Caesar *et al.*, 2008**).

Lactate is released from astrocytes through transmembrane monocarboxylate transporters, a high-capacity cation channel and pannexins (**Sotelo-Hitschfeld *et al.*, 2015; Karagiannis *et al.*, 2016**) and may act as a signaling molecule in the brain through hydroxycarboxylic acid receptor 1 (HCAR1). This particular receptor is found mainly in the neurons of the hippocampus, neocortex and cerebellum (**Morland *et al.* 2015**). When

HCAR1 is activated, neuronal activity decreases as a result of a decrease in cyclic adenosine monophosphate (cAMP) concentration (**Mosienko *et al.* 2015**). Lactate has the ability to diffuse away from where it was released, which allows it to act as a volume transmitter (**Rinholm and Bergersen, 2014**). All of these influences of lactate dehydrogenase reflected the energetic activities of Purkinje and granular cells in response to both pre-and spawning function of the studied teleost fishes.

It is known that G6PD is the first enzyme of the pentose phosphate pathway. It is protected the brain from liberation reactive oxygen species via synthesis of NADPH which reduced the oxidized glutathione and scavenge the liberated reactive oxygen species (**Efferth *et al*., 2006**). The enzyme is required for the cellular processes such as the antioxidant pathways, nitric oxide synthase, NADPH oxidase, and cytochrome p450 system (**Stanton, 2012**). Glucose-6-phosphate dehydrogenase is highly expressed in Purkinje cells and neurons of the granular and molecular layer. Electron microscopic investigations showed a remarkable activities in the basket , stellate cells, and Golgi cells (**Biagiotti *et al*., 2001; Biagiotti *et al*., 2003; Ferri *et al*., 2005**). Astrocytes, are supplied with a mitochondrial complement, that produce lactate instead of using pyruvate to supply the tricarboxylic acid cycle and ATP production (**Magistretti and Allaman , 2018**).

Genetically, the examined fishes differed. The primer OPB-3 expressed the production of nine unique bands between the study fishes, Neverthless, OPA-2 and OPB-7 led to the production of eight unique bands, while the primers of OPA-5, OPA-7, OPA-9 and OPB-8 exhibited seven unique bands. These results agree with the studies carried out by **Okubara *et al.* (2005)** who found that RAPD was more effective than morphological recognition to differentiate between individuals especially at the sub-species.

The effectiveness of RAPD-PCR in determining the relationship and the variations among fish species of genus Synodontis was stated by **Abu-Almaaty et al. (2018).** It is also a commonly used method for examining genetic polymorphism in closely related species without requiring any prior understanding of the genome (**Ali, 2003**). **Megbowon (2019)** recorded that cichlid fishes can be genetically differentiated into different genera based on bands polymorphisms produced by RAPD analysis. There was also, polymorphism within the same genera implying that fish of the same genus.

Fishes are not completely similar.

The phylogenetic tree between the studied species showed two clusters, the first cluster includes *Synodontis schall* and *Malapterurus electricus* which have the least genetic distance and *Lates niloticus* and *Labeo niloticus* which have the most genetic distance,. RAPD research was used to find species relatedness in many experiments, including **Zarringhabai *et al*. (2012), Hameed *et al*. (2012)** and **Singha *et al*. (2016)**. Furthermore, RAPD analysis showed differences in bands number and position between

studied species suggesting that the technique's sensitivity to detect differences between fish species. This finding was in agreement with **Ahmed *et al.* (2004)** and **Garg et al. (2009)** who concluded that RAPD markers are effective for assessing genetic diversity in fish species. Genetic diversity in fish species can be manifested as s a result of climatic and geographical distribution (**Mei *et al.*, 2017**) as well as migration, mutations, and species adaptation (**Zarringhabai *et al.*, 2012**). **Steinke *et al.* (2006)** studied 2466 genes from *Danio rerio*, *Takifugu rubripes*, *Tetraodon nigroviridis* and *Oryzias latipes* and found that one of the species exhibited increased rate of ATP binding proteins and transcription factors. **Mahboob *et al.* (2019)** reported varying gene polymorphism in *Oreochromis niloticus* catching from freshwater rivers, streams and canals. **Lind *et al.* (2019)** used 192 single nucleotide polymorphism (SNP) markers to investigate the genetic resources of Nile tilapia in West Africa and reported that fishes harvested from Gambia River , western Niger River and the upper Red Volta River had significantly lower levels of diversity and high genetic differentiation

Growing *Clarias gariepinus* showed more genetic differences comparing to that of slowing growth. The Cga02 locus was found to be the specific locus where significance levels were consistently expressed (**Ola-Oladimeji *et al.*, 2021**).

CONCLUSION

Finally, the authors concluded that fish species varied in genetic expression and isoenzymes activities especially lactic dehydrogenase and glucose-6-phosphate dehydrogenase of the studied freshwater fishes.

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