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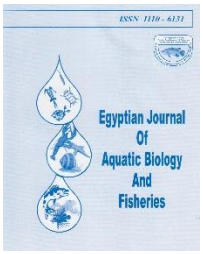

Phylogenetic Relationships among Some Catfishes Assessed by Small and Large Mitochondrial rRNA Sequences

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ABSTRACT

This study was achieved to estimate the phylogenetic relationships of some species of catfish belonging to the order Siluriformes, using small (*12S rRNA*) and large (*16S rRNA*) mitochondrial rRNA genes. The length of small mitochondrial rRNA sequences ranged from 832 to 1191 bp. The overall genetic distance was 0.15. The length of large mitochondrial rRNA sequences ranged from 554 to 564 bp. The overall genetic distance was 0.09. Both genes (*12S rRNA* and *16S rRNA*) showed nearly equal A+T composition (53.42 and 53.03, respectively). In addition, both genes displayed A+T composition higher than the C+G. The phylogenetic trees using (12S rRNA) sequences showed that all understudied catfish species and their related GenBank catfish species were grouped according to their genera and families. The same results were found using (*16S rRNA*) sequences, except for the family Claroteidae which displayed different taxonomic positions. Furthermore, the Monophyly family Claroteidae as well as its position to the other family needs more investigation.

INTRODUCTION

Due to its compact size, maternal inheritance and fast evolutionary rate, the mitochondrial genes has been exceedingly used **(Brown *et al.*, 1979; Wilson *et al.*, 1985)**. Mitochondrial DNA has been considered as a wonderful tool to explore and study the biogeographical events, above or below the species level, and they can resolve terminal taxa because the mitochondrial rRNA genes such as *12S* and *16S* evolve more rapidly than the nuclear rRNA genes **(Avise, 1994; Wang *et al.*, 2000)**.

The *12S rRNA* gene, situated between the *tRNAPhe* and *tRNAVal* genes, is relatively conserved, evolving more slowly than the mitochondrial genome as a whole **(Palumbi, 1996; Di Finizio *et al.*, 2006)**. Several research proclaim the broad use of mitochondrial *12S* rRNA gene in addressing the phylogenetic relationships among different levels of taxa including species, genera and families **(Ledje & Arnason, 1996; Murphy & Collier, 1996, 1997; Gatesy *et al.*, 1997; Halanych & Robinson, 1997**).

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It has been reported that (*16S rRNA*) is useful for analyzing species, population and families due to its slowest mutation and lower substitution rates compared to other mtDNA genes **(Garland & Zimmer, 2002)**. The mitochondrial *16S rRNA* gene is highly conserved and has a slow evolution **(Page & Holmes, 1998)**. Thus, it has been widely used to study the phylogenetic relationships of fishes at different taxonomic levels **(Ortí *et al*., 1996; Moyer, *et al*., 2004; Feng *et al*., 2005; Li *et al*., 2008)**. Among mtDNA genes, *16S rRNA* gene is a special region of mitochondrial genome; it has been considered as one of the most informative regions used in phylogenetic studies **(Bej *et al*., 2012; Patwardhan *et al*., 2014)**.

Catfishes are mainly freshwater fishes with the exception of only two marine families: Plotosidae and Ariidae **(Kailola, 2004)**. Catfishes (Order Siluriformes) are a various group of ray-finned fishes, spreading in all continents and found worldwide **(Diogo, 2004; Nelson, 2006)**. They contain more than 3,088 valid species belonging to 477 genera and 36 families **(Ferraris, 2007)**.

Catfishes have a great intensity to ecologists and evolutionary biologists for their wide-reaching, generally freshwater allocation and diversity. Siluriformes are primary matter in biogeography throughout all ranges from local to international **(Meyer & Van de Peer, 2003; Punhal *et al.*, 2018)**.

The main purpose of our study was to estimate the phylogenetic relationships of some species of catfishes belonging to order Siluriformes using small (*12S rRNA*) and large (*16S rRNA*) mitochondrial rRNA genes.

MATERIALS AND METHODS

Sample collection

Eleven catfishes species (Order Siluriformes) belonging to six families; namely, Bagridae (*Bagrus bajad* and *Bagrus docmak*), Clariidae (*Clarias gariepinus*), Claroteidae (*Chrysichthys auratu*s and *Chrysichthys rueppelli*), Malapteruridae (*Malapterurus electricus*), Schilbidae (*Schilbe mystu*s and *Schilbe uranoscopus*) and Mochokidae (*Synodontis batensoda*, *Synodontis clarias* and *Synodontis* schall) were collected from the River Nile in Upper Egypt. The samples were identified according to **Bishai and Khalil (1997**). The muscles' tissues were secluded and conserved at -20ºC until used.

DNA extraction and PCR amplification

QIAamp DNA Mini kit (Qiagen, Germany) was used to extract the total genomic DNA from the separated muscles' tissues. To amplify small mitochondrial rRNA (*12S rRNA*) gene in the eleven catfishes we used primers according to **Jin *et al.* (2013)**. While, forward and reverse primers **(Simon *et al*., 1991)** were used to amplify large mitochondrial rRNA (*16S rRNA*) gene. The PCR reactions consists of 25µL PCR master mix, 1µL from each of genomic DNA in addition to forward and reverse primers in a final reaction volume of 50µL. The PCR cycling conditions were done with the following

stes: an initial denaturation for 5 minutes at 94ºC, followed by 30 cycles of denaturation for 60s at 94ºC, annealing for 60s at 50ºC (*12S rRNA*) and 48 (*16S rRNA*) and an extension at 72°C for 60 sec, with post cycling extension at 72ºC for 5min. 1.5% agarose gel stained with ethidium bromide was used to separate the PCR products.

The sequencing of PCR products and phylogenetic tree construction

The DNA sequencing was done by Macrogen (Seoul, South Korea). The sequences of small and large mitochondrial rRNA genes were submitted to GenBank/NCBI for obtaining accession numbers. Sequence alignment was performed using MUSCLE **(Edgar, 2004)** with default settings. To implement the phylogenetic trees, analyses were conducted by two phylogenetic methods; Neighbour Joining (NJ) and Minimum Evolution (ME). we used Molecular Evolutionary Genetics Analysis (MEGA) version 11.0.11 **(Tamura *et al.*, 2021)**. Bootstrap analysis was determined with 1000 replicates **(Felsenstein, 1985)**. To provide a graphical representation of divergence between catfishes species, the sequence divergences were calculated using Kimura 2-parameter distances **(Kimura, 1980)**.

RESULTS

The nucleotide sequences of both small and large mitochondrial rRNA sequences were submitted to the GenBank under accession numbers (MW449532- MW449534) and (OM949995 - OM950005), respectively.

The length of small mitochondrial rRNA sequences ranged from 832 bp. in *Bagrus bajad* to 1191 bp. in *Chrysichthys rueppelli*. The aligned *12S rRNA* data set contained 1200 characters of which 679 were constant sites, 516 were variable, and 247 were parsimony informative (Table 1). The average genetic distance between the catfishes species based on *mt12S rRNA* sequence was 0.13.

Table 1. Basic sequence alignment characteristics for *12S rRNA* and *16S rRNA* genes in 11 catfishes

Parameter	*12S rRNA*	*16S rRNA*
Number of species	11	11
Number of aligned sites	1200	577
Constant sites	679	466
Variable	516	104
Parsimony informative	247	80
Best fit model	GTR+G+I	GTR+G+I
Evolutionarily invariable (+I)	0.00	0.20
Gamma distribution (+G)	0.59	0.21

The length of large mitochondrial rRNA sequences ranged from 554 bp. in *Schilbe uranoscopus* to 564 bp. in *Malapterurus electricus*. The length of the aligned *16S rRNA*

data set comprised 577 nucleotides of which 466 were constant sites, 104 were variable, and 80 were parsimony informative (Table 1). The average genetic distance between the catfishes species based on *mt16S rRNA* sequence was 0.07. Both genes (*12S rRNA* and *16S rRNA*) showed nearly equal A+T composition (53.42 and 53.03, respectively). Additionally, both genes displayed A+T composition higher than the C+G. More details about nucleotide frequencies, A+T contents, pyrimidines contents and their averages of small and large subunit ribosomal RNA sequences in 11 catfishes are found in Tables (2, 3).

Table 2. Nucleotide frequencies, A+T contents, pyrimidines contents and their averages of small subunit ribosomal RNA (*12S rRNA*) sequence in 11 catfishes

12S Rrna							
		A	**T**	**C**	**G**	**A+T**	Pyrimidines **C+T**
1	832	32.33	22.72	24.40	20.55	55.05	47.12
2	885	32.32	22.71	25.54	19.44	55.03	48.25
3	1176	34.10	18.96	27.21	19.73	53.06	46.17
4	750	32.00	21.60	25.87	20.53	53.60	47.47
5	1191	31.57	19.56	29.14	19.73	51.13	48.70
6	1182	33.25	21.91	25.55	19.29	55.16	47.46
7	915	30.93	22.08	26.45	20.55	53.01	48.52
8	859	30.38	20.84	27.47	21.30	51.22	48.31
9	1166	33.28	20.33	26.50	19.90	53.60	46.83
10	1159	33.05	20.19	26.83	19.93	53.24	47.02
11	1107	33.60	20.23	26.65	19.51	53.84	46.88
Avg.	1020	32.54	20.88	26.60	19.98	53.42	47.48

Table 3. Nucleotide frequencies, A+T contents, pyrimidines contents and their averages of large subunit ribosomal RNA (*16S rRNA*) sequence in 11 catfishes

16S rRNA							
		A	**T**	**C**	**G**	**A+T**	Pyrimidines **C+T**
1	560	30.00	24.82	22.68	22.50	54.82	47.50
2	557	29.80	24.60	22.80	22.80	54.40	47.40
3	558	30.65	22.40	23.84	23.12	53.05	46.24
4	557	30.34	21.90	24.60	23.16	52.24	46.50
5	557	30.52	21.72	24.60	23.16	52.24	46.32
6	564	31.21	22.87	23.94	21.99	54.08	46.81
7	555	31.35	21.98	23.24	23.42	53.33	45.23
8	554	31.41	21.84	23.29	23.47	53.25	45.13
9	563	30.55	21.67	24.69	23.09	52.22	46.36
10	562	29.89	21.89	24.91	23.31	51.78	46.80
11	561	30.30	21.57	24.78	23.35	51.87	46.35
Avg.	559	30.55	22.48	23.94	23.03	53.03	46.42

Phylogenetic analysis

To carry out the phylogenetic analysis using (*12S rRNA* and *16S rRNA*) genes, sequencing of 11 catfishes were submitted to analysis, together with their 30 related catfishes species from GenBank/NCBI (Table 4).

The phylogenetic tree analysis using (*12S rRNA*) sequences shown that, species of the outgroup formed a separate cluster. All catfish families form two main clades; the first includes family Claroteidae while, the second contains the rest families; Bagridae, Clariidae, Malapteruridae, Schilbidae and Mochokidae. Within the second clade family Mochokidae formed a separated clade, and the rest families; Bagridae, Clariidae, Malapteruridae, Schilbidae were grouped together (Fig.1 a and b).

The phylogenetic tree analysis based on (*16S rRNA*) data revealed that, species of the outgroup formed a separate cluster. Also, family Bagridae formed a separate cluster. The rest catfish divided into two main clades; the first includes three species of family Claroteidae (*Chrysichthys sianenna*, *Chrysichthys grandis* and *Chrysichthys platycephalus*) and the second contains the rest species of family Claroteidae and families; Clariidae, Malapteruridae, Schilbidae and Mochokidae. Within the second clade Mochokidae and Clariidae families found in one clade, while the rest species of family Claroteidae and families Malapteruridae, Schilbidae were grouped together. Five species of family Claroteidae (*Chrysichthys nigrodigitatus*, *Chrysichthys brachynema*, *Chrysichthys* sp., *Chrysichthys rueppelli* and *Chrysichthys auratus*) found near to family Malapteruridae (Fig. 2 a and b).

Monophyly of catfish genera and families

The two trees generated (NJ and ME) using (*12S rRNA*) sequences showed that all 11 species and their related GenBank catfish species were grouped according to their genera and families. Bagridae (two species), Clariidae (eight species), Claroteidae (eight species), Malapteruridae (two species), Schilbidae (three species) and Mochokidae (15 species).

The results of (*16S rRNA*) sequences revealed that, all 11 species and their related GenBank catfish species were grouped according to their genera and families, except family Claroteidae displayed different taxonomic positions. Where, three species (*Chrysichthys sianenna*, *Chrysichthys grandis* and *Chrysichthys platycephalus*) formed a separated clade. While five species (*Chrysichthys nigrodigitatus*, *Chrysichthys brachynema*, *Chrysichthys* sp., *Chrysichthys rueppelli* and *Chrysichthys auratus*) found near to family Malapteruridae.

Table 4: The understudied eleven Catfishes with their related catfishes species in addition to the out-group species from the GenBank/NCBI based on small and large subunit ribosomal RNA sequences.

No.	Family	Species	Accession number	
			12S rRNA	*16S rRNA*
1	Bagridae	*Bagrus bajad*	OM976619.1	OM949995.1
2		*Bagrus docmak*	OM976620.1	OM949996.1
3	Clariidae	*Clarias gariepinus*	OM976621.1	OM949997.1
4		*Clarias sp.*	AP012010.1	AP012010.1
5		*Clarias gabonensis*	JX899749.1	JX899749.1
6		*Clarias batrachus*	KM259918.1	JQ699189.1
7		*Clarias fuscus*	KM029965.1	KM029965.1
8		*Clarias theodorae*	MN255575.1	MN255661.1
9		*Clarias dussumieri*	NC_037193.1	JQ699198.1
10		*Clarias macrocephalus*	NC_046749.1	NC_046749.1
11	Claroteidae	*Chrysichthys auratus*	OM976622.1	OM949998.1
12		*Chrysichthys rueppelli*	OM976623.1	OM949999.1
13		*Chrysichthys sp.*	AP012009.1	AP012009.1
14		*Chrysichthys brachynema*	MN255570.1	MN255656.1
15		*Chrysichthys grandis*	MN255571.1	MN255657.1
16		*Chrysichthys platycephalus*	MN255572.1	MN255658.1
17		*Chrysichthys sianenna*	MN255573.1	MN255659.1
18		*Chrysichthys nigrodigitatus*	NC_042721.1	NC_042721.1
19	Malapteruridae	*Malapterurus electricus*	OM976624.1	OM950000.1
20		*Malapterurus tanganyikaensis*	MN255598.1	MN255684.1
21	Schilbidae	*Schilbe mystus*	OM976625.1	OM950001.1
22		*Schilbe uranoscopus*	OM976626.1	OM950002.1
23		*Schilbe intermedius*	MN255629.1	MN255714.1
24	Mochokidae	*Synodontis batensoda*	OM976627.1	OM950003.1
25		*Synodontis clarias*	OM976628.1	OM950004.1
26		*Synodontis schall*	OM976629.1	OM950005.1
27		*Synodontis schoutedeni*	AP006767.1	AP006767.1
28		*Synodontis eupterus*	MT507647.1	MT508828.1
29		*Synodontis ilebrevis*	MN255631.1	MN255716.1
30		*Synodontis irsacae*	MN255633.1	MN255718.1
31		*Synodontis lucipinnis*	MN255634.1	MN255719.1
32		*Synodontis membranacea*	MH286808.1	MH286812.1
33		*Synodontis multipunctatus*	MN255635.1	MN255720.1
34		*Synodontis nigromaculata*	MN255636.1	MN255721.1
35		*Synodontis petricola*	MN255637.1	MN255722.1
36		*Synodontis polli*	MN255638.1	MN255723.1
37		*Synodontis sp.*	LC535220.1	LC535222.1
38		*Synodontis tanganyicae*	MN255632.1	MN255717.1
39	Out group	*Myoxocephalus jaok*	NC_045875.1	MN871873.1
40		*Myoxocephalus quadricornis*	MT303954.1	OM758114.1
41		*Myoxocephalus scorpius*	MT410889.1	KJ128840.1

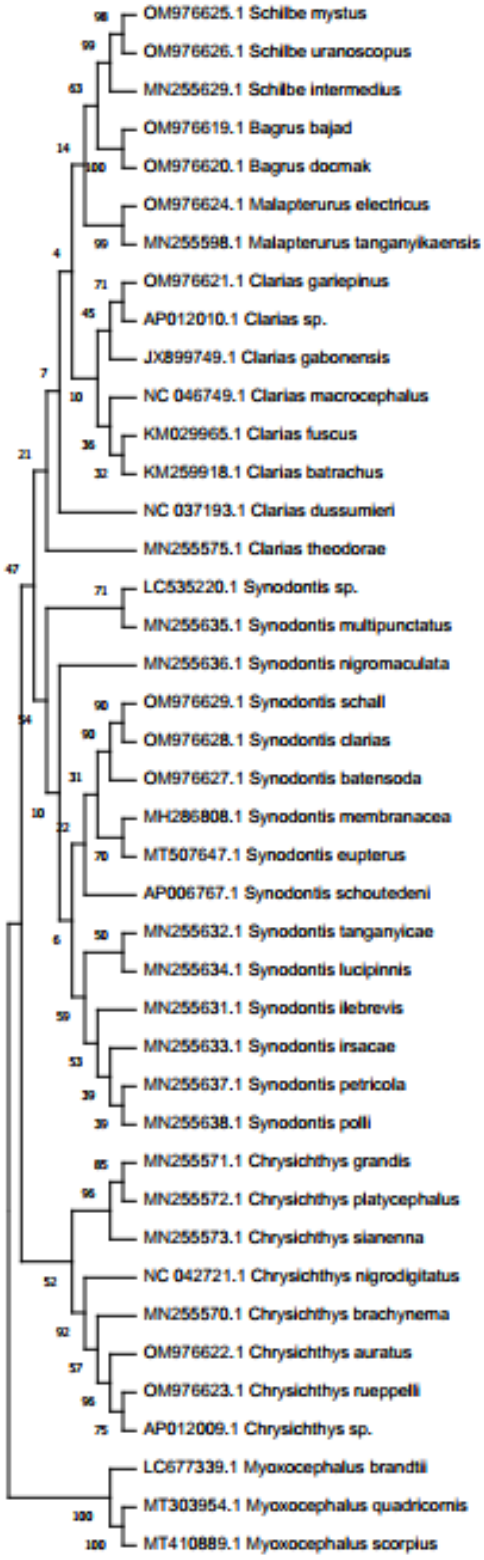

Fig.1a. Neighbour Joining phylogenetic tree among 11 catfishes and their related catfishes species, in addition, the outgroup using (*12S rRNA*) gene.

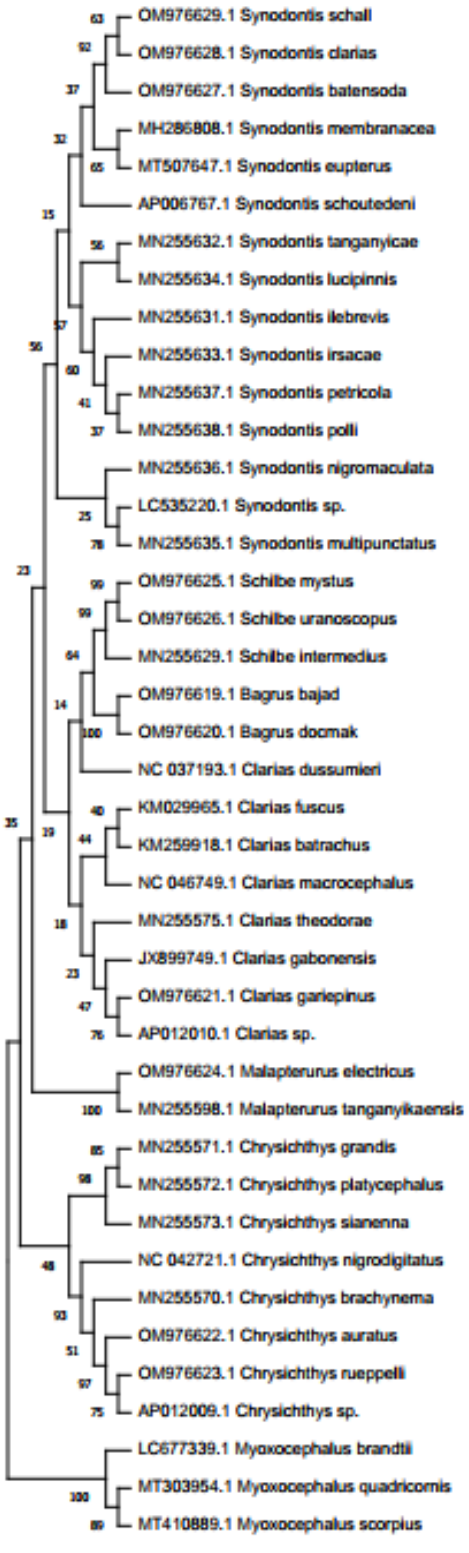

Fig.1b. Minimum Evolution phylogenetic tree among 11 catfishes and their related catfishes species, in addition, the outgroup using (*12S rRNA*) gene.

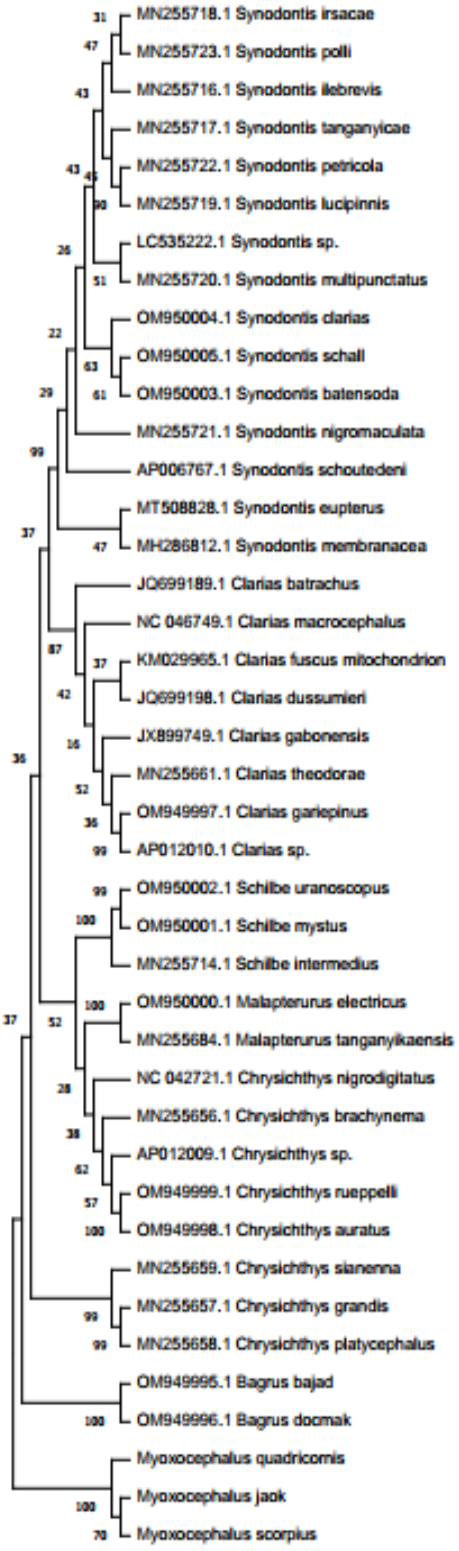

Fig.2a. Neighbour Joining phylogenetic tree among 11 catfishes and their related catfishes species, in addition, the outgroup using (*16S rRNA*) gene.

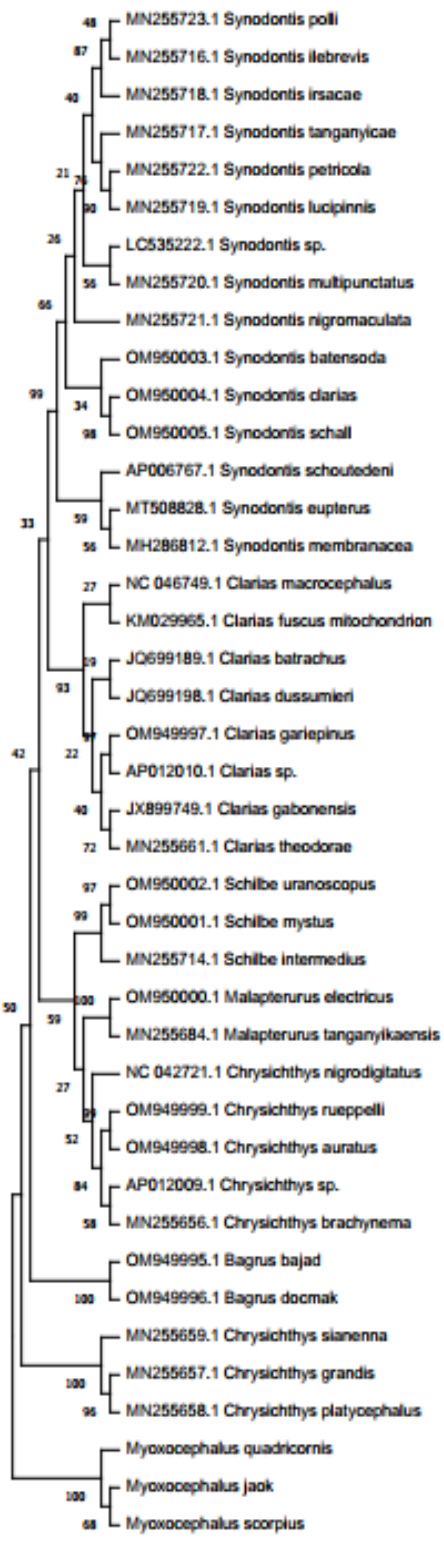

Fig.2b. Minimum Evolution phylogenetic tree among 11 catfishes and their related catfishes species, in addition, the outgroup using (*16S rRNA*) gene.

DISCUSSION

Our study of *12S rRNA* gene revealed high A+T composition than the C+G in all understudied species. This was in agreement with **(Norazila and Ismail, 2002; Sivaraman *et al.*, 2009 and Widayanti *et al.*, 2021)**.

The whole *16S rRNA* gene shows A+T richness, compared to GC **(Bo *et al.*, 2013)**. In this study, the composition of A+T was higher than the C+G in all understudied species. This was in corroboration with many studies **(Lakra *et al.*, 2009; Basheer *et al.*, 2015; Singh *et al.*, 2015 and Mar'ie and Allam, 2019)**.

This study corroborated the family monophyly of some Siluriformes families. **Peng *et al*. (2004)** was recovered bagrid catfishes form a monophyletic clade. This was agreed with our results where two species of family Bagridae (*Bagrus bajad* and *Bagrus docmak*) were shown as monophylectic clade.

The results of (*12S rRNA* and *16S rRNA*) sequences revealed that family Clariidae appeared as monophylectic clade. The monophyly of this family is confirmed by several authors **(Agnese and Teugels, 2005)** by using mitochondrial *cyt b* gene (**Sullivan *et al.*, 2006)** used nuclear genes *Rag1* and *Rag2* **(Pouyaud *et al.*, 2009)** based on *cyt b*, *16S rRNA* and 29 morphometric measurements and **Yu and Quilang (2014)** using mitochondrial and nuclear genes; *COI*, *Cyt b*, *16S rRNA*, *Rag1* and *Rag2*.

The (*12S rRNA*) sequences show family Claroteidae as a monophyletic family. While the data of (*16S rRNA*) sequences shown that family Claroteidae displayed different taxonomic positions. Where, three species of family Claroteidae (*Chrysichthys sianenna*, *Chrysichthys grandis* and *Chrysichthys platycephalus*) formed a separated clade. While five species (*Chrysichthys nigrodigitatus*, *Chrysichthys brachynema*, *Chrysichthys* sp., *Chrysichthys rueppelli* and *Chrysichthys auratus*) found near to family Malapteruridae.

The data of small and large mitochondrial rRNA sequences imply that family Schilbeidae appeared as monophylectic clade. **Vu Dang Ha *et al.* (2018)** applied two mitochondrial genes (*COI* and *16S rRNA*) to study the molecular phylogeny of some catfishes and reported that, at genus level, family Schilbeidae was well resolved as monophylectic clade.

The sequences analysis of the two mitochondrial genes revealed that each of Malapteruridae and Mochokidae families were monophylectic family.

Phylogenetic positions of families such as Siluridae, Schilbeidae, Malapteruridae, Bagridae, Mochokidae and Plotosidae remained undefined **(Hardman, 2005 and Sullivan *et al*., 2006)**.

The data of small mitochondrial rRNA (*12S rRNA*) sequences display Bagridae was closely related to Schilbeidae, this was in agreement with **(Sullivan *et al*., 2006 and Vu Dang Ha *et al.*, 2018)** who confirmed that Schilbeidae was mostly placed closed to Bagridae.

CONCLUSION

This study was achieved to estimate the phylogenetic relationships of Eleven catfish species belonging to six families using small and large mitochondrial rRNA sequences. *12S rRNA* and *16S rRNA* genes seem to be useful in exposing both monophyly and phylogenetic catfish families.

ETHICS STATEMENT

All animal experimental procedures were approved by the Ethics of Animal Experiments Committee of South Valley University, Faculty of Science (Permit No.: 004/11/22)

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