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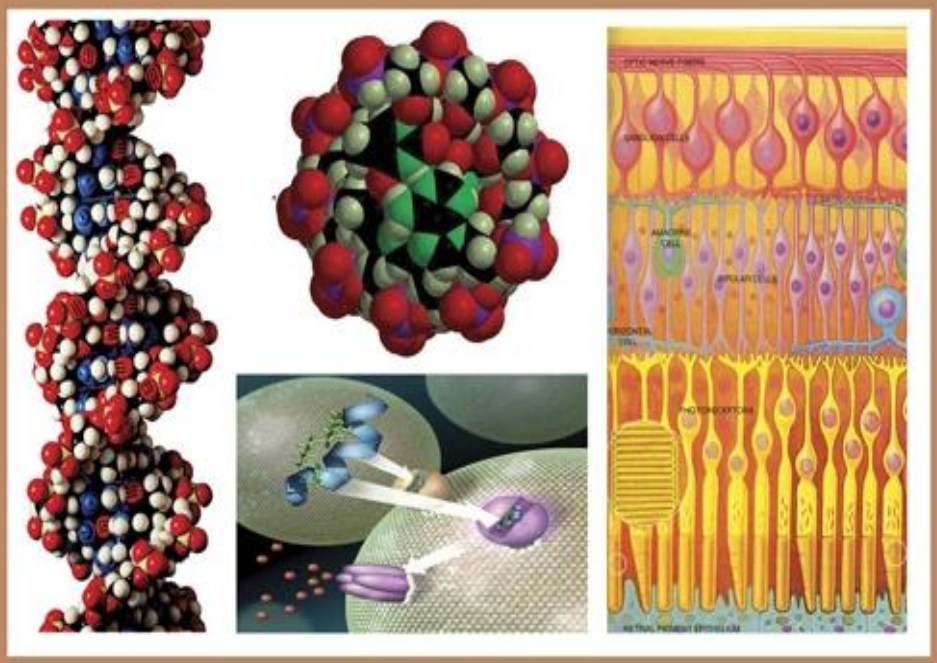

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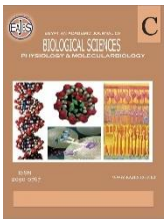

Molecular Phylogenetic Correlation Among Cichlid Fishes (Teleostei: Cichlidae) Based on 18S rRNA Gene Sequencing Analysis

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ABSTRACT

Cichlid fish phylogeny is presented for the most taxonomical approaches. In this study, the phylogeny of cichlid fish correlation was carried out by various analysis based on 18S rRNA gene sequences from GenBank database for 31 species belonging to 13 genera of Cichlid fish (Teleostei: Cichlidae). The alignment of 18S rRNA gene sequences as well as the neighbour-joining tree, distance matrix and phylogenetic tree obtained by using bioinformatics programs. Alignment of 18S rRNA gene sequences, distance matrix and phylogenetic tree results revealed that the majority of species within the same genus were closely related to each other (monophylogenetic) while, some species were polyphylogenetic within the genus showing a close relationship with other genera species. On the other hand, a neighbour-joining phylogenetic tree without a distance correction among cichlid species revealed a variation in phylogenetic relationship between species where most species within the same genus were polyphylogenetic to each other and monophylogenetic to other genera species.

INTRODUCTION

Cichlidae is the most prosperous family, recording 1700 species, belonging to 250 genera. Evolution, distribution and genetic markers of cichlid fishes have been recorded for most of these species in the inland fisheries of Africa (Snoeks *et al*., 2011). Cichlids represent striking examples of fish adaptive radiation, the phenomenon whereby a single phylogenetic lineage diversifies into many ecologically varied species in a short time, especially in eastern African great lakes (Dunz & Schliewen, 2013 and Genner & Turner, 2015). Biodiversity loss has been identified as a major global environmental issue and much attention has been focused on biodiversity conservation (Minelli, 2003). To overlap this problem, genetic data, specifically DNA sequences, has been proposed as a criterion in taxonomic identification (Blaxter, 2003; Tautz *et al*., 2003; Savolainen *et al*., 2005 and Azab *et al*., 2019).

DNA barcoding is a technique for identifying fish that involves the use of a particular gene or genes based on a comparison of a published species marker gene sequence with a reference database of such DNA sequences, which allows the species to be uniquely identified. In general, genetic barcodes are useful for defining unknown fish species, discriminating overlapped species, and determining species boundaries as compared to conventional morphological taxonomy. This molecular approach has been applied worldwide in the field of fish taxonomy due to the availability of facilities and the reduction of the cost of DNA barcoding manipulations (Hebert *et al*., 2003 a, b). Furthermore, improving bioinformatics approaches makes it easier to analyze barcode gene sequences, store them in an online DNA database, and retrieve them. As a result, even monomorphic fish species can now be identified, differentiated, and biogeographically distributed using the DNA barcode sequence data pool (Bhattacharjee *et al*., 2012 and Bhattacharya *et al*., 2016).

Sequence alignment is an inherent issue with using rRNA as barcodes (Lutzoni *et al*., 2000). Since base insertions and deletions are common in rRNA sequences, each sequence with them must be given gaps in order to fit with the others. Since there are no universal alignment criteria, assigning gaps to DNA sequences is arbitrary (Geiger, 2002). As a consequence, even when the alignment process is carried out meticulously by experienced researchers, human errors can occur, particularly in some rRNA sequences for which no closely related sequences are available to serve as a guide. Apart from the complexity inherent in multiple sequence alignment, this procedure must often be repeated if a new sequence (taxon) is added to a dataset prior to analysis. Every year, 200000 barcode records are expected to be added to the database (Hajibabaei *et al*., 2005). Series alignment in the barcode project will become repetitive and time-consuming with such a large dataset.

The multigene families of ribosomal RNA (rRNA) are divided into two groups that are tandemly arrayed in eukaryotic genomes. An external transcribed spacer precedes the transcribing regions of the 18S, 5.8S, and 25S/28S rRNAs, which are separated from one another by two internal transcribed spacers (ITS), ITS1 and ITS2. Multiple copies of a strongly conserved 120-bp transcribing region are isolated by a variable non-transcribed region in the minor class (5S rRNA genes) (NTS) (Eickbush, 2007). Fish cytogenetics is a burgeoning field of study that provides data for taxonomy and the study of phylogenetic relationships among taxa (Carvalho *et al*., 2017; Ferreira *et al*., 2017 and Nirchio *et al*., 2018). Other details on the karyotype include the mapping of 45S or 5S rDNA or the classification of heterochromatin patterns indeed, the sum and distribution of these groups of repetitive sequences that characterize different genomic organization has been linked to neotropical cichlid karyotypic evolution (Feldberg *et al*., 2003 and Poletto *et al*., 2010).

A simple correlation analysis based on 18S rRNA gene sequences from GenBank database for 31 species belonging to 13 genera of Cichlid fish (Teleostei: Cichlidae) is the main purpose of current research. Alignment of 18S rRNA gene sequences, distance matrix and phylogenetic tree may be used as convenient and accurate DNA barcodes for different species.

MATERIALS AND METHODS

The ribosomal RNA (18S rRNA) gene sequences of 31 species belonging to 13 genera of Cichlid fish (Teleostei: Cichlidae) were downloaded from the GenBank database. Partial sequences of 18S rRNA gene from five published rRNA datasets (Booton and Fuerst, 2001; Rodgers *et al*., 2003; Nevado *et al*., 2009; Hardy, 2014 and Ramos *et al*., 2016) were downloaded from GenBank for analysis (Table 1). An unpublished dataset of partial 18S rRNA sequences from 8 cichlid fish species was also included in the analysis.

Clustal Omega is a new multiple sequence alignment program that uses

seeded guide trees and HMM profile-profile techniques to generate alignments between three or more sequences incorporated the common approaches of phylogenetic reconstruction, including neighbor-joining (NJ), maximum parsimony (MJ) and maximum likelihood (ML). The Alignment of 18S rRNA gene sequences as well as the neighbour-joining tree without distance corrections was obtained by using Clustal Omega- Multiple Sequence Alignment **(Sievers** and Higgins, 2018 and Sievers *et al*., 2020). Whereas, the distance matrix and Graphical Phylogenetic Tree with bootstrap values (Topological Algorism) were analysed for 18S rRNA sequences by using GeneBee ClustalW 1.83 (ClustalW with character counts) (Larkin *et al*., 2007).

Table 1. List of Cichlid species, Abbreviations, source references, sequence information and Genbank ACCESSION No. of the 31 studied datasets

Cichlid species	Abbreviation	Reference	rRNA gene	Aligned sequence length (bp)	GenBank ACCESSION No.
Amatitlania nigro fasciata	A. nigrofasciata	Unpublished	18S	1799	KJ774642
Andinoacara pulcher	A. pulcher	Unpublished	18S	1799	KJ774635
Astatotilapialati fasciata	A. latifasciata	Ramos *et al*., 2016	18S	767	KX226400
Geophagus sp. CMH-2014	Geophagus	Unpublished	18S	1800	KJ774680
Haplochromis burtoni	H. burtoni	Unpublished	18S	974	XM_005929941
Lamprologus lemairii	L.lemairii	*Nevado et al., 2009*	18S	116	FJ706346
Lamprologus ocellatus	L.ocellatus	*Nevadoe t al., 2009*	18S	116	FJ706337
Lamprologus ornatipinnis	L.ornatipinnis	*Nevado et al., 2009*	18S	116	FJ706334
Lamprologus signatus	L.signatus	*Nevado et al., 2009*	18S	116	FJ706332
Lamprologus callipterus	L.callipterus	*Nevado et al., 2009*	18S	116	FJ706327
Lepidiolamprologus profundicola	L.profundicola	*Nevado et al., 2009*	18S	116	FJ706347
Lepidiolamprologus elongatus	L.elongatus	*Nevado et al., 2009*	18S	116	FJ706345
Lepidiolamprologus cunningtoni	L.cunningtoni	*Nevado et al., 2009*	18S	116	FJ706344
Lepidiolamprologus attenuatus	L.attenuatus	*Nevado et al., 2009*	18S	116	FJ706340
Maylandia zebra	M. zebra	*Unpublished*	18S	1826	XR_003024145
Maylandia zebra	M. zebra	*Unpublished*	18S	1841	XR_003023994
Neolamprologus leloupi	N. leloupi	*Nevado et al., 2009*	18S	116	FJ706348
Neolamprologus savoryi	N. savoryi	*Nevado et al., 2009*	18S	116	FJ706342
Neolamprologus tetracanthus	N. tetracanthus	*Nevado et al., 2009*	18S	116	FJ706338
Neolamprologus multifasciatus	N. multifasciatus	*Nevado et al., 2009*	18S	116	FJ706335
Neolamprologus calliurus	N. calliurus	*Nevado et al., 2009*	18S	116	FJ706329
Neolamprologus multifasciatus	N. multifasciatus	*Nevado et al., 2009*	18S	116	FJ706328
Neolamprologus fasciatus	N. fasciatus	*Nevado et al., 2009*	18S	116	FJ706308
Neolamprologus similis	N. similis	*Nevadoet al., 2009*	18S	116	FJ706305
Oreochromis aureus	O. aureus	Unpublished	18S	1839	XR_005609725
Oreochromis niloticus	O. niloticus	Unpublished	18S	1841	XR_003216134
Oreochromis mossambicus	O. mossambicus	Rodgers et al., 2003	18S	1085	AF497908
Oreochromis esculentus	O. esculentus	Boot and Fuerst, 2001	18S	1780	AF337051
Pelmatolapia mariae	P. mariae	Hardy, 2014	18S	1691	KJ774766
Rocio octofasciata	R. octofasciata	Hardy, 2014	18S	1813	KJ774653
Variabilichromis moorii	V. moorii	Nevado *et al*., 2009	18S	116	FJ706300

RESULTS

Alignment of 18S rRNA gene sequences of 31 species belonging to 13 genera of Cichlid fish revealed that the species related to the same genus are monophylogenetic. While the species related to different genera are polyphylogenetic (Figs. 1 and 2).

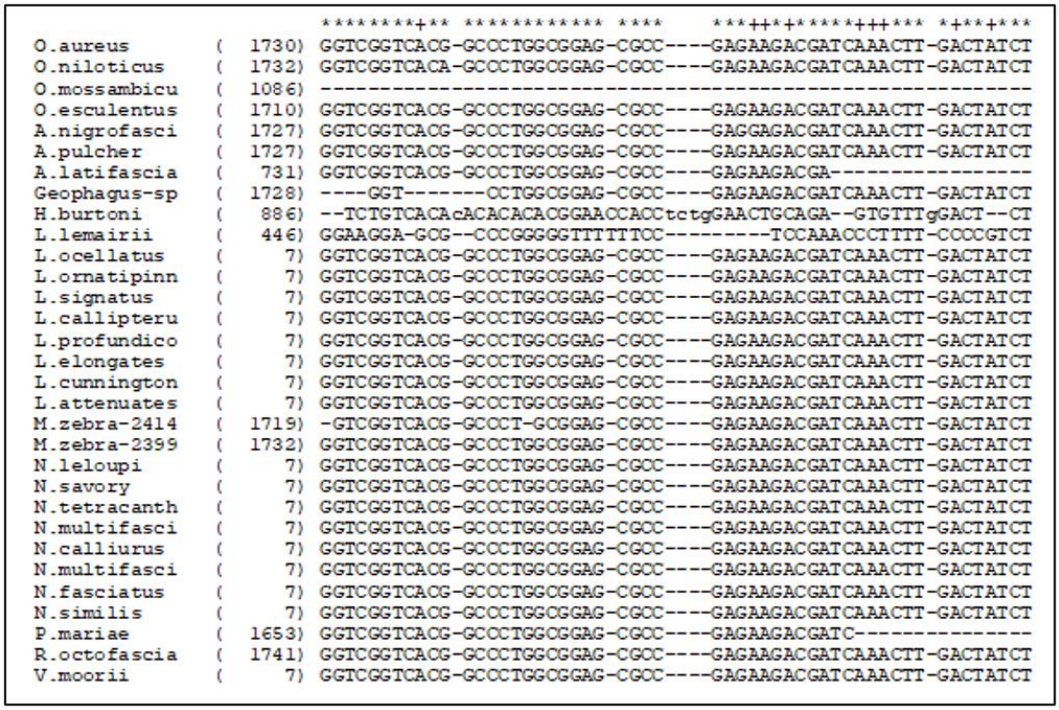

Fig. 1: First variable region of Aligned partial sequences of 18S rRNA gene among the investigated cichlid fishes.

' ' - the average weight of column pair exchanges is less than the weight matrix mean value

'.' - is less than mean value plus one SD

'+' - is less than mean value plus two SD

'*' - is more than mean value plus two SD

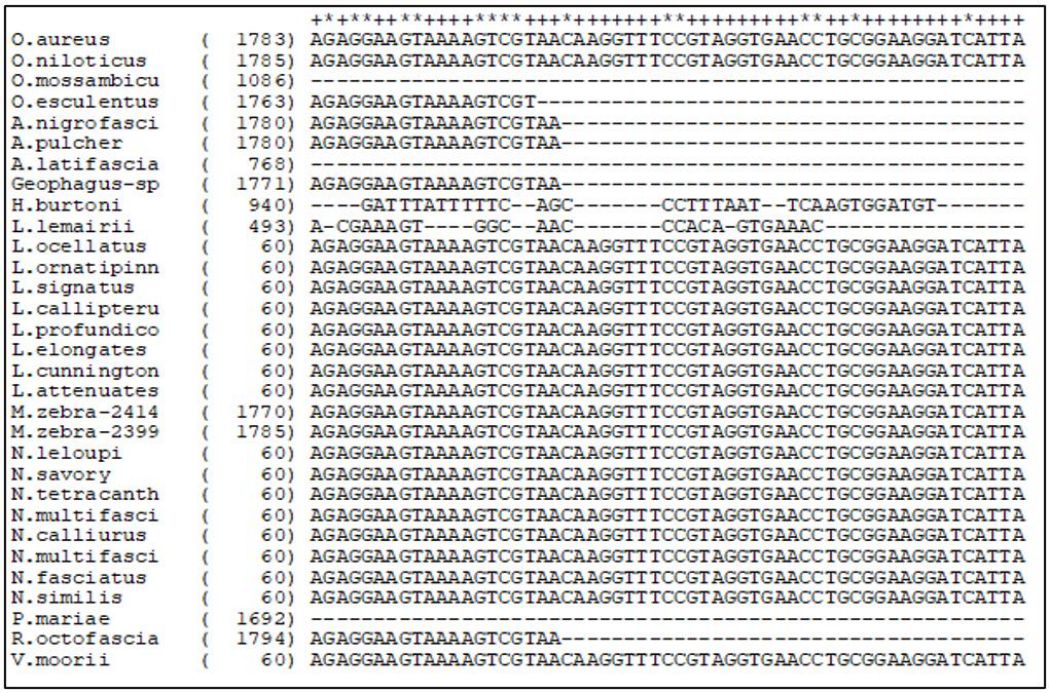

Fig. 2: Second variable region of Aligned partial sequences of 18S rRNA gene among the investigated cichlid fishes.

' ' - the average weight of column pair exchanges is less than the weight matrix mean value

'.' - is less than mean value plus one SD

'+' - is less than mean value plus two SD

'*' - is more than mean value plus two SD

Table (2) and Fig (3) represented the results of a distance matrix and phylogenetic tree with bootstrap values (Topological Algorism) based on the alignment of 18S rRNA gene sequences of cichlid fishes. A closely related species of genus *Maylandia* (*M. zebra*) are monophylogenetic with a distance of 0.144. In the meantime, the phylogeny of genus *Neolamprologus* (*N. leloupi, N. savoryi, N. tetracanthus, N. multifasciatus, N. calliurus, N. multifasciatus, N. fasciatus* and *N. similis)* proved that all species were closely related to each other. On the other hand, the species related to genus *Oreochromis* (O. *aureus, O. niloticus,* and *O. esculentus*) are monophylogenetic to each other apart from O. *mossambicus* was in a distance about 0.529 from other species of the same genus. Similar results were recorded for genus *Lepidiolamprologus* (*L. attenuatus, L. profundicola, L. elongatus* and *L. cunningtoni*) where *L. attenuatus* is polyphylogenetic with other grouped monophylogenetic species. The phylogeny of genus Lamprologus *(L. ocellatus, L. ornatipinnis, L. signatus, L. callipterus*) represented monophylogenetic relationship between the species except *L. lemairii* was in a distance with others.

Polyphylogenetic relationship with varied distance matrix was recorded between different genera where genus *Andinoacara* (*A. pulcher*) was found in a distance of 0.253 with genus *Amatitlania* (*A. nigrofasciata*) and distance of 0.211 with genus *Geophagus* (*Geophagus sp*.) indicated that these genera were relatively closed. While the distance with genus *Astatotilapia* (*A. atifasciata*) was 0.877 indicated the polypgylogenetic relationship between two genera. A similar relationship with a distance of 0.326 was recorded between genera *Pelmatolapia* (*P. mariae*) and Rocio (*R. octofasciata*).

Generally, the species within the same genus were monophylogenetic, while the species from different genera were found to be polyphylogenetic as represented in current results. These data were in contrast to that recorded by the neighbour-joining phylogenetic tree without a distance correction based on alignment of 18S rRNA gene sequences among cichlid species which revealed a great confusion in the phylogenetic relationship between species where some species were polyphylogenetic within the same genus and monophylogenetic with other genera (Fig. 4).

Table 2. Matrix of genetic distances based on alignment of 18S rRNA gene sequences of the studied species.

	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
1 O.aureus	0.000																														
2 O.niloticus	0.153	0.000																													
3 O.mossambicus	0.529	0.564	0.000																												
4 O.esculentus	0.182	0.218	0.520	0.000																											
5 A.nigrofasci	0.292	0.327	0.525	0.280	0.000																										
6 A.pulcher	0.268	0.303	0.531	0.255	0.253	0.000																									
7 A.latifascia	0.883	0.891	0.870	0.872	0.880	0.877	0.000																								
8 Geophagus-sp	0.280	0.312	0.528	0.267	0.245	0.211	0.874	0.000																							
9 H.burtoni	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000																						
10 L.lemairii	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000																					
11 L.ocellatus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000																				
12 L.ornatipinn	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000																			
13 L.signatus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000																		
14 L.callipteru	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000																	
15 L.profundico	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000																
16 L.elongates	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000															
17 L.cunnington	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000														
18 L.attenuates	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000													
19 M.zebra-2414	0.137	0.173	0.549	0.202	0.311	0.289	0.891	0.295	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000												
20 M.zebra-2399	0.124	0.160	0.544	0.189	0.299	0.275	0.887	0.287	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.144	0.000											
21 N.leloupi	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000										
22 N.savory	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000									
23 N.tetracanth	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000								
24 N.multifasci	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000							
25 N.calliurus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000						
26 N.multifasci	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000					
27 N.fasciatus	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
28 N.similis	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
29 P.mariae	0.283	0.319	0.474	0.261	0.321	0.312	0.847	0.324	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.303	0.290	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000		
30 R.octofascia	0.282	0.316	0.537	0.269	0.287	0.242	0.883	0.234	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.301	0.289	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.326	0.000	
31 V.moorii	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000

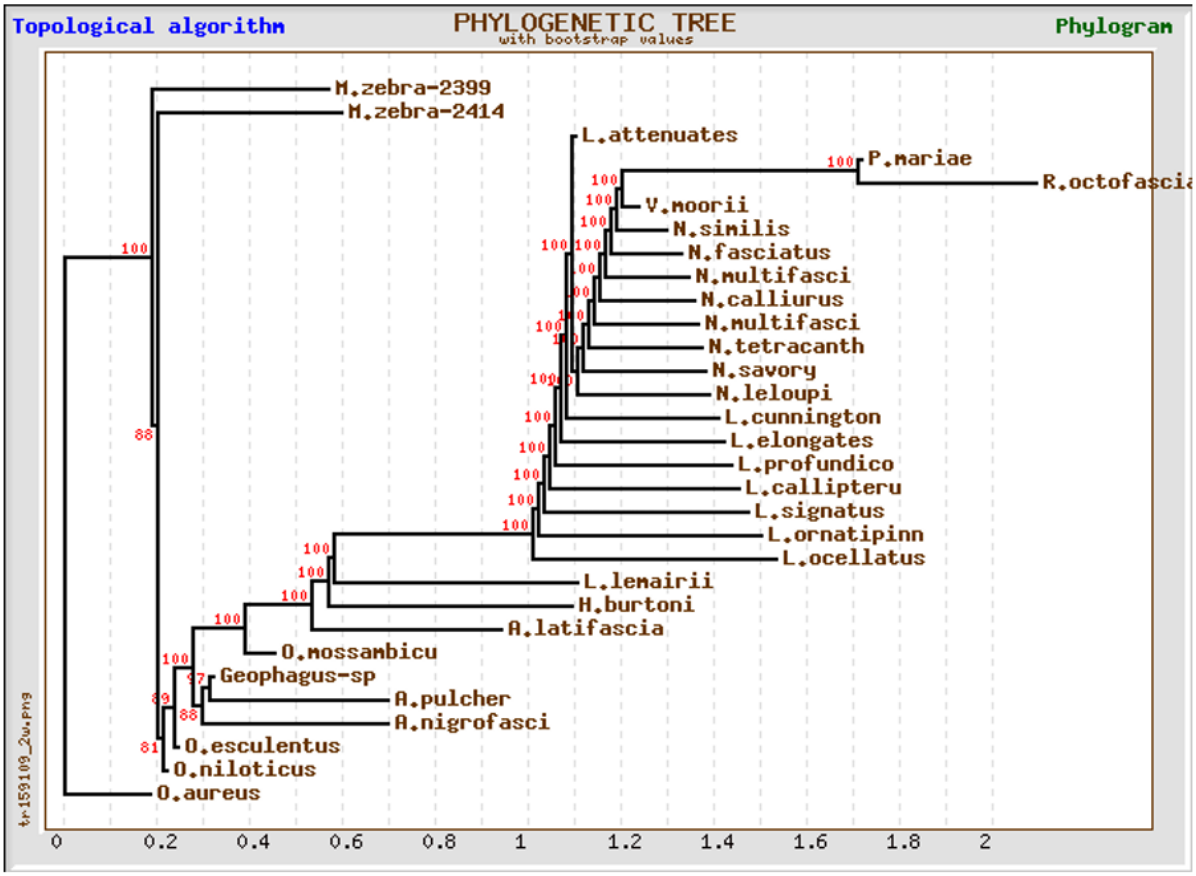

Fig. 3: Phylogenetic tree with bootstrap values (Topological Algorism) based on alignment of 18S rRNA gene sequences among the investigated cichlid fishes.

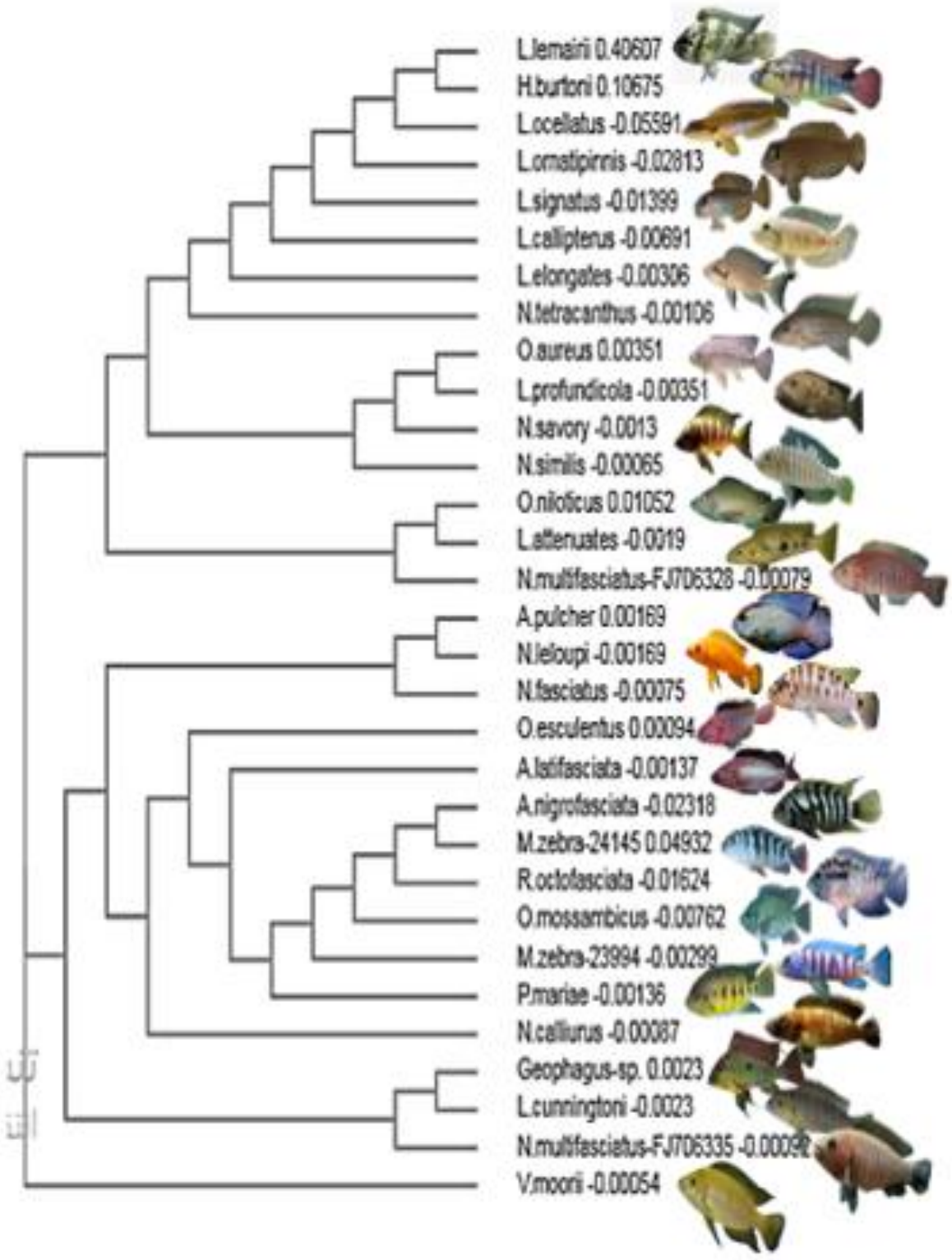

Fig. 4: Neighbour-joining phylogenetic tree without a distance correction based on alignment of 18S rRNA gene sequences among the investigated cichlid fishes.

DISCUSSION

Comprehensive phylogenetic analysis of the cichlid fish using multi-marker molecular datasets comprising nuclear and mitochondrial loci revealed high levels of incongruence between them (Elserafy *et al*., 2007; Genner and Turner, 2012; Willis *et al*., 2013; Meier *et al.,* 2017 and Ford *et al*., 2019). The 18S rRNA gene is considered as evidence of significantly different phylogeny in higher organisms (Elserafy *et al*., 2007 and Nirchio, *et. al*., 2020).

The current alignment of 18S rRNA gene sequences of 31 species belonging to 13 genera of Cichlid fish revealed that the species related to the same genus were monophylogenetic, while the species from different genera were found to be polyphylogenetic. These results were Compatible with Shull *et al.* (2001), who discovered the phylogenetic relationships of 36 adephagan species and 13 outgroup species depend on alignment of 18S rRNA sequences. Furthermore, Marescalchi (2005) proved that molecular data demonstrated the Andinoacara Rivulatus (Cichlidae: Cichlasomatini) defined within the genus as a monophyletic group.

Our analysis of the distance matrix and phylogenetic tree based on alignment of 18S rRNA gene sequences of cichlid fishes proved that species of genus *Maylandia* are monophylogenetic. Conversely, some species of genus *Oreochromis* are monophylogenetic to each other apart from O. *mossambicus* was polyphytogenetic with other species of the same genus. These results resembled that found by Poletto *et al.*, (2010), who detected a variable number of clusters among species (one Asian, 22 African, and 30 South American cichlid species) based on the genetic mapping of 18S ribosomal RNA genes.

Chu *et al*., (2006) used 18S ribosomal RNA datasets from a wide variety of organisms (from archaea to tetrapods) at taxonomic levels ranging from class to species. His suggestion was in agreement with our results where a phylogenetic relationship with varied distance matrix was recorded between different genera i.e., genus *Andinoacara* was found in a distance of 0.253 with genus *Amatitlania* and distance of 0.211 with genus *Geophagus* indicated that these genera were relatively closed. While the distance with genus *Astatotilapia* was 0.877 indicated the polyphylogenetic relationship between two genera.

The present data recorded by the neighbor-joining phylogenetic tree without a distance correction based on alignment of 18S rRNA gene sequences among cichlid species revealed a great confusion in phylogenetic relationship between species where some species were polyphylogenetic within the same genus and monophylogenetic with other genera. Heeg and Wolf (2015) reviewed the analysis using primary sequences simultaneously in inferring neighbor-joining, maximum parsimony and maximum likelihood trees, with increasing robustness and accuracy of reconstructed phylogenies. It was concluded that neighbor-joining and maximum parsimony analyses failed in inferring a robust phylogenetic tree, while the maximum likelihood tree provides a supported phylogeny.

In conclusion, alignment of 18S rRNA gene sequences among cichlid species as well as phylogenetic tree with bootstrap values revealed a great accuracy in phylogenetic relationship among species.

REFERENCES

Azab, M. S.; Zowail, M. E. M.; Nassif, S. A.; Elsadek G. M.; Mohamed S. Z. (2019): Sequencing and Phylogenetic Analysis of the Salmonella Enterotoxin (stn) Gene of Salmonella spp. Isolated from Egyptian Broiler Breeder Chickens Farms. *Egyptian Academic Journal of Biological Sciences C. Physiology & Molecular Biology,* 11(3): 139-147.

Bhattacharjee, M. J; Laskar, B.A.; Dhar, B. and Ghosh SK. (2012): Identification and reevaluation of freshwater catfishes through DNA barcoding. *Plos One* 7. 7(11): e49950.

Bhattacharya, M.; Sharma, A.R.; Patra, B.C.; Sharma, G.; Seo, E.M.; Nam, J.S.,

Chakraborty, C., and Lee, S.S. (2016): DNA barcoding to fishes: current status and future directions. *Mitochondrial DNA* Part A 27, 2744–2752.

Blaxter, M. (2003): Counting angels with DNA. *Nature,* 421, 122–123.

Booton, G. C. and Fuerst, P.A. (2001): Direct Submission J. Molecular Genetics, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210, USA.

Carvalho, P.C.; de Oliveira E.A.; Bertollo, L.A.C; Yano, C.F.; Oliveira, C.; Decru, E. et al. (2017): First chromosomal analysis in Hepsetidae (Actinopterygii, Characiformes): insights into relationship between African and Neotropical Fish Groups. *Frontiers in Genetics* , 8:203.

Chu, K. H.; Li, C. P. and Qi, J. (2006): Ribosomal RNA as molecular barcodes: a simple correlation analysis without sequence alignment. *Bioinformatics,* 22, (14): 1690 –1701 doi:10.1093/ bioinformatics/btl146.

Dunz, A.R. and Schliewen, U. K . (2013): Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as "*Tilapia*". *Molecular Phylogenetics and Evolution*, 68(1): 64-80.

Eickbush, T. H. and Eickbush, D.G. (2007): Finely orchestrated movements: evolution of the ribosomal RNA genes. *Genetics*, 175:477–485.

Elserafy, S. S.; Abdel-Hameid, N. H.; Awwad, M. H. and Azab, M. S. (2007): DNA riboprinting analysis of Tilapia species and their hybrids using restriction fragment length polymorphisms of the small subunit ribosomal DNA. *Aquaculture Research*, 38(3):295 - 303.

Feldberg, E.; Porto, J.I.R. and Bertollo, L.A.C. (2003): Chromosomal changes and adaptation of cichlid fishes during evolution. *Fish Adaptation*; 285:308.

Ferreira, M.; Garcia, C.; Matoso, D.A.; de Jesus I.S., Cioffi M de B, Bertollo LAC, et al (2017): The *Bunocephalus coracoideus* species complex (Siluriformes, Aspredinidae). signs of a speciation process through chromosomal, genetic and ecological diversity. *Frontiers in Genetics*; 8:120.

Ford, A. G. P.; Bullen, T. R.; Pang L.; Genner M. J.; Bills R; Flouria T; Ngatunga B. P. and Rübere L.; *et al.* (2019): Molecular phylogeny of Oreochromis (Cichlidae: Oreochromini) reveals mito-nuclear discordance and multiple colonisations of adverse aquatic environments. *Molecular Phylogenetics and Evolution,* 136: 215-226.

Geiger, D.L. (2002): Stretch coding and block coding: two new strategies to represent questionably aligned DNA sequences. *Journal of Molecular Evolution*. 54, 191–199.

Genner, M. J. and Turner G. F. (2015) Timing of population expansions within the Lake Malawi haplochromini cichlid fish radiation. *Hydrobiologia* 748: 121–132.

Genner, M. J. and Turner, G. F. 2012. Ancient hybridization and phenotypic novelty in Lake Malawi's cichlid fish radiation. *Molecular Biology and Evolution* 29: 195–206.

Hajibabaei , M. ; deWaard, J.R.; Ivanova, N.V; Ratnasingham, S.; Dooh R. T ; Kirk ,S. L ; Mackie P. M and Hebert P. D. N. (2005): Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B: Biological Sciences*.360, 1959–1967.

Hardy, C.M. (2014): DNA barcoding the freshwater fishes of southern Australia. CSIRO, Clunies Ross. CSIRO, Canberra, ACT 2601, Australia.

Hebert, P.D.; Cywinska A.; Ball, Sh. L. and deWaard J. R. (2003a): Biological identifications through DNA barcodes. *Proceeding Biological Science,* 270, 313–321.

Hebert, P.D.; Ratnasingham S. and deWaard J. R. (2003b): Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceeding Biological Science.*, 270 (Suppl. 1), S96–S99.

Heeg, J. S. and Wolf, M. (2015): ITS2 and 18S rDNA sequence-structure phylogeny of Chlorella and allies (Chlorophyta, Trebouxiophyceae, Chlorellaceae). *Plant Gene*, 4: 20-28.

Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; McGettigan, P.A.; McWilliam, H.; Valentin, F.; Wallace, I.M., Wilm, A.; Lopez, R.; Thompson, J.D.; Gibson, T.J. and Higgins, D.G. (2007): Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947-2948.

Lutzoni, F.; Wagner, P.; Reeb, V. and Zoller, S. (2000): Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology*, 49, 628–651.

Marescalchi O. 2005. Karyotype and mitochondrial 16S gene characterizations in seven South American Cichlasomatini species (Perciformes, Cichlidae). *Journal of Zoological System*, 43:22–28.

Meier, J. I.; Marques, D. A.; Mwaiko, S.; Wagner, C. E.; Excoffier, L. and Seehausen, O. (2017): Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nature Communications*, 8 :14363.

Minelli, A. (2003) The status of taxonomic literature. *Trends in Ecology & Evolution*. 18, 75–76.

Nevado, B.; Koblmuller, S.; Sturmbauer, C.; Snoeks, J.; Usano-Alemany, J. and Verheyen, E. (2009): Complete mitochondrial DNA replacement in a Lake Tanganyika cichlid fish *Journal Molecular Ecology*, 18 (20), 4240-4255.

Nirchio, M.; Paim F. G.; Britzke R.; Rossi A. R.; Milana, V. and Oliveira, C. (2020): Molecular Analysis and Chromosome Mapping of Repetitive DNAs in the Green Terror *Andinoacara rivulatus* (Cichlidae: Cichlasomatini) *Zebra Fish*. 17(1): P38-47.

Nirchio, M.; Paim, F.G.; Milana, V.; Rossi AR, Oliveira C. (2018) Identification of a new mullet species complex based on an integrative molecular and cytogenetic investigation of Mugil hospes (Mugilidae: Mugiliformes). *Frontiers in Genetics*;9:17.

Poletto, A.B.; Ferreira, I.A., Cabral-de-Mello, D.C.; Nakajima, R.T.; Mazzuchelli, J.; Ribeiro, H.B. et al. (2010) Chromosome differentiation patterns during cichlid fish evolution. *BMC Genetics*; 11:1–12.

Ramos, E.; Cardoso, A.L.; Brown, J.; Marques, D.F.; Fantinatti, B.E., Cabral-de-Mello, D.C.; Oliveira, R.A.; O'Neill, R.J. and Martins, C. (2016): The repetitive DNA element Bnc DNA, enriched in the B chromosome of the cichlid fish *Astatotilapia latifasciata*, transcribes a potentially noncoding RNA. *Journal of Chromosoma*. 126:313–323.

Rodgers, B.D.; Weber, G.M.; Kelley, K.M. and Levine, M.A. (2003): Prolonged fasting and cortisol reduce myostatin mRNA levels in tilapia larvae; short-term fasting elevates *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 284 (5), R1277-R1286.

Savolainen,V.; Cowan, R. S.; Vogler, A. P.; Roderick, G. K. and Lane, R. (2005): Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Science*, 360, 1805–1811.

Shull, V.L.; Vogler, A .P. ; Baker, M. D.; Maddison D. R. and Hammond, P. M (2001): Sequence alignment of 18S ribosomal RNA and the basal relationships of Adephagan beetles: evidence for monophyly of aquatic families and the placement of Trachypachidae. *Systematic Biology*,

50, 945–969.

Sievers, F. and Higgins, D.G. (2018): Clustal Omega for making accurate alignments of many protein sequences. *Protein Science,* 27:135-145.

Sievers, F.; Barton, G.J. and Higgins, D.G. (2020): Multiple Sequence Alignment. Bioinformatics 227, pp 227-250, AD Baxevanis, GD Bader, DS Wishart (Eds).

Snoeks, J.; Harrison, I. and Stiassny, M. (2011): The status and distribution of freshwater fishes. In: Darwall, W., Allen, D., Holland, R., Harrison, I. & Brooks, E. (Eds), The diversity of life in African freshwaters: under water, under threat. An analysis of the status and distribution of freshwater species throughout mainland Africa. IUCN, Cambridge and Gland, pp. 42–91.

Tautz, D.; Arctander, P.; Minelli, A., Thomas R.H. and Vogler, A. P. (2003): A plea for DNA taxonomy. *Trends in Ecology & Evolution*, 18, 70–74.

Willis, S.C.; Farias, I.P. and Ortí, G. (2013): Testing mitochondrial capture and deep coalescence 851 in Amazonian cichlid fishes (Cichlidae: Cichla). *Evolution,* 68:256–68. 852 https://doi.org/10.1111/evo.12230.