

STUDY OF OPERATIONAL TAXONOMIC UNITS IN *CLARIAS SPP.* IN COI GENE.

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ABSTRACT

The *Clarias spp* being air breathing have got their greater evolutionary significance hence in terms of molecular taxonomy. Four species of the genus placed themselves closest to each other on world wide data base NCBI on blast of one of the COI gene sequence. COI based study although is proposed to be an effective tool for identification of species, however appears to have failed in this case. The use of one more additional nuclear gene has been suggested in the present study.

KEYWORDS: air breathing, BLAST, COI, molecular taxonomy, NCBI.

INTRODUCTION

Clarias spp are called walking catfishes because of their ability to walk on land and breathe in air. The word *Clarias* has been derived from Greek word Chlaros which means lively. Presence of accessory breathing organ enables this species to breath air when very active or under dry conditions. Fishes belonging to genus *Clarias* are fresh and brackish water fish found demersal in habitat (Riede, 2004).

The species of the genus are found in Africa and some parts of Asia (Nelson 1994, Teugels *et al.*, 2001). Several countries, and have reported adverse ecological impact of the species after introduction and even in Hingoli District (Yede *et al.*, 2016) by one of the species *Clarias gariepinus*. There are 61 species of fishes belonging the genus have been recorded by fish base. It is the most diverse group of the family Clariidae (Ng *et al.*, 2011). The fishes are diagnosed by the unique structure of the suprabranchial organ, which are formed by extensions of the second and fourth epibranchials (Teugels and Adriaens 2003). Elongated body, long dorsal and anal fins, absence of adipose fin, jaws bearing a band of villiform teeth and a band of villiform or granular teeth across the vomer, small eyes with a free orbital margin, upper and lateral parts of the head osseous, or covered with only a very thin skin, 4 pairs of circumorbital barbells, ventral fins 6-rayed, and only pectoral fins possess a spine are the other characters possessed by the individuals belonging to genus *Clarias* (Gunther 1864).

The adult fishes occur in standing stagnant waters are preferring shallow muddy areas to live in (Teugels, 1986). They can also occur in fast flowing water of rivers (Seegers, 2008). Their colour varies according to turbidity of water. The fishes of the genus are widely tolerant extreme environment (water) (de Moor and Bruton, 1988). The taxa belonging to this genus have been extensively studied from Africa however their study in Asia is limited.

DNA barcoding is proposed to be a novel system designed to provide rapid, accurate and automatable species identifications by using short, standardized gene regions as internal species tags (Herbert and Gregory, 2005). Many successful attempts in DNA barcoding have been done by Hebert *et al.* (2002); Ward *et al.* (2005), Hubert *et al.* (2008), Wong and Hanner (2008), and in India by Khedkar *et al.* (2014); Lakra *et al.* (2015).

MATERIALS AND METHODS

In the present study mutations occurring in four species of *Clarias* genus have been related to their morphological taxonomy. The study has been part of DNA barcoding and emphasis on the mechanism of morphological taxonomy working with molecular taxonomy at deeper nucleotide levels. The gene bank accession numbers of the fish names have been noted below:

Table 1: The genebank accession numbers and the names of *Clarias spp.*

No	Name of fish	Gene bank accession number
1	<i>Clarias macrocephalus</i>	JF292324.1
2	<i>C. macrocephalus</i>	JF292336.1
3	<i>C. macrocephalus</i>	JF292337.1
4	<i>C. bartachus</i>	MF769378.1
5	<i>C. bartachus</i>	KF604646.1
6	<i>C. bartachus</i>	JQ667518.1
7	<i>C. fuscus</i>	KF011504.1
8	<i>C. fuscus</i>	KF011505.1
9	<i>C. fuscus</i>	KM029965.1
10	<i>C. gariepinus</i>	MK074106.1
11	<i>C. gariepinus</i>	MK074109.1

The sequences were aligned using software MEGA X (Sudhirkumar *et al.*, 2018). The alignment used for sequence alignment was ClustalW. For uniformity in understanding the sequences were trimmed to 644 bases only.

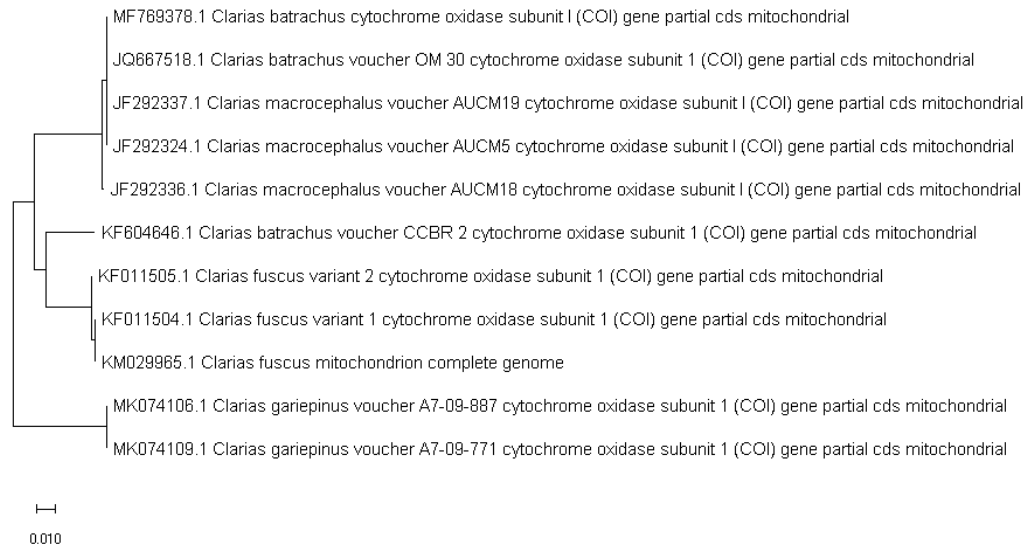
Their corresponding morphological identification have been referred from www.fishbase.org

RESULTS

Table 2: Showing nucleotide composition of COI gene with every single base pair.

Accession	C	T	A	G	T	A	T	T	T	G	G	T	G	C	C	T	G	A	G	C	C	G	G	A	A	T	A	G	T	C
JF292324.1 <i>Clarias macrocephalus</i>
JF292336.1 <i>C. macrocephalus</i>
JF292337.1 <i>C. macrocephalus</i>
MF769378.1 <i>C. batrachus</i>
KF604646.1 <i>C. batrachus</i>	T
JQ667518.1 <i>C. batrachus</i>
KF011504.1 <i>C. fuscus</i>	T
KF011505.1 <i>C. fuscus</i>	T
KM029965.1 <i>C. fuscus</i>	T
MK074106.1 <i>C. gariepinus</i>
MK074109.1 <i>C. gariepinus</i>
JF292324.1	G	G	C	A	C	A	G	C	C	C	T	A	A	G	C	C	T	A	C	T	A	A	T	C	C	G	A	G	C	A
JF292336.1
JF292337.1
MF769378.1
KF604646.1	G
JQ667518.1	G
KF011504.1	G
KF011505.1	G
KM029965.1	G
MK074106.1	G
MK074109.1	G
JF292324.1	G	A	A	C	T	G	G	C	A	C	A	G	C	C	T	G	G	G	G	C	T	C	T	T	C	T	A	G	G	A
JF292336.1
JF292337.1
MF769378.1
KF604646.1	A	A	.	.	A	.	.	A	.	.	A	T	.	.	.
JQ667518.1
KF011504.1	A	A	.	.	.	C	T	.	.	.
KF011505.1	A	A	.	.	.	C	T	.	.	.
KM029965.1	A	A	.	.	.	C	T	.	.	.
MK074106.1	A	A	.	.	.	C	.	.	A	.	.	C	T
MK074109.1	A	A	.	.	.	C	.	.	A	.	.	C	T

Image 1: Cladogram showing the tree based identification of *Clarias spp* drawn using minimum evolution tree at MEGA X software.



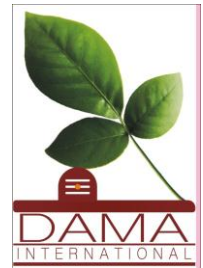
The nucleotide composition table shows very much similarity in intra-specific COI gene sequences except in *Clarias batrachus* individuals. Similarly interspecific difference in COI gene sequences for *C. macrocephalus* and *C. batrachus* can also be observed in table 2. The result of the same can be reflected in the cladogram (Image 1).

DISCUSSION

Although the DNA barcoding based on COI gene has been stated to be an effective tool for species identification, the statement is not apparently followed in *Clarias spp.* uploaded on world wide web data base of NCBI and will certainly not help in satisfying criteria of Biological concept of species. Adding one more gene like RAG 1, (nuclear) might bring deeper insight in the field of molecular taxonomy.

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