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SHORT COMMUNICATION

Genetic identification and phylogenetic relationships of Indian clariids based on mitochondrial COI sequences

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Abstract

Mitochondrial cytochrome C Oxidase I (COI) sequence variation among the clariid fishes of India (*Clarias magur, C. dussumieri* and *C. gariepinus*) and their relationship with other representative clariids was studied in this work. Three species were sampled and together with 23 COI sequences from GenBank were used to reconstruct phylogenetic relationships in the family Clariidae. The study revealed two clades: one consisting of the African species with *C. dussumieri*, and the other of Asian species suggesting the prevalence of intra-continental diversification of catfishes. This study further revealed that the genus *Clarias* is monophyletic. For the *COI* gene, the interspecies genetic divergence ranged from 0.056 to 0.182. The mean genetic difference between *C. dussumieri* and other selected African species in this study is 12.1%. It was also observed that the morphological similarity of *C. dussumieri* and *C. magur* was not replicated in the genetic level. *Clarias dussumieri* was more close to African catfish *C. gariepinus* thus indicating the utility of COI phylogeny to identify the well-known African-Asian relationships within catfishes. The results also showed that *C. magur* and *C. batrachus* are genetically distinct from each other.

Keywords

Catfish, *Clarias*, COI, mitochondrial genes

History

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Introduction

Catfishes of the family Clariidae are known as air breathing fishes, capable of respiring atmospheric oxygen. It is one of the largest families in the order Siluriformes comprising 113 species in 16 genera (Ferraris, 2007; Ng et al., 2011). Out of the 16 genera of the family Clariidae, the genus *Clarias* alone has 57 species (Ferraris, 2007). The capacity to survive in poorly oxygenated water makes these fishes popular for cultivation. Among the genus *Clarias*, four species are seen in India. Among these four varieties, two of them are endemic – *C. dussumieri (Valenciennes)* and *C. dayi. Clarias dayi* is a very rare species being known only by a single specimen collected by Dr. Francis Day in 1877. *Clarias dussumieri* is listed as a threatened fish (Padmakumar et al., 2010). *Clarias magur* is a commercially cultivated species found all over India. The fourth one is the exotic species, introduced surreptitiously in the recent past, *C. gariepinus* (African Catfish).

The main method of taxonomy in the field of aquatic biology is morphology based. Considerable ambiguity exists in the identification of *Clarias* species by morphological feature due to morphological similarity and overlapping meristic counts (Bridge & Haddon, 1893; Nawar, 1954; Nelson, 1948; Tilak, 1963). The exact identification of the species is difficult in such situation. Moreover, in a phylogenetic study of Clariids, Agnese & Teugels (2001, 2005) concluded that the current systematics of the clariid catfishes can no longer be maintained; however, more clariid species will need to be studied before introducing new nomenclature. Moreover, very few genetic studies has been conducted among endemic species of *C. dussumieri* except for the development of captive breeding techniques for conservation management of the species *C. dussumieri* by the Regional Agricultural Research Station, (RARS, KAU), Kumarakom, Kottayam, India (Padmakumar et al., 2004). The reports are scanty on barcoding of clariids found in India. Genetic variability in three clariid species, *C. batrachus*, *C. gariepinus,* and *C. macrocephalus*, were investigated using allozyme electrophoresis and mitochondrial DNA (ND 5/6) RFLP markers by Mohindra et al. (2007). Mitochondrial DNA has been extensively studied in fish phylogenetics since mitochondrial 16S rRNA gene and the protein coding cytochrome C Oxidase subunit I (COI) genes are highly conserved (Lakra et al., 2009). Mitochondrial DNA (mtDNA) has been generally employed in phylogenetic studies because it evolves much more rapidly than nuclear DNA, which will result in the accumulation of differences between closely related species (Timm et al., 2008). The present investigation is an attempt to estimate the phylogenetic relationships among clariid catfishes seen in India, i.e., *C. dussumieri*, *C. magur*, and *C. gariepinus*, and also to relate this phylogeny to the wider context of the genus worldwide which is very important from the point of view of their utilization in conservation and management plan of these commercially important species.

Materials and methods

The fish samples were collected from different locations of Indian subcontinent and Andaman and Nicobar Islands. *Clarias gariepinus* was collected from local fish farms of Cochin,

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Table 1. Mean genetic distance between 10 *Clarias* species included in the study based on the mitochondrial *COI* gene.

		1	2	3	4	5	6	7	8	9	10
1	*C. gabonensis*	0.000									
2	*C. angolensis*	0.056									
3	*C. jaensis*	0.096	0.084								
4	*C. camerunensis*	0.113	0.112	0.101							
5	*C. gariepinus*	0.119	0.104	0.124	0.093						
6	*C. macrocephalus*	0.162	0.160	0.164	0.159	0.180					
7	*C. magur*	0.138	0.150	0.154	0.159	0.162	0.146				
8	*C. batrachus*	0.162	0.182	0.156	0.158	0.159	0.148	0.120			
9	*C. dussumieri*	0.108	0.107	0.108	0.121	0.126	0.156	0.144	0.158		
10	*C. fuscus*	0.129	0.142	0.139	0.135	0.162	0.124	0.079	0.097	0.125	0.000

Kerala. Live specimens of *C. dussumieri* were obtained from Regional Agricultural Research Station (KAU), Kumarakom, Kottayam. *Clarias magur* was collected from local fish market of Lucknow and Andaman. *Clarias magur* and *C. dussumieri* were identified by the literature of Talwar & Jhingran (1991), Misra (1976), and Jayaram (2006). *Clarias gariepinus* was identified using published guides (FAO 2010–2015). Caudal fin clippings of all the samples (non destructive sampling for *C. dussumieri*) were collected and kept in absolute alcohol till DNA isolation.

Total genomic DNA was isolated using (Miller et al., 1988) with minor modifications. Concentration of the total genomic DNA was estimated using UV spectrophotometer. Then it was diluted to get a final concentration of 40–50 ng/µl. Mitochondrial cytochrome C oxidase I was amplified in a 25µl reaction mixture with 2.5µl of 10× assay buffer with 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP's, 0.1 µM of forward and reverse primers, 0.5 mM of $MgCl_2$, 1.5 units of *Taq* DNA polymerase, and 1 µl of DNA using the thermal Cycler. The primers used for the amplification of the COI gene were Fish F1–5′-TCAACCAACCACAAAGACATTG GCAC-3′ and Fish R1-5′-TAGACTTCTGGGTGGCCAAAGAA TCA-3′ (Ward et al., 2005). The thermal regime consisted of initial denaturation of 95°C for 5 min followed by 29 cycles of 94°C for 45 s, 56°C for 30 s, and 72°C for 45 s followed in turn by a final extension of 72°C for 5 min. Amplified products were visualized on 1.5% gel and documented using a BioRAD Gel Documentation System (Bio-Rad Laboratories, Hercules, CA). Further the products were labeled using the BigDye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems, Inc., Waltham, MA) and sequenced bidirectionally using an AB I 3730 capillary sequencer following instructions of the manufacturer (Applied Biosystems, Inc., Waltham, MA).

Sequences were aligned using Bioedit Version 7.0.5.2 (Hall, 1999) and submitted to GenBank under the accession numbers JQ699199–JQ699213. In order to find out the taxonomic position of Indian clariids with other representative clariids, phylogenetic reconstruction was made using the 23 sequences from Genbank (*C. magur* – KF 598807; *C. batrachus* – GQ 466401 and GQ 466403; *C. fuscus* – KF 011504 and KF 011505; *C. batrachus* – JF 292308, JF 292309, KC 789523, KC 789525, and KF 604648; *C. macrocephalus* – JF 292321 and KF 604662; *C. dussumieri* – HM 579862; *C. gabonensis* – HM 880231; *C. angolensis* – HM 880232; *C. jeaensis* – HM 882819 and HM 882818; *C. camerunensis* – HM 882808; *C. gariepinus* – HM 882809, JF 292311, KC 500413, GU 701826, and KF 604660) with *Ictalurus punctatus* (Genbank accession no. HQ 024944) as an outgroup. Pair-wise evolutionary distance among haplotypes was determined by the Kimura 2-Parameter method (Kimura, 1980) using the software MEGA 6. (Tamura et al., 2013). Maximum Likelihood and Neighbor Joining (NJ) trees were constructed in MEGA 6 using 1000 bootstrap replications.

Results

A total of 20 individuals from three genera (*C. gariepinus* (5), *C. magur* (10), and *C. dussumieri* (5) were used for partial sequence analysis of *COI* gene. Multiple alignments of the *COI* gene resulted in a consensus length of 623 sites. There was no insertion, deletion, or stop codon in the sequences. Of the 623 sites, 425, 197, 163, and 34 were conserved, variable, parsimony informative, and singleton, respectively. The analysis revealed nucleotide frequencies as A = 27.4%, T = 28.4%, G = 17.9%, and C = 26.2%. Average transitional pairs (si = 43) were more frequent than transversional pairs (sv = 20) with an average ratio of 2.17.

Phylogenetic tree constructed using Maximum Likelihood (ML) and Neighbor Joining (NJ) methods showed similar topology. They constantly showed two major groups, i.e., African species included in the study and *C. dussumieri* in one group and all the other Asian species in the other group. Inter-species genetic distance is given in Table 1.

Discussion

The topology of ML and NJ trees was congruent (Figure 1). From the present study, it is found that all the *Clarias* species used in this study is monophyletic. Transitions were more than transversions as expected. Generally for teleost mtDNA, a much larger excess of transitions related to transversion is typically observed (Ward et al., 2005.) Based on the ML and NJ tree, two major clades were consistently observed with African species in one clade along with *C. dussumieri* and Asian species in the other clade. The two large continental clades, ''Asia'' and ''Africa'', suggest a prevalence of intra-continental diversification of catfishes. Thus the COI phylogeny identifies the well-known African-Asian relationships within catfishes (Sullivan et al., 2006).

In the Asian species clade, four subclades were seen consisting of *C. magur*, *C. fuscus*, *C. batrachus*, and *C. macrocephalus*. According to Ng & kottelat (2008), the name *C. magur* should be used for the species of *Clarias* occurring in north-eastern India previously identified as *C. batrachus.* This argument is proved in this study as *C. magur* from Indian mainland and Andaman Island forms one subclade and *C. batrachus* of South East Asian origin forms another subclade in the Asian clade with 100 bootstrap values. The African clade differentiates into two sub clades with *C. dussumieri* in one sub-clade and *C. gariepinus* in the other with other African species such as *C. camerunensis* as near immediate sister taxa and *C. jaensis*, *C. angolensis*, and *C. gabonensis* as other members.

Clarias dussumieri is showing strong relation with *C. gariepinus*. The simplest explanation for this phenomenon is to consider that the ancestor species of *C. dussumieri* and *C. gariepinus* originated in Africa and both of them got separated during continental drift and differentiated into two different species. But, Africa and Asia were disconnected about 160

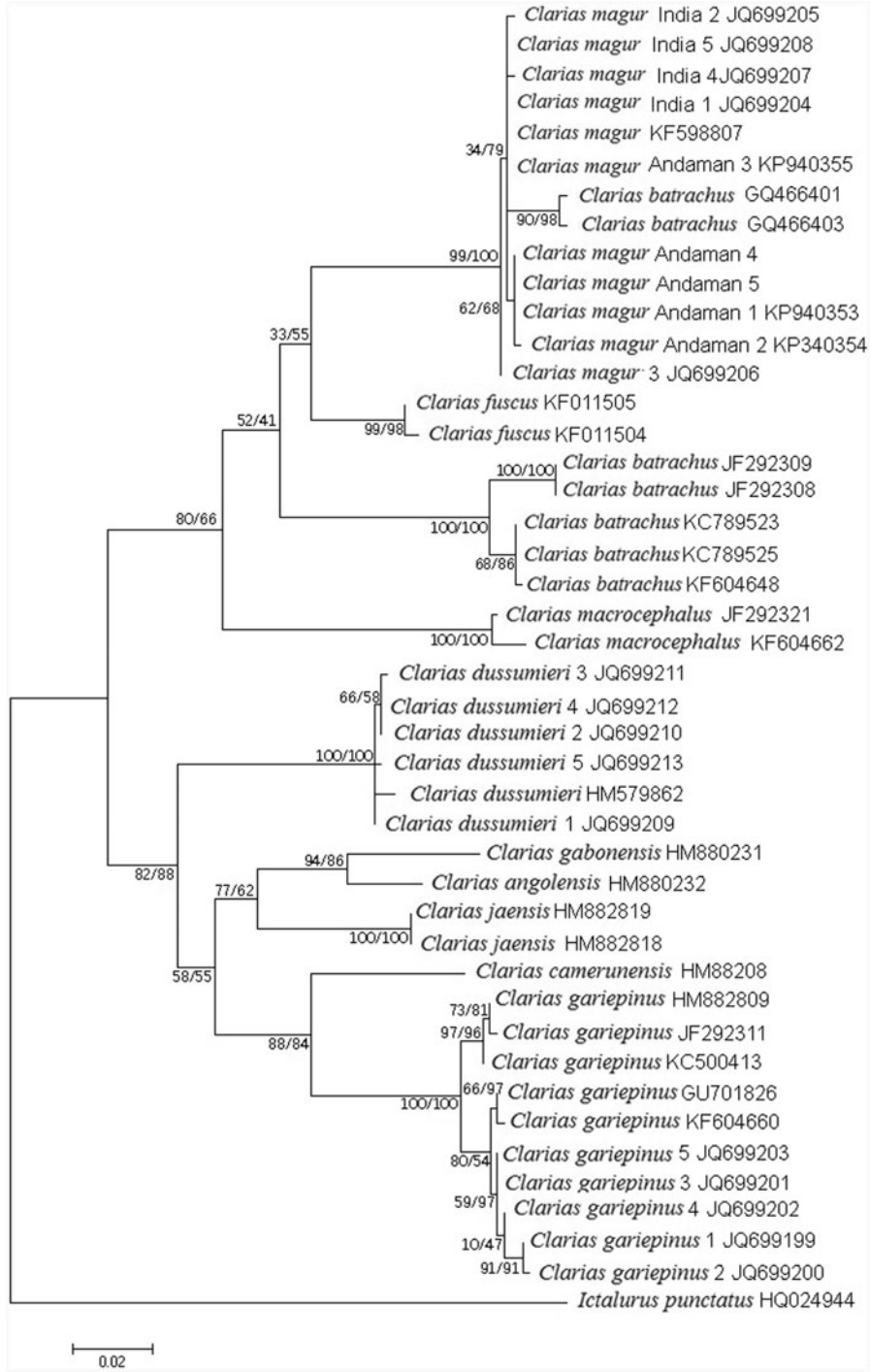

Figure 1. Consensus tree of Maximum Likelihood and Neighbor Joining trees based on the mitochondrial COI gene using the software MEGA 6.0. The numbers by the nodes indicate maximum likelihood and neighbor joining bootstrap supports.

million years ago (Agnese & Teugels, 2005). The rate for mitochondrial gene evolution estimated for fishes is 1% sequence divergence per million years (MY) (Dowling et al., 2002; Smith et al., 2002). The mean genetic difference between *C. dussumieri* and other selected African species in this study is 12.1%. Had the two groups separated during continental drift then the genetic difference would have been more than what was observed. The oldest African fossils are from Middle Miocene (10–15 MY) (Van Couvering, 1977). As per the observations of Otero & Gayet (2001), migration of freshwater fishes between Asia and Africa should have happened on early Miocene through early terrestrial connections between the two continents. The brackish water bridges like lagoons should have preceded the first terrestrial connections then; some Clariidae species should have been able to easily cross these bridges. Combining the fossil data, molecular clock data, and brackish water connections between the continents, it can be postulated that the common ancestor for *C. dussumieri* and other African species included in this study might have originated in Africa and later got separated into different species through brackish water connections.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. The authors greatly acknowledge the funding from KSCSTE, Trivandrum and the Director,

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