Invasion of African Clarias gariepinus in Bangladesh

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The African catfish Clarias gariepinus has been introduced for aquaculture in Bangladesh due to the scarcity of indigenous *C. batrachus* fingerlings. The African catfish Clarias gariepinus is a highly carnivorous species and predates small indigenous freshwater fishes when escaping into natural water bodies. However, the government of Bangladesh has banned the farming of *C. gariepinus* due to the carnivorous nature of this species. The introduction of *C. gariepinus* caused native biodiversity loss due to its predatory nature.

catfish | Claris batrachus | C. gariepinus | aquaculture | Bangladesh

1. Introduction

The walking catfish *Clarias batrachus* belonging to the Clariidae family, commonly known as walking catfish, is a popular food fish in the Indian sub-continent, including Bangladesh [1][2][3]. Catfish are a diverse group of fishes distributed from freshwater to marine environments, such as *Clarias batrachus* in freshwater [4] and *Arius maculatus* in brackish water [5]. Three species of the *Clarias* genera are important aquaculture candidates in Asia, namely *C. batrachus* in the Indian subcontinent [6], *C. fuscus* in Taiwan region [7][8], and *C. macrocephalus* in South-East Asia [9]. The native range of *C. batrachus* is Asia but *Clarias* aff. *Batrachus* from Indochina and Sundaland has been incorrectly identified as *Clarias batrachus* from Java [10]. The widely distributed African catfish *C. gariepinus* expanded its native range from the South to the Middle East and Eastern Europe [11].

Many exotic species have been introduced in Bangladesh for aquaculture in the past few decades, and some species are playing important roles in aquaculture production. A few of the exotic species have been proven detrimental to aquatic biodiversity and eventually banned for aquaculture in Bangladesh. The African magur, *Clarias gariepinus*, was introduced in Bangladesh in 1989 from Thailand by the Ministry of Fisheries and livestock (MoFL), but banned from aquaculture since 2014. The introduction of *C. gariepinus* caused native biodiversity loss due to its predatory nature [12]. The feasibility of hybrid vigor production between *C. batrachus* × *C. gariepinus* has been assessed but attained limited success [13]. Hybridization of *C. gariepinus male* × *C. macrocephalus* female in Vietnam has been widely practiced [14]. However, the potential of hybrids for a decline in the abundance of *C. batrachus* in the Chao Phraya and Mekong Basins has been considered [13]. Although aquaculture of *C. gariepinus* has been banned in Bangladesh [15], the availability of this species has been reported by consumers and farmers. Hybridization of *C. batrachus* × *C. gariepinus* has been known to occur in India [16] and in the Mekong Basin of Vietnam and Thailand [13]. Unplanned hybridization between *C. gariepinus* and *C. batrachus* is considered illegal in Bangladesh. Hybrids are less accepted by consumers due to differences in appearance and taste. In

addition, the escape of the hybrids in natural water bodies could cause irreversible loss of the native biodiversity. If the purity of native stock is compromised in the natural population, it would be an irreversible problem for the future.

The mitochondrial genes were analyzed in this entry to identify the native and hybrid *Clarias* species in Bangladesh. DNA sequencing was used to identify animal species because the mutation rate is low enough to allow distinguishing of closely related species [17]. This approach is used in evolutionary biology, phylogenetic systematics, and population genetics. Mitochondrial genes based on cytochrome C oxidase subunit I (COI) and cytochrome b (cytb) have been targeted for analysis in this entry. These mitochondrial genes have been used in evolutionary and phylogenetic analyses of fishes, including genetic variation assessment of native and exotic climbing perch *Anabas testudineus* in Bangladesh [18]. The COI nucleotide sequences are widely used to authenticate the purebreds of different fish species and families, including the Sparidae family [19], groper fishes [20], Cyprinidae family [21], *Ompok* genus [22], and Clariidae and Pangasiidae families [23]. The mitochondrial cytochrome b (Cytb) has been found effective in fish species identification and authentication [24][25], the identification of catfishes in Korea [26], *Puntius* genus in Indian rivers [27], and fishes of the South China Sea [25]. In this entry, COI and Cytb based DNA barcoding techniques have been targeted to identify *C. batrachus, C. gareipinus*, and the suspected hybrids in Bangladesh.

2. Sequence Analysis of COI and Cytb Genes

Validation of morphological identification by geometric morphometry: Based on geometric morphometry, the scattered plot of the landmark points showed that native *C. batrachus*, suspected hybrid and exotic *C. gariepinus* were morphologically distinguishable (**Figure 1**). The principal component 1 (PCA 1)_and PCA 2 of landmark points showed that native *C. batrachus*, suspected hybrid exotic *C. gariepinus* formed a separate cluster.

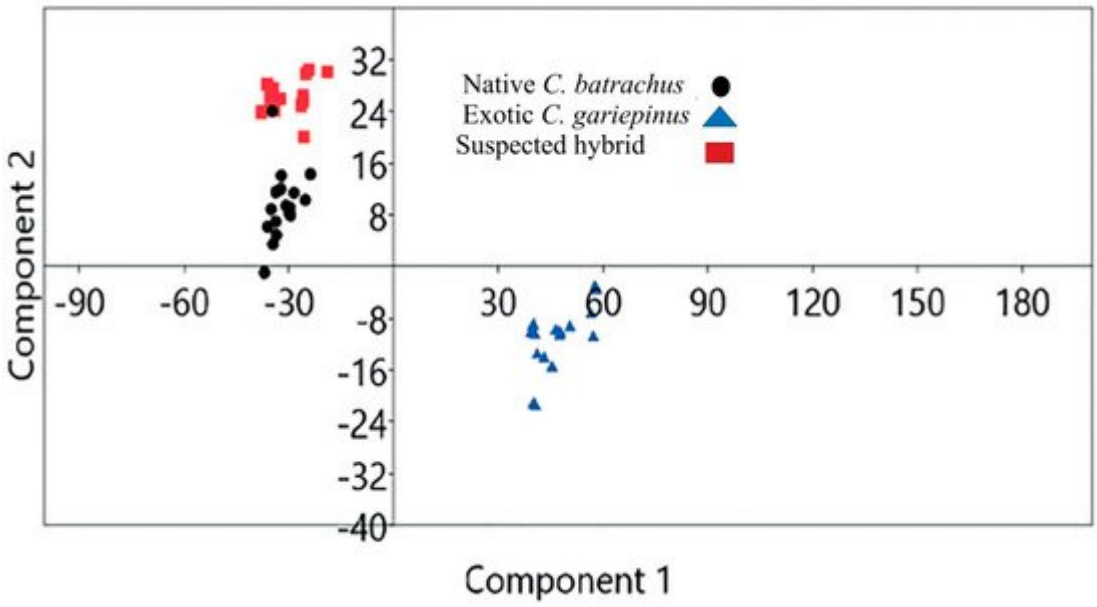

Figure 1. Scatter plot generated 13 landmark landmark points based on geometric morphometry of native *Clarias batrachus,* suspected hybrid and exotic *C. gariepinus*.

3. Sequence Analysis of COI and Cytb Genes

In the COI gene, 751 sites were identified where 76 (10.12%) were conserved; 670 (89.88%) were variable, which indicated a high level of variation among *C. batrachus, C. gariepinus*, and the hybrid. The average nucleotide composition of the COI gene was 29.3%, 26.6%, 24.6%, and 19.5% for T, C, A, and G, respectively. The AT content was higher than GC content in all the samples. Higher differences were observed between the sequence pair of *C. batrachus* of Bangladesh and reference *C. batrachus* from the Philippines and Indonesia, Sequenced *C. gariepinus*, and reference *C. batrachus* from the Philiphines and Indonesia. Higher differences were not observed between the sequenced *C. gariepinus* and suspected hybrid (**Table 1**).

Table 1. The estimated net base composition bias disparity between COI nucleotide sequences of native *Clarias batrachus* suspected hybrid andexotic *C. gariepinus.*

No	Species Name	1	2	3	4	5	6	7
1	*C. batrachus* 1 (MG988399)							
2	*C. batrachus* (Philiphine)	0.189						
3	*C. batrachus* (Indonesia)	0.691	0.000					
4	*C. batrachus* (India)	0.000	0.000	0.000				
5	*C. gariepinus* (MG988400)	0.000	1.328	1.961	0.900			
6	*C. gariepinus* (Indonesia)	0.318	0.000	0.000	0.000	1.370		
7	*C. gariepinus* (Nigeria)	0.146	0.000	0.000	0.000	1.068	0.000	
8	Suspected hybrid (MG988401)	0.000	1.258	1.909	0.923	0.000	1.440	1.235

ed bases between the studied COI nucleotide sequences [28].

4. Transition/Transversion Bias

The estimated transition/transversion bias (*R*) among the COI gene sequences of the *Clarias* genus was 1.12. The rates of transitional and transversional substitutions are presented in bold and italics, respectively (**Table 2**). The nucleotide frequencies were A = 24.91%, T/U = 29.34%, C = 26.39% and G = 19.36%. The rates of transitional substitution from A to G, T to C, C to T, and G to A were 8.76%, 15.39%, 17.11%, and 11.27%, respectively (**Table 1**). The rates of transversional substitution from A to T, A to C, T to A, T to G, C to A, C to G, G to T, and G to C were 6.96%, 6.26%, 5.9107%, 4.59%, 6.96%, and 6.26%, respectively (**Table 2**). The rates of transitional substitution of the COI nucleotide sequences were higher (52.547%) than transversional substitution (47.453%).

Table 3. The estimated maximum likelihood pattern of nucleotide substitutions.

	A	T	C	G
A	-	*6.9605%*	*6.2622%*	**8.7613%**
T	*5.9107%*	-	**15.3969%**	*4.5926%*
C	*5.9107%*	**17.1140%**	-	*4.5926%*
G	**11.2759%**	*6.9605%*	*6.2622%*	-

The rates of different transitional substitutions are presented in bold, and transversions substitutions are presented in italics.

5. Homogeneity of Substitution Patterns of COI Sequences

The estimated *p* values smaller than 0.05 (considered significant and marked with an asterisk) are presented above the diagonal in **Table 3**. The estimated disparity index per site is presented for each sequence pair above the diagonal in **Table 3**. The *p*-values were larger than 0.05 between sequence pairs of reference *C. batrachus* from the Philippines and Indonesia; the reference *C. batrachus* from India and Bangladesh relative to the reference from the Philippines and Indonesia; the Bangladeshi sequence of native *C. batrachus* and *C. gariepinus*; the *C. gariepinus* from Nigeria, Indonesia and *C. batrachus* from India, Indonesia, and the Philippines; the sequence of suspected hybrid and native *C. batrachus*and exotic *C. gariepinus*. The sequences of the COI gene in native *C. batrachus* evolved with the same pattern of substitution like the species originating from other counties (**Table 3**).

Table 3. Test of the homogeneity of substitution patterns between COI sequences of native *Clarias batrachus*, suspected hybrid and exotic *C. gariepinus.*

Sl.No	Species Name	1	2	3	4	5	6	7	8
1	*C. batrachus* 1 (MG988399)		0.189	0.691	0.000 *	0.000 *	0.318	0.146	0.000 *
2	*C. batrachus* (Philiphine)	0.194		0.000 *	0.000 *	1.328	0.000 *	0.000 *	1.258
3	*C. batrachus* (Indonesia)	0.108	1.000		0.000 *	1.961	0.000 *	0.000 *	1.909
4	*C. batrachus* (India)	1.000	1.000	1.000		0.900	0.000 *	0.000 *	0.923
5	*C. gariepinus* (MG988400)	1.000	0.044	0.012	0.100		1.370	1.068	0.000 *
6	*C. gariepinus* (Indonesia)	0.234	1.000	1.000	1.000	0.032		0.000 *	1.440

Sl.No	Species Name	1	2	3	4	5	6	7	8
7	*C. gariepinus* (Nigeria)	0.332	1.000	1.000	1.000	0.050	1.000		1.235
8	Suspected hybrid (MG988401)	1.000	0.024	0.022	0.082	1.000	0.026	0.046	

st genetic distance (6.238) was found between *C. gariepinus* from Indonesia and Bangladeshi *C. batrachus* . Low levels of genetic divergence (0.295–6.339) were found among sequenced native *Clarias batrachus*, suspected hybrid and exotic *C. gariepinus* in Bangladesh, whereas interspecies genetic divergence was found between *C. batrachus* and *C. gariepinus* from other countries (**Table 4**). The pairwise genetic distances based on nucleotide sequence divergences are presented in **Table 4**.

Note: * means the p values is smaller than 0.05 ($p < 0.05$) are considered as significant, which indicating the rejection of the null hypothesis that the sequences have evolved with the same pattern of substitution.

Table 4. Pairwise genetic distances of *Clarias* species from different countries based on a Kimura-2 parameter (KP2) model.

No	Species Name	1	2	3	4	5	6	7	8
1	*C. batrachus 1 (MG988399)*								
2	*C. batrachus (Philiphine)*	4.931							
3	*C. batrachus (Indonesia)*	3.283	3.110						
4	*C. batrachus (India)*	4.122	2.137	2.302					
5	*C. gariepinus((MG988400)*	0.339	5.005	4.400	4.635				
6	*C. gariepinus (Indonesia)*	2.837	4.987	4.638	6.238	2.594			
7	*C. gariepinus (Nigeria)*	4.055	4.636	5.095	3.147	4.025	2.315		
8	*Suspectred hybrid (MG988401))*	0.311	4.706	4.297	4.763	0.295	2.631	4.629	

7. Phylogenetic Tree Using COI Nucleotide Sequences by Maximum Likelihood Methods

The phylogenetic tree inferred from COI nucleotide sequences based on the maximum likelihood method showed that all the sequenced native *C. batrachus* and exotic *C. gariepinus* formed a single clade, where *C. gariepinus* and suspected hybrid and *C. gariepinus* formed a sister clade. The sequenced *C. batrachus* of Bangladeshi did not cluster with any of the reference sequences *C. batrachus* originated from other countries. However, *C. batrachus* from India, Indonesia, and the Philippines formed one clade. *C. gariepinus* from Nigeria and Indonesia formed a sister clade (**Figure 2**).

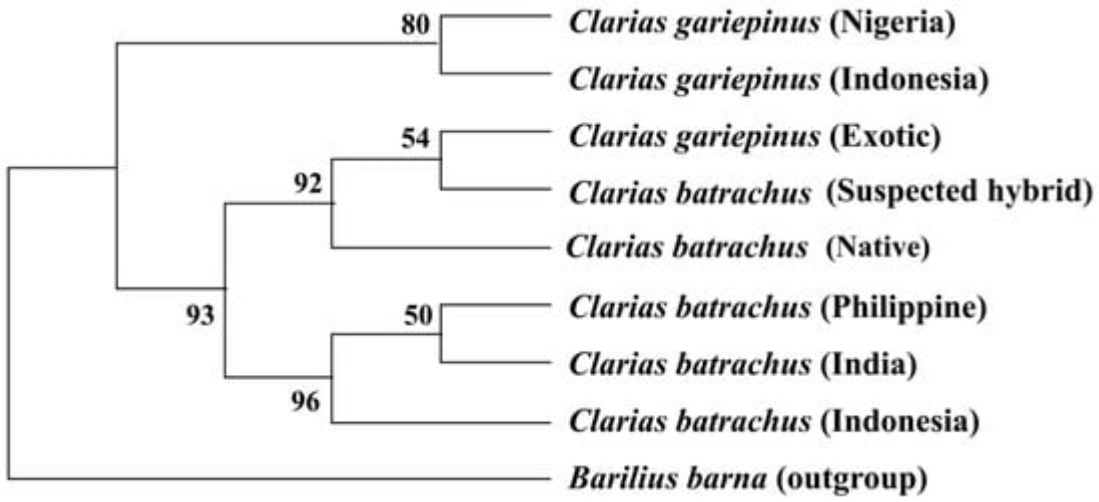

Figure 2. Rooted phylogenetic tree inferred from COI nucleotide sequences using maximum likelihood method. The numbers indicate the bootstrap value, which validates the sister taxa and clade formation in the phylogenetic tree.

8. Phylogenetic Tree Using Cytb Nucleotide Sequences by Maximum Likelihood Method

The phylogenetic tree based on the Cytb nucleotide sequences was used to validate the phylogenetic tree inferred from the COI nucleotide sequence (**Figure 3**). The results showed that sequenced suspected hybrid formed sister taxa with the sequenced *C. gariepinus*. The native *C. batrachus* formed a clade with *C. batrachus* of originated from different countries (Nigeria, France, Germany, and Malaysia) except suspected hybrid and *C. gariepinus*. The formation of sister clade of suspected hybrid with native *C. batrachus* and *C. gariepinus* confirmed the occurrence of hybridization of *C. batrachus* and *C. gariepinus* in Bangladesh, which validated the results based on COI gene.

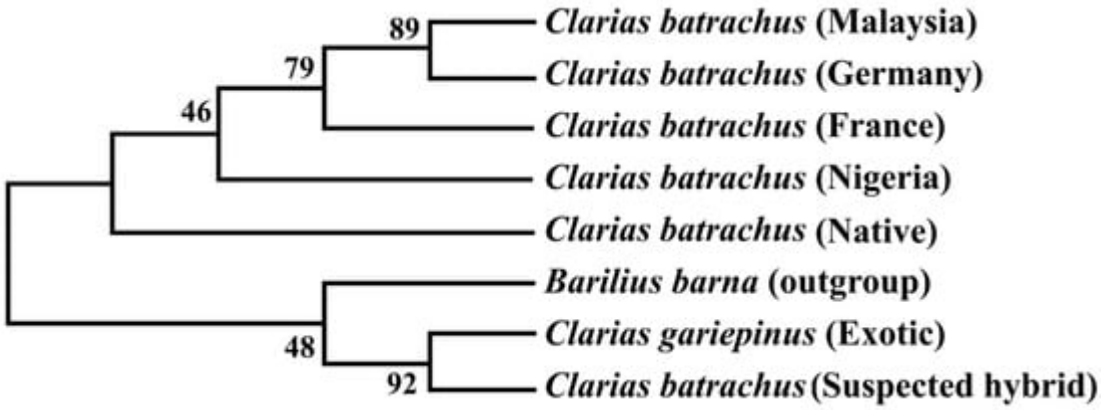

Figure 3. Unrooted phylogenetic tree inferred from Cytb nucleotide sequences using a maximum likelihood method. The numbers indicate the bootstrap value, which validates the sister taxa and clade formation in the phylogenetic tree.

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