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Molecular characterization of *Clarias gariepinus* from the Nile and an inland Lake, Sudan

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Abstract

Genetic diversity and population structure of *Clarias gariepinus* were evaluated by analyzing genetic distances among Sudanese populations and comparing them with reference sequences obtained from GenBank. Pairwise genetic distance (π) estimates indicated substantial variation, ranging from complete genetic identity (π = 0.000) to moderate divergence (π = 0.056). The greatest genetic distance was recorded between the Al-Rahd 2 population and an Indian reference sequence (KX946613), reflecting notable genetic differentiation. Similarly, Al-Rahd 2 exhibited relatively high divergence (π = 0.048) from populations originating in North Korea, Thailand, Congo, Brazil, and Bangladesh, suggesting possible historical isolation, limited gene flow, or distinct evolutionary trajectories. Conversely, several Sudanese populations demonstrated very low to zero genetic divergence when compared with global reference sequences. Abu-Gasba 1, Khashm Al-Girba 3, and Sennar 2 were genetically identical (π = 0.000) to sequences from Egypt and Indonesia. In addition, Abu-Gasba 1 and Khashm Al-Girba 3 showed complete genetic similarity with populations from a broad geographic range, including Nigeria, Ethiopia, Uganda, China, Turkey, Bangladesh, and India. This widespread genetic uniformity suggests strong connectivity and dispersal across regions. A slightly elevated but still minimal genetic distance (π = 0.002) was observed between Khashm Al-Girba 4 and several Asian and African reference populations. The findings reveal a combination of localized genetic differentiation and extensive genetic homogeneity in *C. gariepinus*, highlighting patterns of dispersal, introduction, and population connectivity.

Keywords: *Clarias gariepinus*; Population Structure; Genetic divesity; Phylogeney

Introduction

The Nile fauna is rich in fish species [1], including *Clarias gariepinus* which covers most of the African countries [2]. The taxonomic status of *C. gariepinus,* in the Sudan, needs to be clarified for better utilization of the fish and identification of a candidate population to develop in aquaculture and exploit on a sustainable manner.

Genetic diversity is a critical measure in fish population studies [3,4], in determining the survival of species and in providing information about the formation of stocks [5]. Recently, molecular tools and techniques were used to refine the taxonomy of some freshwater fish species in Sudan [6-11].

DNA barcoding is a useful and precise identification and characterization tool of species [12]. It has become the technique of choice for identification of fish species, using a short mitochondrial DNA sequence of cytochrome c oxidase I (COI) gene [13]. A region of the CO1 gene is approved by the Consortium for Barcode of Life (CBOL) for assembling DNA barcodes. This is the only gene sequence currently approved for DNA barcoding [12]. Polymorphic DNA markers provide genetic link to traits of interest useful in identification and in genetic improvement programmes [7]. They also establish relationships with other populations of the same species in other geographical areas [14]; differentiate between species in a specific country [2] and can establish the origin of an introduced species.

The objective of the present work was to study the systematic status of three populations of *C. gariepinus* from the Nile and one from an inland lake using molecular mitochondrial DNA and to add to the Sudan Gene Bank data.

Material and Methods

Fish specimen

One hundred twenty-three specimens of *Clarias gariepinus* fish were obtained from 4 sites. Collection sites were Sennar (Blue

Nile), Abu-Gasaba (White Nile), Khashm Al Girba (Atbara River) and Al Rahad (an inland lake in the Midwest).

Tissue sampling

Tissue samples from the left pectoral fin and from the second left gill of each specimen were taken and preserved individually in 5.0ml autoclaved plain labeled tubes, and kept in absolute alcohol at -20ºC, as described by [6].

Molecular techniques for identification

The genomic DNA was extracted from the preserved tissue samples using the Potassium Acetate method following [15]. The PCR amplification was conducted following the procedure described by [16]. The PCR machine (G-STORMA S/N 482-0642) was used to generate the reaction. For the amplification of DNA of *C. gariepinus* species, two CO1 primers (KF929769F and KF929769R, size and 560bp) were used following [17].

In the initial denaturation step, the contents of the PCR tubes were heated to 95°C for 5 minute to separate the two strands of the template DNA, followed by 35 cycles of denaturation at 95°C for 0.3 second. The temperature was decreased rapidly, and the annealing temperature was 60°C for 0.45 seconds. The final step was achieved at 72°C for 1 minute. A final extension cycle was 72°C for 5 minutes, and the machine was programmed to store the reaction at 4°C for 4 minutes until collected. A Nano-Drop (ND-1000) Spectrophotometer Apparatus was used to assess the quality, quantity and the purity of the samples. Gel electrophoresis, using (Bio RAD CE S/N 62S54249), was used to insure the reaction was successful and gave the proper bands which were photographed. The product size was estimated using a 100bp DNA ladder.

Sequencing

For sequencing the best 10 CO1 fragments with proper bands (2 from Sennar, 2 from Abu Gasaba, 2 from Al-Rahd ad 4 from Khashm Al-Girba) were used. The nucleotide sequences obtained were used to search for homology with mitochondrial genes in the National Centre for Biotechnology Information (NCBI) database, using the BLAST programme to identify the species following [18]. Accession numbers were obtained for the most similar hit with the lowest e-value and highest score. Sequences were visualized using Finch TV Version 1.4.0 and trimming was done using BioEdit version 7.0.3.5.).

NCBI BLAST and BOLD Identification systems analysis

The obtained sequences were blasted to the National Center of Biotechnology Information (NCBI) and (Bold systems) to infer the similarity and differences with the global *C. gariepinus*.

Statistical Analysis

The evolutionary distances were computed using Tamura 3-parameter method described by [19]. Sequence's analysis was used to model evolutionary rate differences among the four populations with the highest log likelihood. The exploratory search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of estimated pairwise distances using the Maximum Likelihood method and Jukes-Cantor model (1969) following [20]. A discrete Gamma distribution was used to model evolutionary rate differences among sites model, and then selecting the topology with superior log likelihood value. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were automatically shown next to the branches. There was a total of 447 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [21]. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated. This analysis involved 29 nucleotide sequences. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees following [20].

Results

Molecular analysis results

PCR amplification of the mitochondrial cytochrome c oxidase subunit I (CO1) gene produced clear and consistent amplicons of approximately 560bp across all individuals from the four sampled populations. All positive samples exhibited identical banding patterns, indicating successful and specific amplification of the target CO1 fragment. No amplification was observed in the negative control, confirming the absence of contamination and the reliability of the PCR assay (Figure 1).

Phylogenetic relationship analysis

A phylogenetic tree was constructed to evaluate the genetic relationship differences among the four population samples (Figure 2). All sequences of *C. gariepinus*, formed two major clusters. Khashm Al Girba samples cluster separately in one clade. Sennar 2 and Abu- Gasaba 1 formed a sister taxon, and they formed one clade with Abu- Gasaba 3. Sennar 1 and Al-Rahd 2 formed a sister taxon, and together they form one clade with Al-Rahd 1.

Pairwise genetic distances in native C. gariepinus

In the Sudan, the highest genetic distance (Table 1) was (π = 0.048) between Khashm Al-Girba 2 and Al-Rahad 2, followed by (π = 0.045) between Al-Rahad 2 and Abu-Gassaba 3. The lowest genetic distance was found between Sinnar 2 and from Abu-Gassaba1 sample (π = 0.000).

NCBI BLAST and BOLD identification systems analysis results

A comparison of 10 studied sequences to infer the similarity and differences with the global *C. gariepinus* was made. Based on Blasting, NCBI search Bold systems of known identity from GenBank, 19 similar sequences were selected. The values showed that the identity between countries varied between 79.48% and 100% (Table 2).

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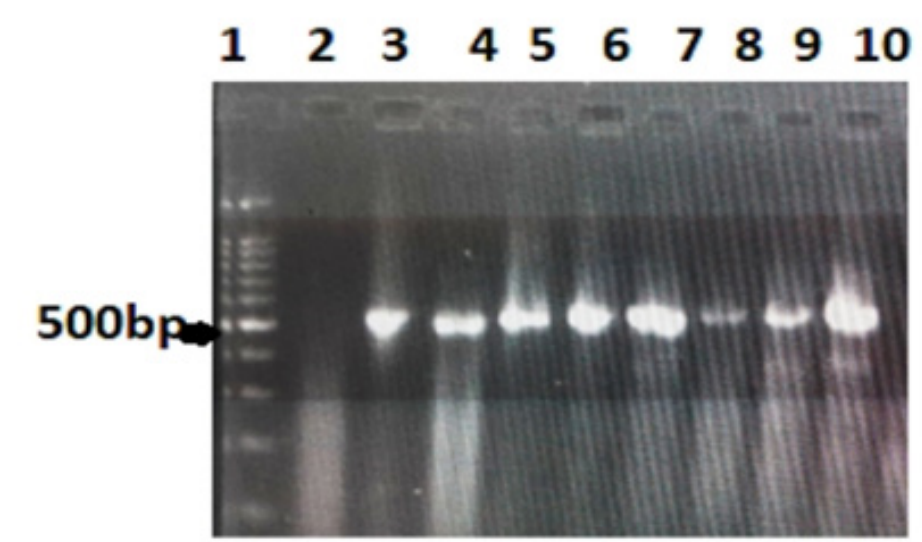

Figure 1: PCR amplification of the mitochondrial CO1 gene (~560bp). Lane 1: 100 bp ladder; Lane 2: negative control; Lanes 3–10: positive samples

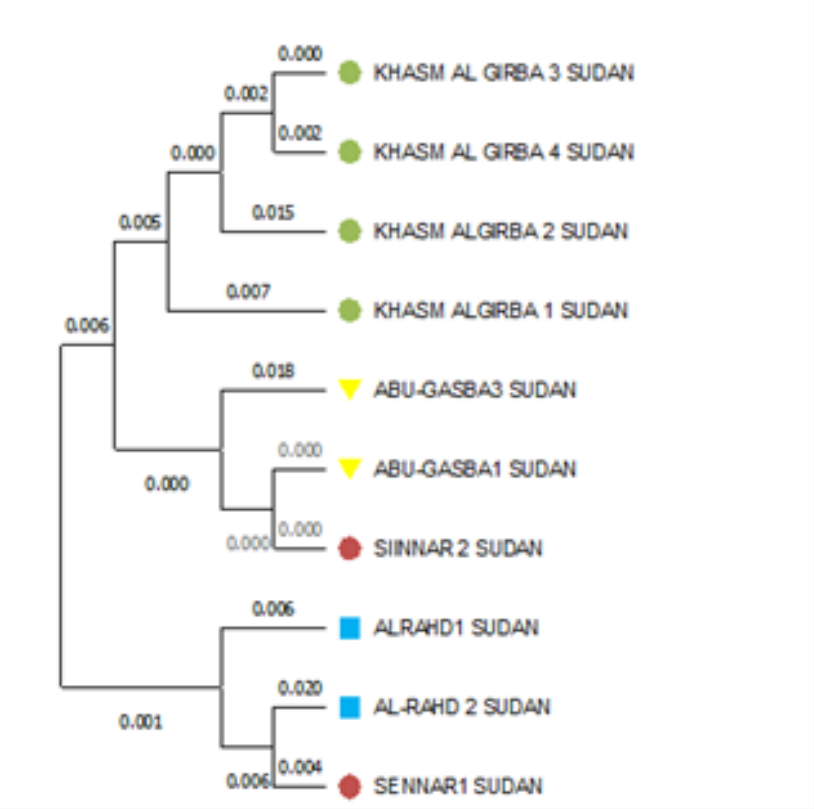

Figure 2: Neighbor Joining (NJ) Phylogenetic tree.

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Table 1: Pairwise genetic distances of *C. gariepinus* from different place based on a Kimura-2 parameter Model (JC+G).

Location	1	2	3	4	5	6	7	8	9
Abu-Gassaba 3	0								
Al-Rahd 2	0.045	0							
Al-Rahd 1	0.031	0.023	0						
Abu-Gassaba 1	0.016	0.031	0.014	0					
Khashm Al-Girba 3	0.021	0.033	0.016	0.007	0				
Khashm Al-Girba 4	0.018	0.035	0.018	0.009	0.002	0			
Khashm Al-Girba 1	0.031	0.035	0.018	0.014	0.011	0.014	0		
Khashm Al-Girba 2	0.035	0.048	0.03	0.023	0.018	0.016	0.021	0	
Sennar 1	0.031	0.021	0.014	0.016	0.016	0.018	0.021	0.033	0
Sennar 2	0.016	0.03	0.014	0	0.007	0.009	0.014	0.023	0.016

Table 2: Accession numbers for the similar sequences retrieved from GenBank supported with identity percentage (Studied sp= studied species; Pub sp= published species).

Studied sp	Pub sp	E-value	Identity%	Accession No.	Country
C. gariepinus	*C. gariepinus*	0	96.47	MG988400.	Bangladesh
C. gariepinus	*C. gariepinus*	0	96.94	KX657715.	Bangladesh
C. gariepinus	*C. gariepinus*	0	97.83	GU701825.	Brazil
C. gariepinus	*C. gariepinus*	0	97.38	KM610469	China
C. gariepinus	*C. gariepinus*	0	97.12	KT193342	Congo
C. gariepinus	*C. gariepinus*	0	79.48	LC487119	Egypt
C. gariepinus	*C. gariepinus*	0	100	MK335909.	Egypt
C. gariepinus	*C. gariepinus*	0	97.4	HM345934	Indonesia
C. gariepinus	*C. gariepinus*	0	99.2	KF929769.	Ethiopia
C. gariepinus	*C. gariepinus*	0	99.02	OK001827.	India
C. gariepinus	*C. gariepinus*	0	100	KX946613.	India
C. gariepinus	*C. gariepinus*	0	98.57	KU692441	Indonesia
C. gariepinus	*C. gariepinus*	0	99.02	MT571809.	Jinsha River China
C. gariepinus	*C. gariepinus*	0	97.38	HM882809.	Nigeria
C. gariepinus	*C. gariepinus*	0	99.2	KM261768.	North Korea
C. gariepinus	*C. gariepinus*	0	98.37	JF292318.	Thailand
C. gariepinus	*C. gariepinus*	0	98.56	KC500413.	Turkey
C. gariepinus	*C. gariepinus*	0	97.41	MT523545	Uganda
C. gariepinus	*C. gariepinus*	0	99.4	OL804284.	Sudan

Global phylogenetic tree analysis with native C. gariepinus

The Neighbor-Joining phylogenetic tree (Figure 3) of partial mtCO1 *C. gariepinus* from the four populations was compared with the reference sequences based on Blast, NCBI search of known identity. The GenBank Values at the nodes indicated bootsrap support of 1000 replication. An evolutionary relationships of *C. gariepinus* using MEGA11 was made. All sequences of *C. gariepinus* formed two major clusters. Cluster I consisted of the sequences of Abu-Gassaba 3 Sudan sample and reference Ol804284 Atbara River of the Sudan branched as sister taxa from the same node, while, Khashm Al-Girba 3 and 4, Abu-Gassaba 1 and 2, Sennar 2, showed sister taxa with MK335909 and LC487119 Egypt, KU692441 and HM345934 Indonesia, OK001827 India, MT523545 Uganda, MG988400 Bangladesh, KF929769 Ethiopia, HM882809 Nigeria, KM610469 China, KC500413 Turkey, MT571809 China.

However, Cluster II formed *C. gariepinus* from Sennar 2 and Al-Rahd 2 formed a-sister-taxa, and they formed one clade with Al-Rahd 1. This result from the phylogenetic tree indicated that three of the studied native *C. gariepinus* populations were closely related to each other than to one of Sennar and the two sequencing from Al-Rahd population.

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Figure 3: Evolutionary relationships of *C. gariepinus* taxa, Neighbor Joining (NJ) Phylogenetic tree (1000 bootstraps) based on comparing its sequences with sequences from the database.

Global phylogenetic tree analysis with native *C. gariepinus*

The Neighbor-Joining phylogenetic tree (Figure 3) of partial mtCO1 *C. gariepinus* from the four populations was compared with the reference sequences based on Blast, NCBI search of known identity. The GenBank Values at the nodes indicated bootsrap support of 1000 replication. An evolutionary relationships of *C. gariepinus* using MEGA11 was made. All sequences of *C. gariepinus* formed two major clusters. Cluster I consisted of the sequences of Abu-Gassaba 3 Sudan sample and reference Ol804284 Atbara River of the Sudan branched as sister taxa from the same node, while, Khashm Al-Girba 3 and 4, Abu-Gassaba 1 and 2, Sennar 2, showed sister taxa with MK335909 and LC487119 Egypt, KU692441 and HM345934 Indonesia, OK001827 India, MT523545 Uganda, MG988400 Bangladesh, KF929769 Ethiopia, HM882809 Nigeria, KM610469 China, KC500413 Turkey, MT571809 China.

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Global genetic distance of *C. gariepinus* versus native *C. gariepinus*

The genetic distance among the analyzed *C. gariepinus* populations ranged from 0.000 to 0.056 (Table 3). The highest genetic divergence (π = 0.056) was observed between the Al-Rahd 2 population and the reference *C. gariepinus* sequence from India (GenBank accession KX946613). This was followed by a genetic distance of π = 0.048 between Al-Rahd 2 and sequences from North Korea (KM261768), Thailand (JF292318), Congo (KT193342), Brazil (GU701825), and Bangladesh (KX657715).

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Table 3: Pairwise genetic distances of *C. gariepinus* from different sites.

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 Abu-Gas-ba3-Sudan	0.000														
2 AlRahd_2_Su-dan	0.046	0.000													
3 Alrahd1_Su-dan	0.028	0.026	0.000												
4 LC487119_ Egypt	0.016	0.030	0.011	0.000											
5 ANGBF9223-_ Indonesia	0.016	0.030	0.011	0.000	0.000										
6 ABU-GASBA1_ Sudab	0.016	0.031	0.012	0.000	0.000	0.000									
7 KT193342(2)_ Cango	0.033	0.048	0.028	0.016	0.016	0.016	0.000								
8 GU701825._ Brazil	0.033	0.048	0.028	0.016	0.016	0.016	0.000	0.000							
9 HM882809._ Nigeria	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000						
10JF292318._ Thailand	0.033	0.048	0.028	0.016	0.016	0.016	0.005	0.005	0.016	0.000					
11 KC500413. Turkey	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000				
12 KF929769._ Ethiobia	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000			
13 Khashm_Al_ Girba3_Sudan	0.016	0.031	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000	0.000		
14 Khashm_Al_ Girba4 Sudan	0.013	0.033	0.014	0.002	0.002	0.002	0.019	0.019	0.002	0.019	0.002	0.002	0.002	0.000	
15 Khashm_Al Girba1_Sudan	0.031	0.035	0.019	0.014	0.014	0.014	0.031	0.031	0.014	0.033	0.014	0.018	0.011	0.014	0.000
16 KM261768._ North_Korea	0.033	0.048	0.028	0.016	0.016	0.016	0.000	0.000	0.016	0.005	0.016	0.016	0.016	0.019	0.031
17 Khashm Al Girba2 Sudan	0.035	0.048	0.031	0.023	0.023	0.023	0.041	0.041	0.023	0.041	0.023	0.023	0.021	0.018	0.021
18 KM610469_ China	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000	0.000	0.002	0.014
19 KU692441_ Indonesia	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000	0.000	0.002	0.014
20 KX-657715Bangla-desh	0.033	0.048	0.028	0.016	0.016	0.016	0.005	0.005	0.016	0.000	0.016	0.016	0.016	0.019	0.031
21 KX946613._ India	0.041	0.056	0.036	0.024	0.024	0.024	0.016	0.016	0.024	0.016	0.024	0.024	0.024	0.026	0.038
22 MG988400. LBangladesh	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000	0.000	0.002	0.014
23 MK335909._ Egypt	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000	0.000	0.002	0.014
24 MT523545_ Uganda	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000	0.000	0.002	0.014
25 MT571809. Jinsha Riv-er-China	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000	0.000	0.002	0.014
26 OK001827. India	0.016	0.031	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000	0.000	0.002	0.014

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27 OL804284._ Sudan	0.014	0.033	0.019	0.002	0.002	0.002	0.019	0.019	0.002	0.019	0.002	0.002	0.002	0.000	0.016
28 Sinnar 1_ Sudan	0.031	0.021	0.014	0.016	0.016	0.016	0.033	0.033	0.016	0.033	0.016	0.016	0.016	0.019	0.021
29 Sinnar 2_ Sudan	0.016	0.030	0.011	0.000	0.000	0.000	0.016	0.016	0.000	0.016	0.000	0.000	0.000	0.002	0.014

Site	16	17	18	19	20	21	22	23	24	25	26	27	28	29
16 KM261768._North_ Korea	0.000													
17 Khashm Al Girba2 Sudan	0.018	0.000												
18 KM610469_CHINA	0.023	0.041	0.000											
19 KU692441_Indonesia	0.023	0.000	0.016	0.000										
20 KX657715Bangaladesh	0.041	0.016	0.016	0.016	0.000									
21 KX946613._India	0.049	0.024	0.024	0.016	0.005	0.000								
22 MG988400.LBangaladesh	0.023	0.000	0.000	0.016	0.024	0.016	0.000							
23 MK335909._Egypt	0.023	0.000	0.000	0.016	0.024	0.000	0.016	0.000						
24 MT523545_Uganda	0.023	0.000	0.000	0.016	0.024	0.000	0.000	0.016	0.000					
25 MT571809.Jinsha River-China	0.023	0.000	0.000	0.016	0.024	0.000	0.000	0.000	0.016	0.000				
26 OK001827._India	0.023	0.000	0.000	0.016	0.024	0.000	0.000	0.000	0.000	0.016	0.000			
27 OL804284._Sudan	0.021	0.002	0.002	0.019	0.026	0.002	0.002	0.002	0.002	0.002	0.019	0.000		
28 Sinnar 1_Sudan	0.033	0.016	0.016	0.033	0.041	0.016	0.016	0.016	0.016	0.016	0.018	0.031	0.000	
29 Sinnar2_sudan	0.023	0.000	0.000	0.016	0.024	0.000	0.000	0.000	0.000	0.000	0.002	0.016	0.016	0.000

In contrast, very low levels of genetic divergence (π = 0.000) were detected between the Sudanese populations Abu-Gasba 1, Khashm Al-Girba 3, and Sennar 2 and reference sequences from Egypt (LC487119) and Indonesia (ANGBF9223). Similarly, Abu-Gasba 1 showed zero genetic distance (π = 0.000) with sequences from Nigeria (HM882809), Turkey (KC500413), Ethiopia (KF929769), Indonesia (KU692441 and ANGBF9223), Bangladesh (MG988400), Egypt (MK335909), Uganda (MT523545), China—Jinsha River (MT571809 and KM610469), and India (OK001827).

Zero genetic divergence (π = 0.000) was also observed between Khashm Al-Girba 3 (Sudan) and sequences from Bangladesh (MG988400), Egypt (MK335909), Nigeria (HM882809), Uganda (MT523545), China—Jinsha River (MT571809 and KM610469), India (OK001827), Indonesia (KU692441), and Turkey (KC500413).

A slightly higher genetic distance (π = 0.002) was recorded between Khashm Al-Girba 4 (Sudan) and reference sequences from China (KM610469), Bangladesh (MG988400), Egypt (MK335909), Indonesia (ANGBF9223), Nigeria (HM882809), and Uganda (MT523545).

Discussion

In terms of abundance and economic importance, *C. gariepinus* is the second most common and widely distributed freshwater fish species in the Sudan [22,23]. The present study aimed to contribute to *C. gariepinus* taxonomy and add to its molecular biology using PCR. It indicated that the four populations belong to one species. The sequences of the cytochrome oxidase subunit 1 (CO1) mitochondrial gene are extensively used to determine inter and intra species relationships and to draw their evolutionary trees [11,24,25].

Sequences of CO1 was used in this study to draw the phylogenetic tree to allow for comparison of the results with similar studies retrieved from GenBank database showed that the interspecies genetic divergence based on the substitution pattern ranged from 0.000 to 0.056 for the COI gene. This indicates that the native *C. gariepinus* are closely related with most of the reference groups. It seems that *C. gariepinus* originated from Africa, and due its high environmental tolerability it spread to almost all continents and gaining popularity in aquaculture [26]. According to [27] in Brazil and since its market interest was low, the species was used as sport fish. They reported that uncontrolled escapes

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happened and the fish propagated to the Amazon River branches and lakes and became the top invader there. Molecular genetics was used to follow the origin and movement of the *C. gariepinus* out of Africa [4,14,28,29]. The phylogenetic results drawn by [28], showed that *C. gariepinus* from Africa, Asia and Turkey are closely related. None of the Sudanese studied individuals was found closely related to GU701825 from Brazil, KX657715 from Bangladesh, KX946613 from India, KM261768 from North Korea, JF292318 from Thailand and KT193342 from Congo. The results indicated that the variation in this species is very wide internationally.

The phylogeny analyses identified identical groups between native Khashm Al-Girba 3 and 4, Abu-Gassaba 1 and Sennar 2 and most of the members of the two groups from the Genbank. The first group, a reference of African Sudanese *C. gariepinus,* mainly clustered with Uganda, Ethiopia, Nigeria and Egypt. The second one related to a reference of Asian *C. gariepinus* found in Thailand, China, Bangladesh, India and Indonesia. All these groups were closely related to each other and derived from the same clade. The sequences of Abu-Gassaba 3 Sudan sample and Ol804284 Sudan retrieved from genbank branched as sister taxon from the same node. Sennar 2 is showing the greatest diversion from the most of the native *C. gariepinus*.

Another important aspect in the population genetics of *C. gariepinus* is its capacity to hybridize with other species from the same genus, or with other genera belonging to its family [30,31]. Some of these hybrids are well-known to grow faster than the parent native species [32-35]. Some cases of escapes from farms [27] of these hybrids to the wild were recorded. This may cause some degree of genetic introgression and hence possible changes on the distribution of different conservation units of *C. gariepinus* in the wild as normally expected by preference to certain environmental conditions over the others.

Conclusion

The findings showed that the populations of *C. gariepinus* are phenotypically the same. The native *C. gariepinus* fish populations are closely related with most of the reference groups reported from Africa and Asia. The bioinformatics analyses of phylogenetic trees, and the median joining network data show a close proximity between the Sudanese *C. gariepinus* samples and the Indonesian ones.

Ethics: Ethics approval and consent to participate, human and animal rights, consent for publication and availability of data and material are not applicable.

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