

Original Article

Genetic homogeneity across geographically isolated populations of *Coptodon guineensis* in Southern Morocco: Insights from the mitochondrial Cytochrome b gene

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Abstract: This study examined the genetic differentiation of the Guinean tilapia, *Coptodon guineensis*, from two geographically isolated localities in southern Morocco: the arid-zone watercourse Oued Aabar (Smara Region) and the hypersaline Sebkhah Imlili (Dakhla City), using the mitochondrial cytochrome b gene (1140 bp) as a molecular marker. Our results showed low genetic diversity and homogeneity between the two populations, indicating a lack of significant genetic differentiation and supporting the treatment of the two populations as a single conservation management unit. The neighbor-joining phylogenetic analysis, including other conspecific sequences and the haplotype network, confirmed this pattern. The observed genetic homogeneity may be attributed to the species' euryhaline dispersal ability and tolerance to extreme environments, which can facilitate gene flow or reflect historical connectivity among fragmented habitats. Further research should involve more comprehensive sampling with broader spatial and ecological coverage to address the overall genetic structure of *Coptodon guineensis* across its full distribution range in Morocco.

Article history:

Received 22 April 2026

Accepted 30 May 2026

Available online 25 August 2026

Keywords:

Coptodon guineensis

Cytochrome b gene

Genetic homogeneity

Morocco

Introduction

The Guinean tilapia, *Coptodon guineensis* (Günther, 1862), is classified as a benthopelagic, euryhaline cichlid species and is native to West Africa, occurring primarily along the Atlantic coast from Senegal to Angola and inhabiting various freshwater and brackish environments (Stiassny et al., 2008). Within Morocco, the species is known to occur in five geographically and ecologically distinct sites in the southern part of the country: the Sebkhah of Imlili (a hypersaline depression located south of Dakhla), Oued Aabar (a river in the Smara region), and three gueltas (Guelta Mrimima, Guelta Kheng El Mekraz, and Guelta Kehla) draining into the Drâa basin watershed (Qninba and Mataame, 2009; Qninba et al., 2012; Yayhaoui et al., 2020; Louizi et al., 2022; Ouassal, 2022). Each of these habitats represents a unique, isolated aquatic environment within arid and semi-arid landscapes. More recently, *C. guineensis*

has been unexpectedly recorded in the Nador Canal in northern Morocco (Ainou et al., 2021), significantly expanding the species' known range and raising questions about its dispersal mechanisms and potential anthropogenic translocations.

Given its restriction to a small number of isolated populations across a handful of extreme environments, understanding the genetic connectivity among populations is relevant to any informed conservation planning for this species. Therefore, the present study provides a preliminary assessment of mitochondrial genetic variation across two Moroccan populations of *C. guineensis*. This contribution aims to serve as a baseline for future studies on the population genetics and conservation of *C. guineensis* in Moroccan aquatic ecosystems.

Materials and Methods

Field collections were carried out at two localities in

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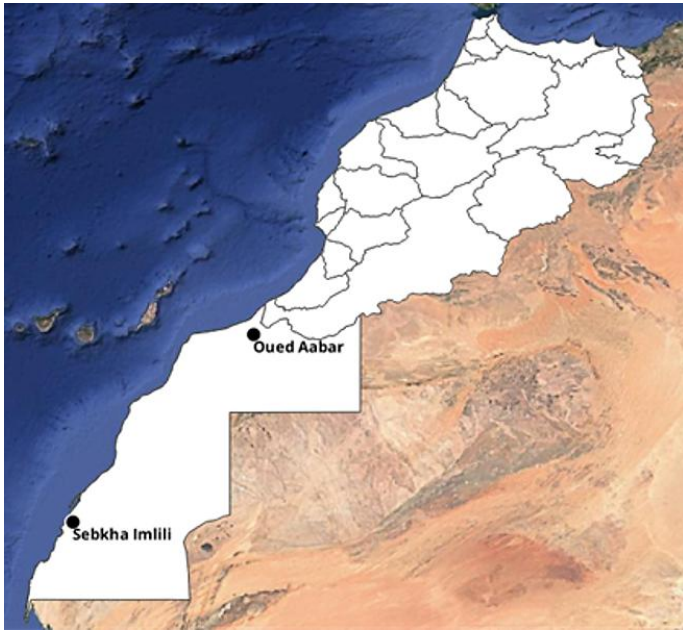


Figure 1. Sampling locations of *Coptodon guineensis* in southern Morocco: Oued Aabar (Smara) and Sebkhla Imlili (Dakhla).

southern Morocco: Oued Aabar, situated in the Smara Province, and Sebkhla Imlili, located approximately 30 km south of Dakhla city (Fig. 1). Coordinates for both locations were recorded using a GPS receiver and projected in QGIS for cartographic representation. Fish were captured using standard electrofishing procedures, and a tissue sample consisting of a fin clip was preserved individually in 94% ethanol for subsequent DNA extraction.

Genomic DNA was extracted from the fin clip using the Qiagen Biosprint15 kit. The complete mitochondrial cytochrome b gene (1140 bp) was amplified using primers from Doadrio and Perdices (2005) (H16460: CGAYCTTCGG ATTA CAAGA CCG) and Palumbi et al. (1991) (GluDGL: TGA CTGAAR AACCA YCGTGG), and sequenced by Macrogen Inc. (Amsterdam). Sequences were aligned in MEGA v7.0.26 (Kumar et al., 2015) using Clustal W (Thompson et al., 1994) and refined manually. In total, 23 sequences were studied. Sequences are submitted to the GenBank platform, and the accession numbers are listed in Table 1. In addition, six *Coptodon* sequences were retrieved from GenBank (Table 1). All sequences were aligned using ClustalW and trimmed to a common length prior to phylogenetic reconstruction.

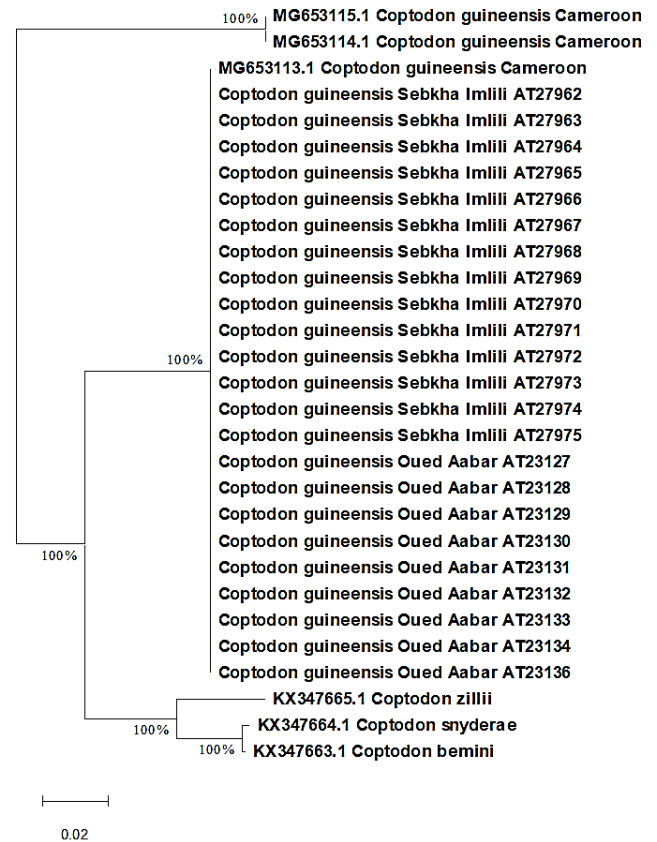


Figure 2. Neighbor-joining phylogenetic tree of *Coptodon guineensis* based on cytochrome b gene.

A neighbor-joining tree was generated with 1000 bootstrap replications in MEGA v7.0.26 (Kumar et al., 2015).

A haplotype network was built using the Median-Joining algorithm in PopArt (Leigh and Bryant, 2015). Genetic diversity indices (H_d , π) and fixation index (F_{st}) were calculated using DnaSP v6.12.01 (Rozas et al., 2017).

Results

The phylogenetic tree of *C. guineensis* based on the cyt b gene revealed a well-supported monophyletic clade (Bootstrap = 100%) including all Moroccan specimens and sequences from Cameroon (Fig. 2). Within this clade, most Moroccan samples from Sebkhla Imlili and Oued Aabar clustered closely together, showing very low genetic differentiation, which indicates genetic homogeneity among populations despite geographic isolation.

Interestingly, one Cameroonian sequence

Table 1. Details of target species: sampling localities, coordinates and GenBank accession numbers.

Species	MNCN DNA Code	Locality	Latitude	Longitude	Genbank accession
<i>Coptodon guineensis</i>	AT27962	Sebkha Imlili	23.276444	-15.915417	PZ294408
<i>Coptodon guineensis</i>	AT27963	Sebkha Imlili	23.276444	-15.915417	PZ294409
<i>Coptodon guineensis</i>	AT27964	Sebkha Imlili	23.276444	-15.915417	PZ294410
<i>Coptodon guineensis</i>	AT27965	Sebkha Imlili	23.276444	-15.915417	PZ294411
<i>Coptodon guineensis</i>	AT27966	Sebkha Imlili	23.276444	-15.915417	PZ294412
<i>Coptodon guineensis</i>	AT27967	Sebkha Imlili	23.276444	-15.915417	PZ294413
<i>Coptodon guineensis</i>	AT27968	Sebkha Imlili	23.276444	-15.915417	PZ294414
<i>Coptodon guineensis</i>	AT27969	Sebkha Imlili	23.276444	-15.915417	PZ294415
<i>Coptodon guineensis</i>	AT27970	Sebkha Imlili	23.276444	-15.915417	PZ294416
<i>Coptodon guineensis</i>	AT27971	Sebkha Imlili	23.276444	-15.915417	PZ294417
<i>Coptodon guineensis</i>	AT27972	Sebkha Imlili	23.276444	-15.915417	PZ294418
<i>Coptodon guineensis</i>	AT27973	Sebkha Imlili	23.276444	-15.915417	PZ294419
<i>Coptodon guineensis</i>	AT27974	Sebkha Imlili	23.276444	-15.915417	PZ294420
<i>Coptodon guineensis</i>	AT27975	Sebkha Imlili	23.276444	-15.915417	PZ294421
<i>Coptodon guineensis</i>	AT23127	Oued Aabar	27.941668	-11.424738	PZ294422
<i>Coptodon guineensis</i>	AT23128	Oued Aabar	27.941668	-11.424738	PZ294423
<i>Coptodon guineensis</i>	AT23129	Oued Aabar	27.941668	-11.424738	PZ294424
<i>Coptodon guineensis</i>	AT23130	Oued Aabar	27.941668	-11.424738	PZ294425
<i>Coptodon guineensis</i>	AT23131	Oued Aabar	27.941668	-11.424738	PZ294426
<i>Coptodon guineensis</i>	AT23132	Oued Aabar	27.941668	-11.424738	PZ294427
<i>Coptodon guineensis</i>	AT23133	Oued Aabar	27.941668	-11.424738	PZ294428
<i>Coptodon guineensis</i>	AT23134	Oued Aabar	27.941668	-11.424738	PZ294429
<i>Coptodon guineensis</i>	AT23136	Oued Aabar	27.941668	-11.424738	PZ294430
<i>Coptodon guineensis</i>	-	Cameroon	-	-	MG653113
<i>Coptodon guineensis</i>	-	Cameroon	-	-	MG653114
<i>Coptodon guineensis</i>	-	Cameroon	-	-	MG653115
<i>Coptodon snyderae</i>	-	-	-	-	KX347664
<i>Coptodon bemini</i>	-	-	-	-	KX347663
<i>Coptodon zillii</i>	-	-	-	-	KX347665

clustered within the Moroccan group, whilst the two remaining Cameroonian sequences formed a slightly distinct lineage, although still closely related. The outgroup species (*Coptodon zillii*, *C. snyderae*, and *C. bemini*) were clearly separated, supporting the validity of the tree topology.

Analysis of 23 *C. guineensis* sequences revealed four haplotypes with low overall genetic diversity ($H_d = 0.557$; $\pi = 0.00056$). The Oued Aabar population showed the highest diversity ($H_d = 0.694 \pm 0.147$; $\pi = 0.00073 \pm 0.00021$). The low and non-significant F_{ST} value (0.014 ; $P > 0.05$) suggests the absence of genetic differentiation between Sebkha Imlili and Oued Aabar (Table 2).

The haplotype network displayed four closely related haplotypes, with Hap_1 being the most common, shared by 15 individuals from both Oued Aabar and Sebkha Imlili. The remaining three haplotypes, including one private to Oued Aabar,

differed from Hap_1 by a single mutation (Fig. 3).

Discussions

The haplotype network structure and the low F_{ST} value indicate little to no genetic differentiation between the Oued Aabar and Sebkha Imlili populations. The small number of specimens used in this study may not be sufficient to detect subtle differences in population differentiation. However, our result is consistent with the phylogenetic study of Louizi et al. (2019) based on the cytochrome oxidase gene, which did not reveal strong genetic differentiation between the two populations analyzed. This finding is particularly surprising given the ~700 km geographic distance that isolates the two populations. Our results, therefore, confirm that geographical isolation may occur despite genetic homogeneity among populations, as observed in other freshwater fishes (Waters and Wallis, 2001).

Table 2. Number of haplotypes (H), number of polymorphic sites (S), Haplotype diversity (Hd) and nucleotide diversity (π), standard deviation (SD) and F_{ST} values (underlined value).

	Total	Sebkha Imlili	Oued Aabar
N	23	14	9
H	4	3	3
S	3	2	2
Hd $\pm$ SD	0.557 $\pm$ 0.108	0.484 $\pm$ 0.142	0.694 $\pm$ 0.147
$\pi\pm$ SD	0.00056 $\pm$ 0.00013	0.00046 $\pm$ 0.00015	0.00073 $\pm$ 0.00021
K	0.64	0.527	0.833
Sebkha Imlili	-	-	
Oued Aabar	-	<u>0.014</u>	-

In addition, the phylogenetic tree based on the

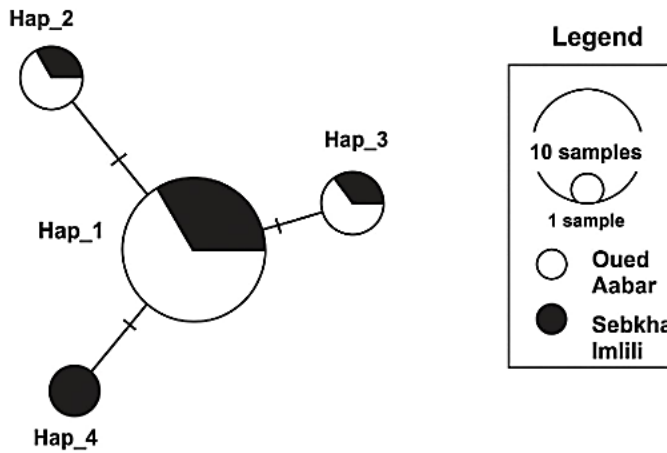


Figure 3. Haplotype network (based on Median-joining) of *Coptodon guineensis* based on the cytochrome b gene

cytochrome b gene showed that all Moroccan specimens cluster within a well-supported clade together with sequences from Cameroon, further supporting the low level of genetic structuring within the species. Notably, one Cameroonian sequence grouped closely with Moroccan samples, while the remaining sequences from the same river (Memé) (Bitja Nyom et al., 2021) formed a slightly distinct lineage, suggesting the presence of multiple haplotypes within a single locality.

We hypothesize that the low genetic differentiation detected between Oued Aabar and Sebkha Imlili, and the lack of evidence for isolation by distance, suggest the absence of strong barriers to gene flow in *C. guineensis*, despite their morphological differentiation (Louizi et al., 2020). This species' euryhaline nature and ability to tolerate extreme environments may promote dispersal and occasional connectivity between distant habitats. Historical

geological events may have facilitated rare long-distance dispersal events among *C. guineensis* populations, disrupting the development of an isolation-by-distance pattern across drainages. Rare transfers between the two drainages mediated by contemporary human activities may also occur.

The absence of genetic differentiation is commonly interpreted as evidence of panmixia (Ben Chehida et al., 2020). Thus, the two populations surveyed would exchange enough individuals to homogenize the genotypes (efficient migration). In addition, the low genetic diversity indicates limited genetic variation, suggesting either a recent demographic expansion, a founder effect, or a bottleneck event (Grant and Bowen, 1998; Avise, 2000). The slightly higher diversity observed in Oued Aabar (Hd = 0.694) than in Sebkha Imlili (Hd = 0.484) may reflect the retention of ancestral haplotypes or secondary contact.

Coptodon guineensis individuals in the Sebkha Imlili are found in different ponds and form a metapopulation rather than a single continuous population (Agnèse et al., 2018). Our sampling approach did not allow us to test for genetic differentiation among these ponds to evaluate a metapopulation structure.

Conclusion

This preliminary assessment revealed no genetic differentiation between the two populations of *C. guineensis* studied. The phylogenetic results further support this pattern, highlighting the close genetic relationships among Moroccan populations and their affinity with other conspecific lineages. However, our

limited sampling, both within Sebkha Imlili's multiple ponds and across the species' broader range, restricts interpretations. Future studies should include more localities, particularly the Nador canal, and better represent the sebkha's internal structure to clarify the genetic dynamics of the species in Morocco. The apparent genetic homogeneity across approximately 700 km suggests that the two populations may constitute a single management unit, with implications for conservation prioritization of this restricted-range species.

Acknowledgements

Many thanks to Morocco's Department of Water and Forestry for granting approval for fish collection. Funding provided by The Moroccan MENFPESRS and CNRST to Ahmed Yahyaoui (PPR/2015/1) is gratefully acknowledged.

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