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Original Article

Genetic diversity of populations of the endemic species *Coptodon walteri* from the Cavally River, Western Côte d'Ivoire

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Abstract: *Coptodon walteri* is a fish species of particular ecological importance due to its endemic status within the Cavally River basin. However, this hydrological basin is experiencing increasing environmental pressures, notably from gold mining, which may alter both the basin and the fish communities inhabiting it. Therefore, the objective of this study was to assess the genetic diversity of *C. walteri* populations, which to date have not been the subject of any genetic study in this region. A total of 120 individuals (30 per sampling station) were collected through experimental and artisanal fishing using gill nets. DNA from each specimen was extracted from a fragment of the caudal fin following a standard 2% CTAB protocol. To evaluate genetic diversity and population structure, seven microsatellite markers (GM005, UNH142, UNH146, UNH159, UNH174, UNH189, and UNH190) were analyzed. Genetic analyses were conducted using the software GenAlex, MEGA12, and STRUCTURE. The number of alleles per locus ranged from 2 to 9, with an average of 2. A total of 41 alleles were identified, corresponding to a mean polymorphism rate of 54% at a 95% confidence level. Despite this moderate level of polymorphism, the studied populations exhibited a pronounced heterozygote deficit (Ho = 0.01-0.10) and high inbreeding coefficients (Fis = 0.53-0.73), indicating strong genetic depression. High genetic differentiation across all analyzed loci was observed (Fst = 0.93), suggesting an almost complete absence of gene flow (Nm = 0.11) among sampling stations. Phylogenetic and population structure analyses revealed a clear separation of populations into three and four distinct groups, corresponding to their geographic locations. These results highlight severe genetic fragmentation and increased vulnerability in *C. walteri*, underscoring the urgent need to implement conservation measures.

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Introduction

Biodiversity conservation is a major ecological challenge in the 21st century, amid a global acceleration in the loss of living organisms (IPBES, 2019). The alarming increase in species extinction rates, affecting both animal and plant taxa, is a clear manifestation of the biodiversity crisis and is largely attributed to anthropogenic pressures (Mehdi, 2023). In response to this urgency, conservation biology relies on fundamental concepts to assess species vulnerability and to guide preservation strategies. Among these, genetic diversity, defined as the variability of genes within a species or population, plays a central role. It is considered the fundamental raw material of evolution, underpinning essential mechanisms such as adaptation and speciation (Gaillard et al., 2023).

The assessment of genetic diversity has therefore become an indispensable tool for evaluating the viability of threatened populations, their adaptive potential in the face of environmental fluctuations, and, more broadly, ecosystem resilience (Ellegren and Galtier, 2016; Dalongeville et al., 2022). Indeed, a diverse gene pool enhances a species' ability to cope with and adapt to biotic stresses (e.g., diseases and predation) and abiotic stresses (e.g., climate change and pollution) (Gaillard et al., 2023). Conversely, low genetic diversity is generally regarded as a limiting

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factor for short- and medium-term adaptive capacity, thereby increasing the risk of extinction (Romiguier et al., 2014; Ellegren and Galtier, 2016).

These issues are particularly relevant in regions subjected to intense anthropogenic pressures. In Côte d'Ivoire, mining activities constitute a significant threat to aquatic biodiversity. This activity has intensified in the upper catchment of the Cavally River, particularly near the locality of Ity (Konan et al., 2015; Kouassi et al., 2017), leading to habitat degradation and contamination with chemicals, such as mercury and cyanide. This fluvial ecosystem hosts an exceptional endemic species, *Coptodon walteri*, a fish listed as "Endangered" on the IUCN Red List (Richard et al., 2019; IUCN, 2020). Exposure to chemical contamination and environmental changes may increase this species' vulnerability and jeopardize its long-term survival (Sanogo et al., 2012).

In this critical context, it is imperative to obtain robust scientific data to assess the impact of these disturbances on *C. walteri* and to develop well-informed conservation plans. To date, scientific knowledge regarding this species remains particularly limited. Only one study, focusing on the length-weight relationship and condition factors, has been published (Yoboué et al., 2025). Although these parameters provide valuable insights into the immediate health status of individuals, they do not inform on the population's genetic resilience and adaptive capacity in response to environmental stressors.

Consequently, a detailed molecular characterization is essential to understand the genetic structure and diversity of *C. walteri* populations. The use of molecular markers, such as microsatellites, enables a fine-scale analysis of genetic polymorphism, offering unmatched resolution for assessing genetic variability within (intra-population diversity) and among (inter-population diversity) groups of individuals. Accordingly, the present study aims to examine genetic variation within and among populations of *C. walteri* in the Cavally River basin by analyzing polymorphisms at seven microsatellite markers. The results of this work are intended to provide an accurate diagnosis of the genetic health status of this threatened species, thereby supporting the formulation of scientifically grounded recommendations for its sustainable conservation.

Materials and Methods

Study area: The study was conducted along the Cavally River, located in western Côte d'Ivoire, within the Zouan-Hounien Department. This watercourse serves as a natural boundary between Côte d'Ivoire, Liberia, and Guinea. The river extends over approximately 700 km and drains a catchment area of about 28,800 km² (Doffou et al., 2024; Yoboué et al., 2025).

Selection of sampling sites: Four sampling stations were selected along a gradient of increasing anthropogenic pressure, primarily associated with gold mining activities. Their strategic distribution allows for a comparative assessment of environmental impacts. (1) *Niampleu*: Located upstream of mining areas, this station serves as a reference (control) site. Its presumably undisturbed habitat provides a baseline for the river's initial ecological condition. (2 and 3) *Bakatouo and Daapleu:* Situated immediately downstream of the industrial Ity gold mine, these sites are subject to marked anthropogenic influence. They are exposed to mining effluents and to illegal artisanal gold mining, which involves direct alluvial extraction through dredging within the river channel. This activity causes substantial sediment remobilization and significant alteration of aquatic habitats. (4) *Floleu:* Located farther downstream, this station is subject to intense anthropogenic pressure. Gold mining is carried out intensively both within the riverbed and along the banks, exacerbating erosion, substrate clogging, and the physicochemical contamination of the aquatic environment. Figure 1 shows the geographic locations of all sampling stations.

Sampling: Specimens of *C. walteri* were collected using two complementary approaches: experimental fishing and sampling from artisanal fishers. Experimental fishing involved setting gill nets with different mesh sizes (20-50 mm) in the evening (between 17:00 and 18:00), followed by retrieval the

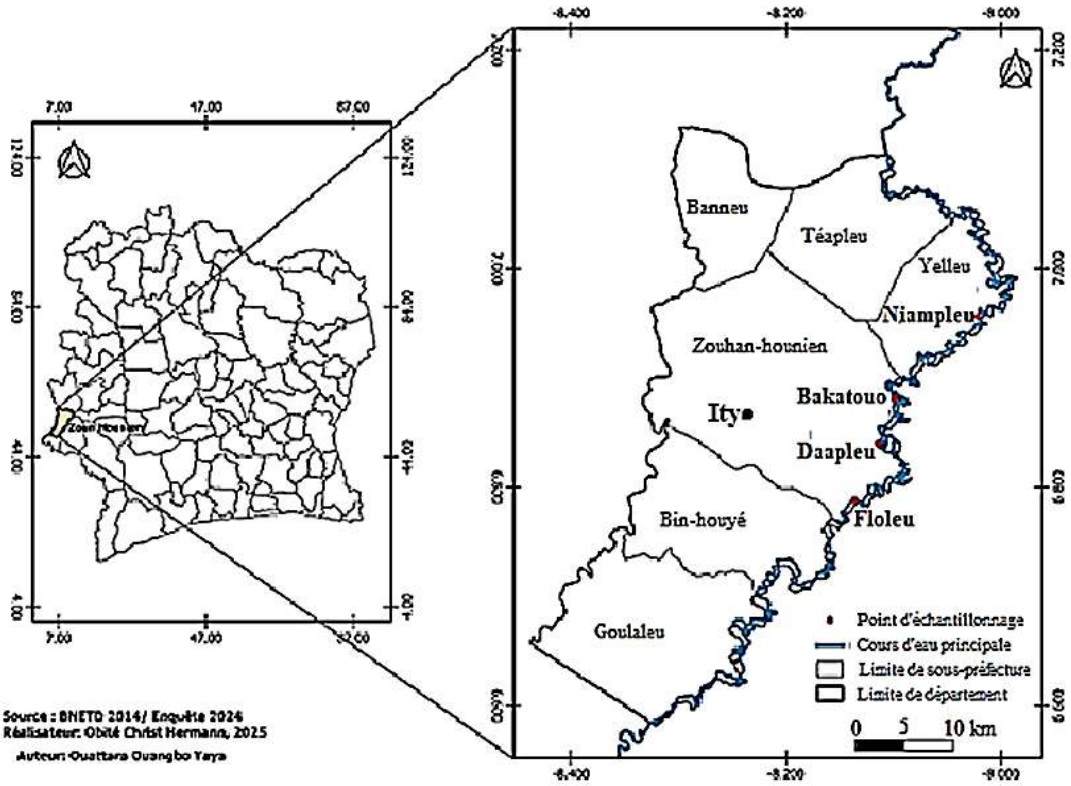

Figure 1. Location of the sampling stations for *Coptodon walteri* from the Cavally River, Western Côte d'Ivoire.

following morning (between 07:00 and 08:00). In addition, supplementary individuals were obtained from local fishers to complete the sampling for each station. A total of 120 individuals were sampled, with 30 specimens collected per station. Sampling was conducted from July 2023 to February 2024, with one sampling session per month at each station. For fish captured by experimental fishing, a fragment of approximately 10 mm from the upper part of the caudal fin was collected from each specimen. These samples were immediately preserved in 1.5 mL Eppendorf tubes and stored in a refrigerated cooler before the fish were released back into their natural habitat. Specimens obtained through artisanal fishing were carefully transported in an ice-filled cooler to the Molecular Genetics and Epidemiology Research Unit (URGEM) laboratory in Daloa. In the laboratory, approximately 9 mm of white muscle tissue was sampled from each individual for subsequent molecular analyses.

DNA extraction and polymerase chain reaction (PCR) amplification: Genomic DNA was extracted from each *C. walteri* individual using a standard 2% CTAB protocol (Mirimin et al., 2015). Seven microsatellite primer pairs (GM005, UNH142, UNH146, UNH159, UNH174, UNH189, and UNH190) were used to amplify genomic DNA. Each 20 µL PCR reaction mixture contained 9 µL of ultrapure water, 4 µL of 5× Ready-to-Load Master Mix (containing 12.5 mM $MgCl_2$), 1 µL of 10% DMSO, 1 µL of 10% BSA, and 3 µL of genomic DNA. After vortex homogenization, amplification was performed using a Bio-Rad thermal cycler under the following conditions: initial denaturation at 95°C for 5 min; 35 cycles consisting of denaturation at 95°C for 30s, annealing at 40-60°C for 45s (depending on the primer pair; Table 1), and extension at 72°C for 30s; followed by a final extension at 72°C for 5 min. For analysis, 5 µL of PCR product were mixed with 3 µL of loading dye, loaded onto a 2% agarose gel, and electrophoresed for 45 min. Amplified products were visualized by transillumination, photographed, and fragment sizes were determined using the semi-logarithmic method.

Table 1. Primers and annealing temperature for each locus.

Locus	Amorce Forward	Amorce Reverse	Tm
GM055	CATGCCTGTAAACCTTTTCC	ATCCAGCGTTTACAGAGTGAA	52
UNH142	CTTTACGTTGACGCAGT	GTGACATGCAGCAGATA	47
UNH146	CCACTCTGCCTGCCCTCTAT	AGCTGCGTCAAACTCTCAAAAG	52
UNH159	TTGTTTTAGGAGCTTCTTTTGTC	ATATTCATCTGGATTTGGCTCTAA	50
UNH174	TGAAAAATGGAATTTGG	TTAGATGAGATATGAAACTGC	40
UNH189	ATCGATGCTTTAAGAATCAG	TTCTCTGACATTTTTCAGC	60
UNH190	CGCGATCGAGCATTCTAA	TGTCTGCACGCGCTTTTGT	56

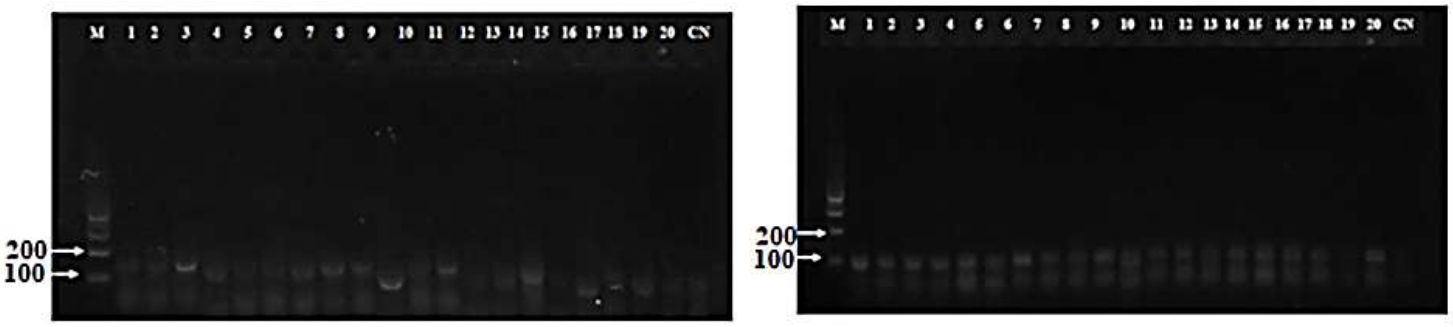

Figure 2. Electrophoretic profile of PCR products.

Data analysis: Genetic diversity within and among *C. walteri* populations was assessed using GenAlex version 6.51. Parameters estimated to quantify intra-population variability included allele frequency, percentage of polymorphic loci (P95%), mean number of alleles per locus (A), allelic richness, inbreeding coefficient (Fis), and observed (Ho) and expected (He) heterozygosity. According to the criterion defined by Mohamed (2010), a locus was considered polymorphic when the frequency of its most common allele was less than or equal to 0.95.

To evaluate inter-population genetic variability, genetic differentiation indices (Fst and Fit) and gene flow (Nm) were calculated. Population genetic structure was subsequently analyzed using STRUCTURE version 2.3.4, which allowed identification of the number of distinct genetic clusters present across the sampled stations. In addition, a matrix of genetic distances was generated using FreeNA software. This matrix was used to construct a phylogenetic tree using the Neighbor-Joining (NJ) method in MEGA version 12.

Results

Genetic diversity analysis

Allelic variability: Analysis of the seven microsatellite loci across all sampled individuals revealed a total of 41 alleles (Fig. 3). All loci were polymorphic, with the number of alleles per locus ranging from 2 to 9. Figure 2 illustrates representative electrophoretic profiles of the genotypes obtained. The UNH146 locus exhibited 2 alleles, UNH189 showed 5 alleles, GM005, UNH159, and UNH174 each displayed 6 alleles, UNH190 presented 7 alleles, and UNH142 exhibited the highest allelic diversity with 9 alleles (Fig. 3). The mean number of alleles per locus across all analyzed samples was 2 (Table 2).

Genetic polymorphism of microsatellite loci: Populations of *C. walteri* exhibited low within-population heterozygosity across all loci (Table 2). Among the four studied stations, 54% of loci were polymorphic at the 95% confidence threshold. Observed heterozygosity (Ho) was low, ranging from 0.01 to 0.10, with a mean of 0.04. Expected heterozygosity (He) was higher, ranging from 0.10 to 0.28, with a mean of 0.18. The inbreeding coefficients (Fis) confirmed this trend, with values ranging from 0.53 to 0.73 and a mean of 0.62.

Genetic differentiation of *C. walteri* populations: The values of genetic differentiation indices (Fst, and Fit) and gene flow (Nm) for all samples from the four stations (Niampleu, Bakatouo, Daapleu, and Floleu)

Table 2. Observed heterozygosity (Ho) and mean number of alleles per locus in the Niampleu, Bakatouo, Daapleu, and Floleu populations across all loci.

Population	Na	Ne	I	Ho	He	P*(95%)*	PHWE	Fis
Niampleu	2.15	1.34	0.30	0.01	0.19	57	0.000***	0.73
Bakatouo	1.29	1.20	0.20	0.03	0.10	29	0.000***	0.54
Daapleu	2.00	1.30	0.30	0.10	0.15	43	0.000***	0.53
Floleu	2.57	1.50	0.50	0.10	0.28	86	0.000***	0.62
Moyenne	2.00	1.30	0.30	0.04	0.18	54	0.000***	0.62
Ecart-type	0.23	0.10	0.10	0.00	0.04	12		0.09

Na: Number of alleles; Ne: Effective number of alleles; I: Shannon's index; Ho: Observed heterozygosity; He: Expected heterozygosity; P (%): Percentage of polymorphism; PHWE: Probability of Hardy–Weinberg equilibrium; Fis: Inbreeding coefficient.

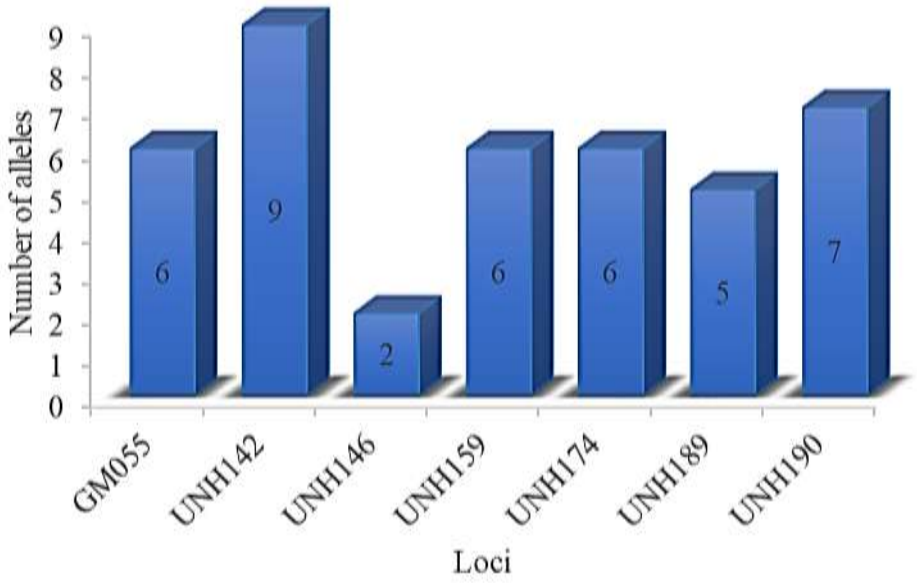

Figure 3. Variation in the mean number of alleles per microsatellite locus across all stations in *Coptodon walteri*. M: Size marker; 1-20: sample numbers; CN: Negative control.

are presented in Table 3. The Fst and Fit indices reveal strong genetic differentiation among populations, with high values observed across all loci. Fst ranged from 0.78 to 1.00, with a mean of 0.93, while Fit ranged from 0.43 to 0.85, with a mean of 0.73. Gene flow (Nm) was low for all microsatellite markers, ranging from 0.04 to 0.33, with a mean of 0.11.

Phylogenetic relationships of *C. walteri* populations: A phylogenetic analysis conducted using MEGA12 with the Neighbor-Joining (NJ) method revealed three main genetic groups (GI, GII, and GIII). Group I consisted entirely (100%) of individuals from Niampleu and Bakatouo, Group II was composed exclusively of individuals from Floleu (100%), and Group III included all individuals (100%) from Daapleu (Fig. 4). This phylogenetic structure shows a clear separation by sampling station, confirming strong genetic differentiation among *C. walteri* populations across the four stations.

Genetic structure of *C. walteri* populations: Population clustering was performed using STRUCTURE version 2.3.4 following the Evanno method. The most significant result was obtained for K = 4 (Fig. 5). This analysis revealed four distinct genetic groups that correspond precisely to the four sampling stations. The first group comprised individuals from Niampleu, the second from Bakatouo, the third from Daapleu, and the fourth from Floleu.

Discussions

A set of seven microsatellite loci was used to assess genetic variation in *C. walteri* across four stations along the Cavally River, which are affected by gold

Table 3. Values of Fit, Fst, and Nm among *Coptodon walteri* populations.

	GM055	UNH142	UNH146	UNH159	UNH174	UNH189	UNH190	Moy
Fit	1.00	0.81	1.00	0.78	0.92	1.00	0.98	0.93
Fst	0.85	0.43	0.74	0.78	0.66	0.91	0.75	0.73
Nm	0.04	0.33	0.08	0.06	0.12	0.024	0.083	0.11

Fit: Total fixation index; **Fst**: Genetic differentiation index; **Nm**: Number of migrants.

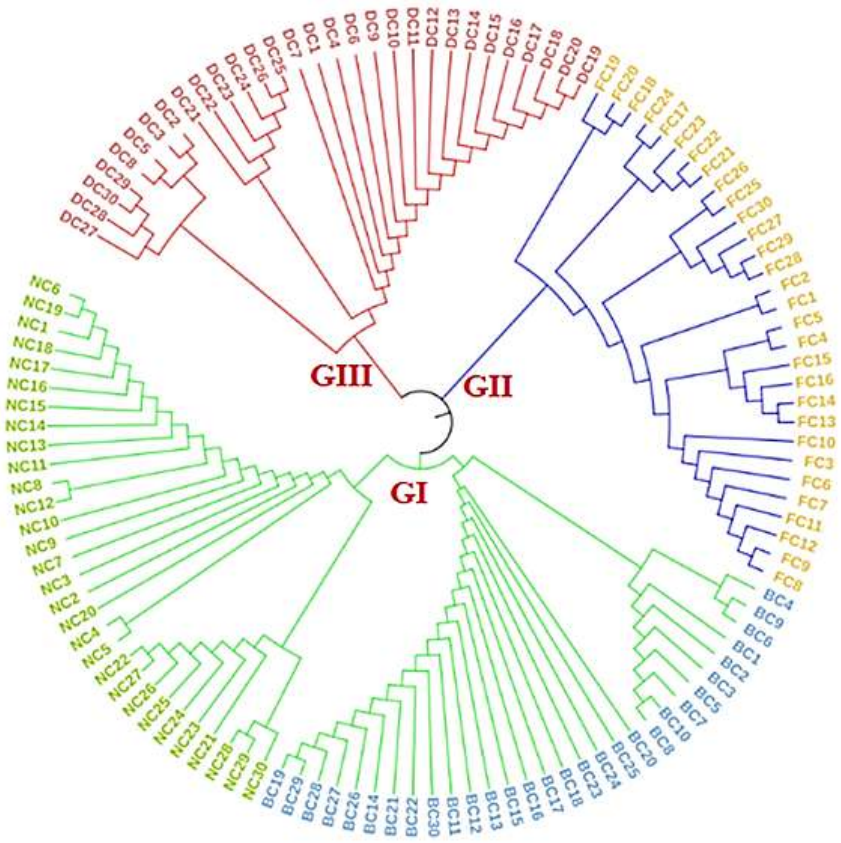

Figure 4. Phylogenetic tree of *Coptodon walteri* individuals based on the Neighbor-Joining (NJ) method.

mining in western Côte d'Ivoire. Each marker was polymorphic, allowing identification of 41 alleles, with a mean of 2 alleles per locus. This allelic diversity is comparable to the 2.9 alleles per locus reported by Dembélé et al. (2023) in a study of *C. zillii* in Mali. A higher value (5.89 alleles per locus) was observed in a study on *Oreochromis niloticus* in Benin (Amoussou, 2017). The low allelic diversity observed in the genus *Coptodon* may therefore be intrinsic to the genus.

The results also revealed a pronounced heterozygote deficit (Ho = 0.01-0.10 vs. He = 0.10-0.28), associated with high inbreeding coefficients (Fis = 0.53-0.73), indicating strong inbreeding within populations. Similar results were reported by Wognin et al. (2020) in six populations of *Heterobranchus longifilis* in Côte d'Ivoire, with Fis values above zero. This imbalance may also result from the presence of null alleles or from difficulty distinguishing genotypes of similar size due to stutter bands on dinucleotide microsatellite electropherograms, potentially leading to an underestimation of heterozygosity (Yoboué et al., 2014). Low genetic variation may also be driven by habitat loss, habitat fragmentation, overexploitation, and other anthropogenic activities (Exposito-Alonso et al., 2021; Sean et al., 2023).

In this study, exceptionally high genetic differentiation was observed (Fst = 0.93), far exceeding values reported by Monique et al. (2019) in *O. niloticus* populations in Madagascar (Fst = 0.038-0.379) and by Dembélé et al. (2023) in *C. zillii* in Mali

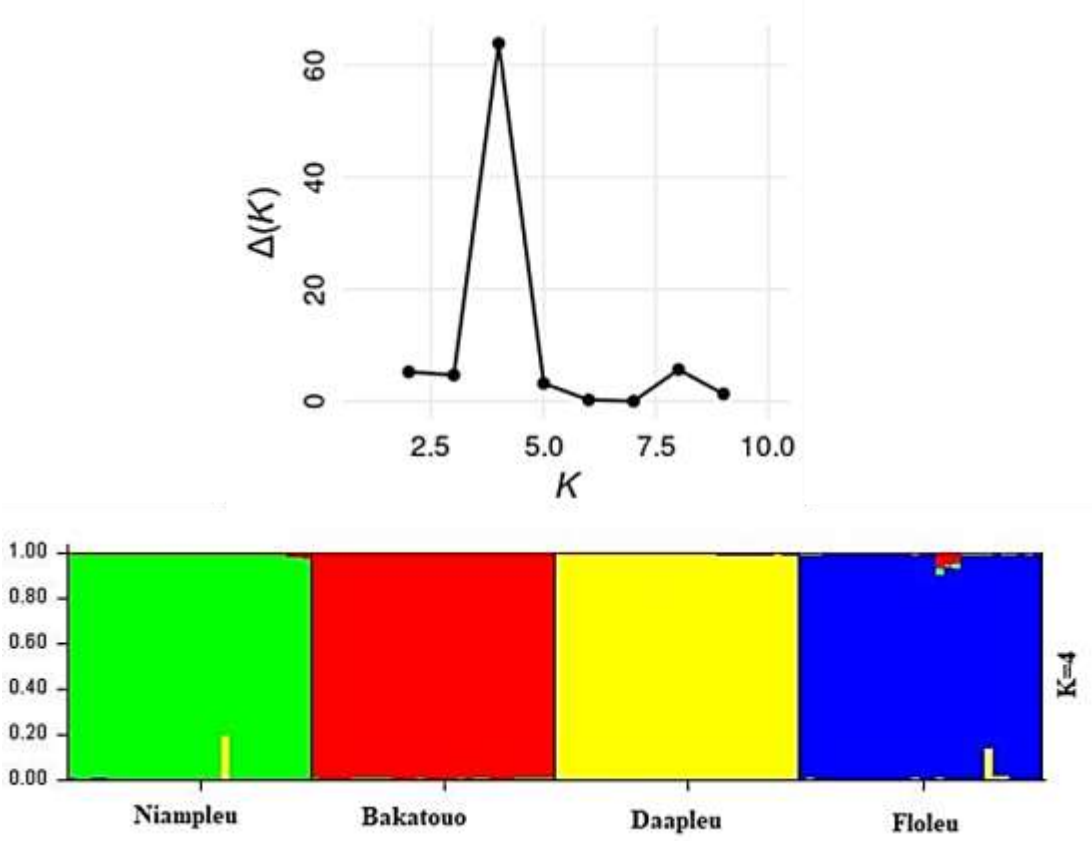

Figure 5. Population structure of *Coptodon walteri* based on the admixture method according to ΔK.

(Fst = 0.02). These results indicate a near-complete absence of genetic connectivity among *C. walteri* populations. Extremely low gene flow (Nm = 0.11) confirms this pattern of near-total genetic isolation, in contrast to patterns observed in *C. zillii* populations from different river basins in Mali (Dembélé et al., 2023).

Phylogenetic analysis separated *C. walteri* populations into three distinct genetic groups: Group I (GI) comprising individuals from Niampleu and Bakatouo, Group II (GII) including individuals from Floleu, and Group III (GIII) comprising individuals from Daapleu. Additionally, STRUCTURE analysis revealed clear genetic structuring among populations, classifying them into four groups consistent with their geographic origin, highlighting limited gene flow between distinct zones (Sbiba et al., 2024). This genetic distribution aligns with observations by Mergeay et al. (2021) and Hodan et al. (2021), which emphasize the stability of genetic signatures in isolated river systems. Overall, these results, which are more extreme than those in most previous reports, raise major concerns about the long-term viability of *C. walteri* populations. Consistent with the recommendations of Chloé et al. (2022) and the IUCN (2023), such high levels of genetic isolation and inbreeding necessitate urgent conservation measures, potentially including controlled genetic transfers among populations. However, these interventions must be carefully managed to preserve local adaptations and prevent erosion of genetic diversity, given the strong differentiation observed among groups.

Conclusion

The analysis of genetic diversity in *C. walteri* from the Cavally River reveals a concerning situation. Although the species exhibits moderate polymorphism (2 alleles per locus), populations show severe heterozygote deficits and high inbreeding rates, indicating substantial genetic depression. High genetic differentiation (Fst = 0.93) and near-zero gene flow (Nm = 0.11) indicate complete population fragmentation, with no connectivity among the

stations studied. Clear genetic structuring into multiple distinct groups, confirmed by phylogenetic and STRUCTURE analyses, highlights the deep genetic isolation of populations, likely exacerbated by anthropogenic pressures from gold mining. Compared with other species studied in West Africa, *C. walteri* exhibits greater genetic vulnerability, raising concerns about its ecological resilience. These findings underscore the urgent need for conservation measures, such as carefully managed genetic transfers, while preserving local adaptations. They provide a solid scientific basis to guide sustainable fisheries management in fragmented river systems. Furthermore, broader phylogenetic studies based on DNA sequencing could offer a more comprehensive view of diversity and relationships within the species.

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