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Morphological and Genetic Differentiation of Fish of the Subgenus *Chrysichthys chrysichthys* from Ivorian Rivers

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Abstract

Fish of the *Chrysichthys* genus are subject to intense exploitation, which is threatening the available stock. The implementation of a sustainable resource management plan requires a good knowledge of the biodiversity of this genus. The aim of this study was to determine the morphological and genetic characteristics of the fish of the subgenus *Chrysichthys chrysichthys* in order to identify descriptors for differentiating the species of this subgenus present in Côte d'Ivoire. The biometric analysis involved forty-one morphological measurements taken from each of the 255 specimens sampled in five hydro systems. The genetic analysis of the D-loop mitochondrial DNA sequence was carried out in 34 specimens. The amplicons obtained by PCR were subjected to sequencing. Individual analysis of morphometric characters divided the subgenus into two different species: *C. maurus* and *C. auratus*. The high classification rate (>90%) of the Discriminant Factor Analysis confirmed the existence of these two species. Wilk's Lambda test revealed thirteen discriminating descriptors. Phylogenetic analysis confirmed the existence of both species. Within the *C. maurus* species, fish from the river Bia are genetically different from those from other rivers. On the other hand, this population is fairly close to that of the *C. auratus* species, which is also divided into two different clades. Finally, molecular analysis corrected the taxonomic status of the fish.

Keywords

Chrysichthys, Morphological, Characterization, Mitochondrial DNA, Genetic Diversity

1. Introduction

For the majority of the human population, fish resources represent one of the main sources of protein for a quality diet. Fish in particular is important for ensuring food security [1]. In Côte d'Ivoire, fish of the genus *Chrysichthys*, commonly known as "mâchoiron", whose flesh is highly prized by the local population, is subject to intense exploitation pressure and could be threatened by overfishing. To avoid the collapse of stocks, a rational and sustainable resource management plan needs to be put in place. This requires a sound knowledge of the biodiversity of fish of this type. In addition, the *Chrysichthys* genus has been used in aquaculture since the early 1980s to meet the very high demand for this fish in the Ivorian market. However, the vast majority of research work on species in this genus has been carried out mainly in the fields of biology, ecology and reproduction [2] [3]. For a long time, work to identify and classify *Chrysichthys* species was based on morphological and biometric characters. Thus, on the basis of anatomical and morphological studies of a large number of specimens in museum collections, Risch [4] revised the taxonomic status of species in the genus *Chrysichthys.* Within this genus, several sub-genera have been determined, including the sub-general *Chrysichthys melanodactylus* and *Chrysichthys chrysichthys*, which have been identified in Ivorian rivers. Within the subgenus Chrysichthys chrysichthys, two species: *Chrysichthys maurus* and *C. auratus* have been described (Risch, 1986). The first work on the population genetics of this siluriform was carried out by Agnèse in 1989, as part of a study on the genetic differentiation of several species of West African Siluriformes of interest to fisheries and aquaculture. Using the results of enzyme electrophoresis, he confirmed the synonymies established by Risch [4]. He demonstrated the phyletic relationships that exist between the different populations of species in the genus *Chrysichthys* [5] [6]. Even today, however, difficulties persist in identifying morphologically related species. Distinguishing between species of the genus *Chrysichthys* is not always easy, because, for individuals of comparable size, interspecific morphological differences are minimal, whereas intra-specific variability can be very great [7]. The aim of this study is therefore to determine the morphological and molecular characteristics of fish of the genus *Chrysichthys* in the rivers of Côte d'Ivoire. In fact, these two methods have been widely used to differentiate certain species of fish [8] [9].

2. Material and Method

2.1. Study Area

Côte d'Ivoire has a vast hydrographic network of rivers and lagoons linked by artificial channels. Fish of the subgenus *Chrysichthys chrysichthys* were sampled in five hydro-systems from East to West: the Bia River, the Aby Lagoon, the Ebrié Lagoon, the Bandama River and the Grand-Lahou Lagoon (**Figure 1**).

2.2. Morphometric Analysis

The morphological analysis included 245 specimens of the subgenus *Chrysichthys*

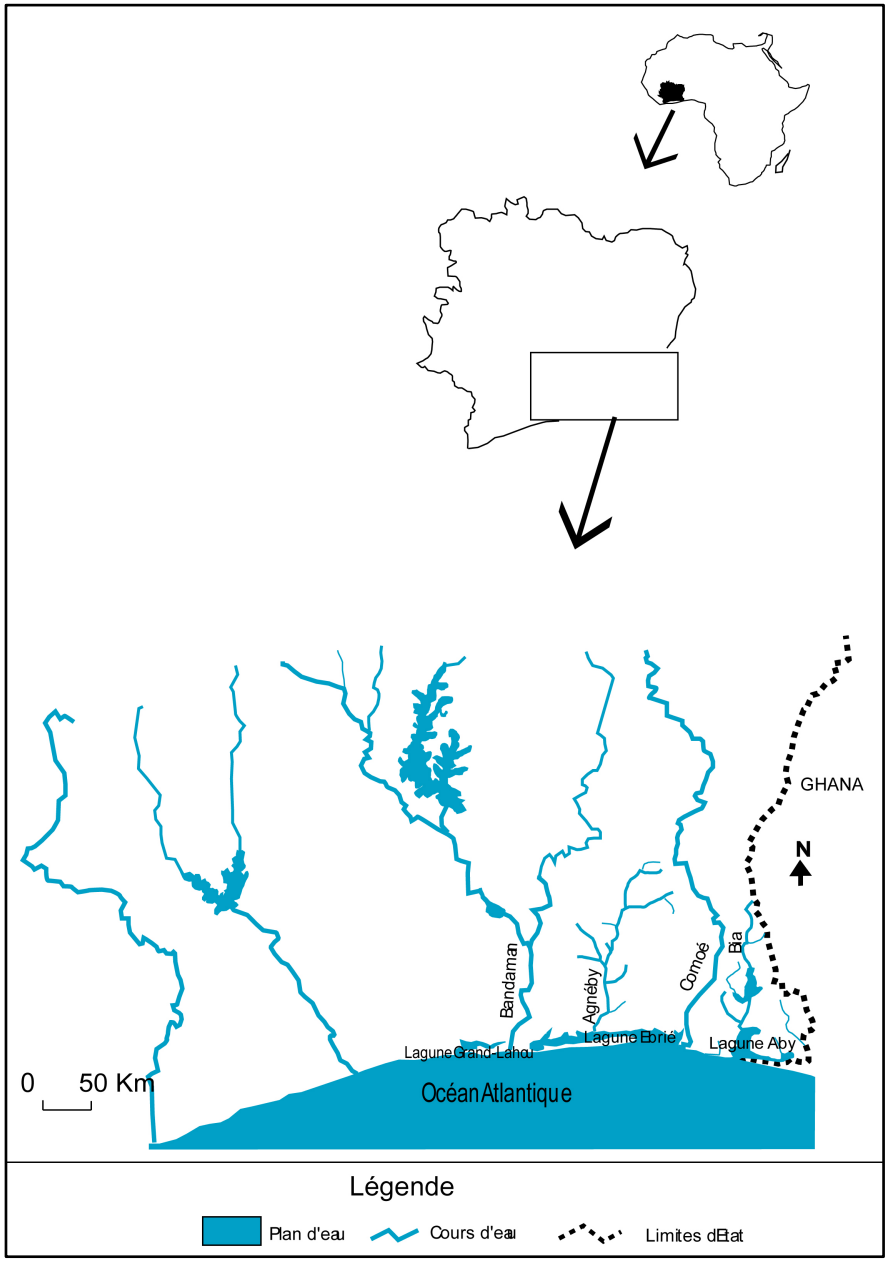

Figure 1. Sampling sites for *Chrysichthys* fish.

chrysichthys. The fishes come from the sampling campaign that took place from 2012 to 2014. Forty-one conventional characters were measured on each specimen with digital callipers on the left side of the specimens and rounded to the nearest 0.05 mm (**Figure 2**).

To reduce the allometric effect, all morphometric characters were transformed into ratio to the head length (HL) for the measurements recorded on the fish's head or into ratio to the standard length (SL) for the measurements performed on fish's body [10] [11] [12].

The species-less method requires that the morphometric parameters be recorded on a large number of individuals from the populations studied. Several

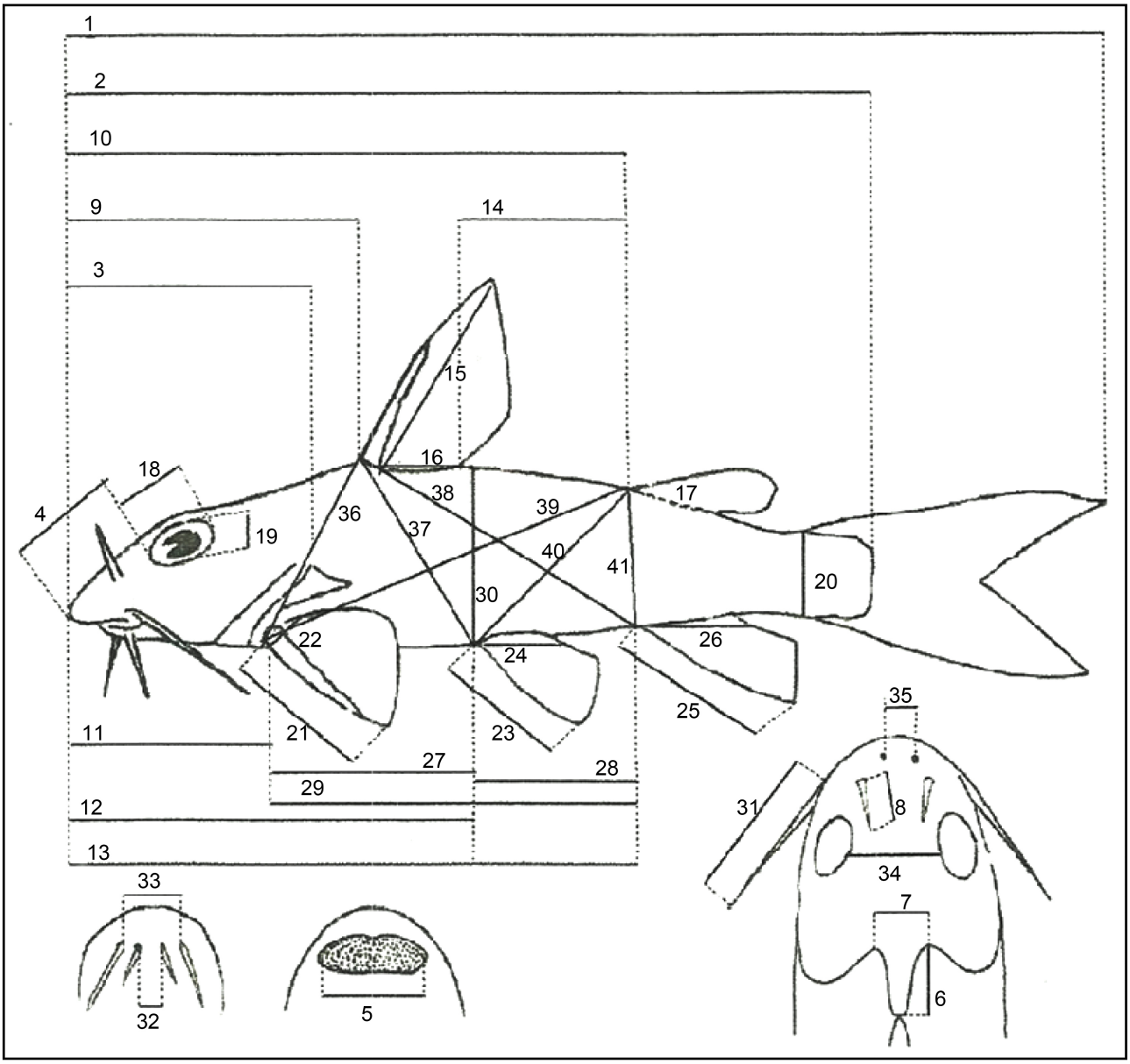

Figure 2. Metrics measurements taken from the individuals of the four species of *Chrysichthys*. **NB**: **1.** total length (TL); **2.** standard length (SL); **3.** head length (HL); **4.** snout length (SnL); **5.** width of premaxillary toothplate (WPm T); **6.** occipital process length (OPL); **7.** occipital process width (OPW); **8**. nasal barbel length (NBL); **9.** predorsal length (DsL); **10.** preadipose length (AdL); **11.** prepectoral length (PtL); **12.** prepelvic length (PlL); **13.** preanal length (AnL); **14.** distance between dorsal and adipose fins (DDsAd); **15.** dorsal fin heigth (DsH); **16.** dorsal base (DsB), **17.** adipose base (AdB), **18.** eye diameter horizontal (ED1); **19.** eye diameter vertical (ED2); **20.** caudal peduncle length (CPcL); **21.** pectoral height (PtH); **22.** pectoral base (PtB); **23.** pelvic height (PlH); **24.** pelvic base (PlB); **25.** anal height (AnH); **26.** anal base (AnB); **27.** distance pectoral/pelvic (DPtPl); **28.** distance pelvic/anal (DPlAn); **29.** distance pectoral/anal (DPtAn); **30.** body height (BdH); **31.** mandible barbell length 1 (MBlL1); **32.** mandible barbell length 2 (MBlL2); **33.** mandible barbell length 3 (MBlL3); **34.** distance inter-orbital (DIO); **35.** distance inter-nostril (DIN); **36.** distance pectoral/dorsal (DPtDs); **37.** distance pelvic/dorsal (DPlDs); **38.** distance anal/dorsal (DAnDs); **39.** distance pectoral/adipose (DPtAd); **40.** distance pelvic/adipose (DPlAd); **41.** distance anal/adipose (DAnAd).

comparisons are first made between the specimens. The differences between them "character by character" are noted. Then, species groups are formed and intra- and inter-specific variations are highlighted. Discriminant Factor Analysis

was run to test the effectiveness of the characters in predicting different species location. For this analysis, a stepwise inclusion procedure was carried out to reduce the number of characters according and to identify the combinations of characters that best separated species [13] [14]. The percentage of correct classification of individuals is determined to assess the effectiveness of the discriminant analysis [15] [16] [17]. These analyses were carried out using the program STATISTICA 7.1.

2.3. Genetic Analysis

The genetic analysis was carried out on 34 specimens of the subgenus *Chrysichthys chrysichthys*. Each of the specimens analyzed was assigned to a species on the basis of the classification made from the morphometric analysis. Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method [18]. The segment of the mtDNA D-loop was amplified using fish primer: 5'ACCCCTAGCTCCCAAAGCTA3' (Forward) and 5'CCTGAAGTAGGACCAGATG3' (reverse). The PCR reaction was performed in a final volume of 50 µl containing: 10 µl template DNA, 25 mM $MgCl_2$, 100 µM of dNTPs, 10 µM of each primer, 10 X reaction buffer and 1 unit of Taq DNA polymerase. Initial denaturation was 4 min at 94˚C, followed by 35 cycles of 30 s at 91˚C for the denaturation, 1 min at 52˚C for annealing, 1 min at 72˚C for the extension and a final extension at 72˚C for 5 min. Direct sequencing of the reverse strand was performed for each amplified fragment.

The sequences were aligned and compared with each other using the Clustal X program in the Geneious software [19]. A genetic distance matrix was constructed using the distance index of Nei [20]. The phylogenetic study provided information on the genetic proximity of specimens of the species and the relationships between individuals are visualized on the phenogram [21] [22] [23].

3. Results

3.1. Morphometric Analysis

The descriptor analysis showed a bimodal distribution for the anal-dorsal distance (DAnD). This character was chosen for the division of specimens of the subgenus *Chrysichthys chrysichthys* into two taxonomic entities: G11 and G12. The anal-dorsal distance of the G11 group is between 34.6% and 46% LS and that of the G12 group is between 46% and 57.8% LS (**Figure 3**).

The result of the discriminant analysis was shown in **Table 1**. The stepwise discriminant analysis identified 13 descriptors that discriminated the studied species. According to the importance of their discriminant power, there are DPtPl (λ = 0.75), DPlAn (λ = 0.84), HL (λ = 0.89), DPtAd (λ = 0.90), DO2 (λ = 0.91), MBL2 (λ = 0.94), AnL (λ = 0.94), BdH (λ = 0.96), CPcl (λ = 0.96), DsB (λ = 0.97), DDsAd (λ = 0.97), DsL (λ = 0.98) and DPtAn (λ = 0.98).

The discriminant analysis confirmed 99.59% of the total classification (**Table 2**). The predicted classification was 99.43% for subgroup G11 and 100% for

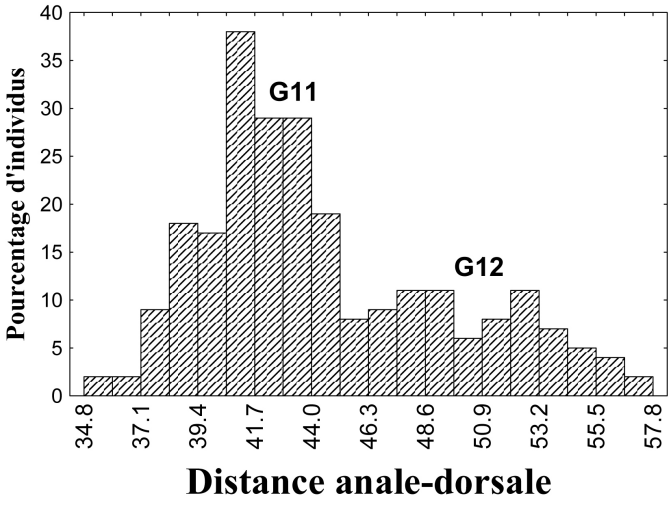

Figure 3. Frequencies of anal-dorsal distance distribution of specimens of the subgenus *Chrysichthys chrysichthys*.

Table 1. Multivariate Wilk's Lambda (λ) significance tests of metric variables in group G1.

Descriptors	λ	F	P
DPtPl	0.75	76.6	***
DPlAn	0.84	43.82	***
HL	0.89	28.30	***
DPtAd	0.90	26.8	***
ED2	0.91	22.46	***
MBL2	0.94	15.04	***
AnL	0.94	13.71	***
BdH	0.96	10.87	**
CPcl	0.96	8.33	**
DsB	0.97	6.55	*
DDsAd	0.97	5.84	*
DsL	0.98	5.08	*
DPtAn	0.98	5.01	*

F: statistical value of the discriminant analysis; λ: statistical value of the test; p: probability, *: $p < 0.05$; **: $p < 0.01$; ***$p < 0.001$. DPtPl: pectoral/pelvic distance, DPlAn: pelvic/anal distance, HL: head length, DPtAd: pectoral/adipal distance, DO2: eye diameter 2, MBl2: mandibular barbel length 2, AnL: pre-anal length, BdH: body height, CPcl: height of caudal peduncle, DsB: length of dorsal base, DDsAd: dorsal to adipal distance, DsL: predorsal length, DPtAn: pectoral to anal distance.

subgroup G12. Only one specimen of subgroup G11 was misclassified.

3.2. Molecular Analysis

After cutting off the primers from both ends prior to alignment, a total of 427

Table 2. Classification matrix of individuals in subgroups G11 and G12 by discriminant analysis of metric traits.

	Percentage of correct classification (%)	Number of well-classified specimens	
		Gr 11	Gr 12
Gr 11 (n = 174)	99.43	173	1
Gr 12 (n = 71)	100	0	71
Total (n = 245)	99.59	173	72

n = number of specimens.

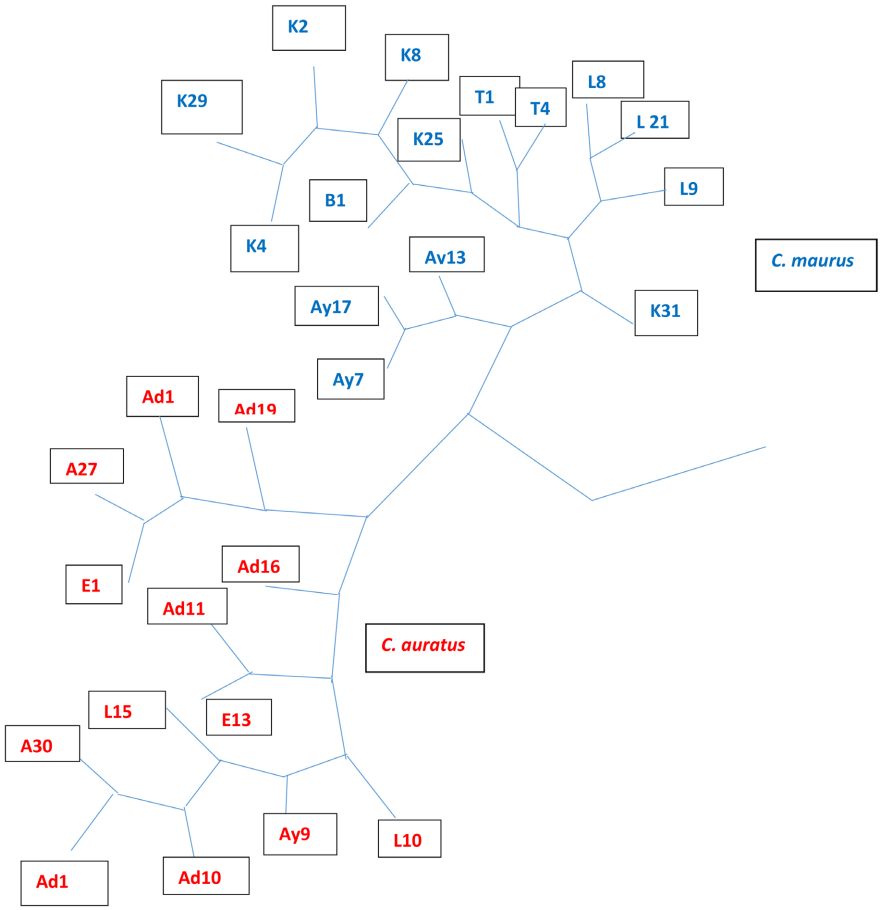

Figure 4. Neighbors-Joining phenogram illustrating the relationships between sample specimens of subgenus *Chrysichthys chrysichthys*.

nucleotides sites were obtained. Eight haplotypes have been detected and are grouped into three haplogroups: HG1, HG2 and HG3. The haplogroup HG1 is found in *C. auratus* and includes the haplotypes H1 (Ad1, A30, L15, Ad10, Ad11, E13, Ay9, L10, Ad16), H2 (Ad19, E1); H3 (A27) and H4 (Ad15). The haplogroups HG2 and HG3 are characteristic of the species *C. maurus*. The haplogroup HG2 comprises the haplotypes H5 (K25, K31, K8, K29, K2, K4) and H6 (L9, L8, L21, B15, T15', T4) while haplogroup HG3 is composed of haplo-

types H7 (Ay7, Ay13) and H8 (Ay17). Within each group, the sequences differ from each other by only one or two nucleotides. HG1 is distinguished by six to eight mutations from HG2 and four to five mutations from HG3 while HG2 and HG3 differ by three to five mutations.

The Hierarchical Cluster Analysis based on Neighbord Joinning was represented in **Figure 4**. The dendrogram revealed that within each species, the specimens were clustered into two distincts groups; the genetic distance between these two entities varies between 0.02 and 0.04.

4. Discussion

Morphometric variables are important elements in the study of species systematics. The biometric analysis, including morphometric characters, has been adopted by many authors to identify different fish races or populations [24] [25] [26]. Despite the low number of discriminant variables in our study, the high percentages of classification (99.43% for G11 and 100% for G12) are obtained for groups, indicating that the morphometric descriptors used have an important taxonomic. The high percentages of correct classification (>99%) confirm that groups G11 and G12 are distinct from each other. This suggests the existence of two distinct species within the subgenus *Chrysichthys chrysichthys*. Individuals of G11 are characterized by a large, elongated body, an elongated head and large eyes. In addition, specimens from the Bandama River are characterized by a long filament on the dorsal fin. This feature was reported by Risch [4] in the description of the species *C. maurus*. Individuals in G12 are defined by a shorter and lower body, a less elongated head and smaller eyes. Based on the descriptions by Risch [4], G11 and G12 are assigned to the species *Chrysichthys maurus* and *C. auratus* respectively.

The genetic variations observed on the mitochondrial marker concerned sequence polymorphism (number and distribution of nucleotide substitutions), genetic diversity within each river and the relationships between individuals visualized on the phenogram. These methods of studying population genetics are subjects that are widely covered in the literature [27] [28]. Analysis of polymorphism and sequence similarity led to the classification of specimens in the subgenus *Chrysichthys chrysichthys*. Three groups of genetically differentiated haplotypes G1, G2 and G3 appear within this subgenus. Groups G1 and G3 contain specimens of the species *C. maurus*, which segregate together. However, these two groups are quite far apart in terms of the number of nucleotide substitutions and genetic distance. In fact, the number of mutations separating these two entities is greater than or equal to four (4) and the genetic distance between them is high. There is therefore a high level of genetic differentiation between these two groups. This suggests that there is little gene flow between them. Group G1 is made up of specimens from the Bandama River and the Ebrié and Grand-Lahou lagoons, while group G3 comes from the Bia River. There are therefore two genetically different populations within this species. This differ-

ence seems to be linked to the geographical distance of the River Bia from the other rivers. During work carried out on populations of *Pteropus marianus* on the Mariana Islands and Palau, the study of microsatellite and mitochondrial markers (D-loop, COI and Cytochrome b) highlighted the genetic isolation of the Palau population from those of the Mariana Islands [29]. Using genetic methods, Meyer [30] highlighted the phenomenon of isolation through distance in his work on tropical bats. Group G2 contains specimens of the species *C. auratus*. This species can also be distinguished into two different populations that are genetically quite close to specimens of the species *C. maurus* from the river Bia. This could be due to the fact that this species is essentially made up of individuals from the Aby lagoon, which is directly linked to this river. This great genetic similarity could also be explained by the fact that these two species are sympatric in the region under consideration [4]. Furthermore, in some specimens, there is no concordance between morphological differentiation and genetic differentiation. Within group G2, with the exception of individuals A30 and A27, the other specimens (Ad1, L15, Ad10, Ad11, E13, Ay9, L10, Ad16, Ad19, E1 and Ad15) are morphologically similar to the species *C. maurus*. However, molecular analysis has shown that they belong to the *C. auratus* species. During work on *Liza abu* stocks in three rivers, genetic data did not confirm the phenotypic variations detected [26].

5. Conclusion

Characterization based on fish morphology demonstrated the validity of the morphometric descriptors traditionally used to describe the different species of fish in the genus *Chrysichthys*. Despite the small number of samples, the genetic analyses demonstrated the value of population genetics in understanding both the biodiversity of species in the subgenus *Chrysichthys chrysichthys* and the genetic structure of the various populations in the rivers sampled. The results of the genetic analysis of fish of the genus *Chrysichthys* provided information on the genetic proximity of the specimens studied. The morphometric study combined with the molecular analysis therefore clarified the taxonomic status of the species of the subgenus *Chrysichthys chrysichthys* in the Ivorian rivers sampled. It would therefore be important to extend sampling to all Ivorian hydrosystems. In addition, the use of several different types of markers (*Cytochrome oxydase*, microsatellites) will enable additional information to be acquired.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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