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Morphological and genetic characterization of *Chrysichthys* species from the Bia river (Cote D'ivoire)

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Abstract
We conducted this study in order to determine the morphometric and the genetic variation of four species of *Chrysichthys* genus (*Chrysichthys nigrodigitatus, C. maurus, C. auratus, C. Johnelsi*) from the Bia River. The discriminant analysis and cluster analysis were used to study the morphological variation. Discriminant analysis with selected 9 morphological parameters showed that the identification accuracy was between 93.33% and 100%, and global identification accuracy was 95, 83%. The results of cluster analysis indicated that the four species were clustered into three distinct groups: the first group included *C. maurus* and *C. nigrodigitatus*; the second group *is composed of C. auratus;* whereas the last group is made of *C. johnelsi*. Mitochondrial DNA sequencing of the nine *Chrysichthys* samples showed that a total of eight haplotypes for the two subgenera (*C. chrysichthys* and *C. melanodactylus*). The genetic diversity among *C. chrysichthys* is determined by haplotype and nucleotide diversities were 1 ± 0.177 and 0.023 ± 0.01, respectively whereas the values for the population of *C. melanodactylus* were 0.9 ± 0.161 and 0.022 ± 0.006. The High genetic diversity values and the non-significant neutrality tests found in this study, suggest that *Chrysichthys* population from the Bia River had a long evolutionary history in a large stable population.

Keywords: *Chrysichthys* species, mitochondria DNA, genetic diversity, Bia river, Côte D'ivoire

1. Introduction
The catfish *Chrysichthys*, a siluroid fish of the family Claroteidae is widely distributed in fresh and brackish waters in West Africa [1]. *Chrysichthys nigrodigitatus* is highly valued food fish in Ivory Coast and is among the dominant fish of commercial catches. The culture of this fish is widely practiced in many areas and constitutes one of the largest groups of farmed freshwater fish. The method of confinement of broodstock for reproduction is well vulgarized, but still poorly controlled by the majority of fish farmers. In addition, deficiency of fry production remains one of the main obstacles limiting the expansion of intensive fish production [2]. Therefore, the collection of juveniles in the natural environment seems to be an alternative for overcoming these obstacles [3]. However, this method remains subject to seasonal and inter annual variations in catches, their size, storage and post-harvest transport [4]. There is also a risk of mixing species with unequal performances. Indeed, the identification of species of the genus *Chrysichthys* is not always easy because at the same size, there are few interspecific morphological differences whereas the intra-specific variability can be very large [5]. Hence, the knowledge on the identity of the species chosen for culture is an impelling necessity to eliminate mixing of species [6]. The choice of the stocks to be improved must be based on a good knowledge of the diversity of the wild populations and of the species likely to possess interesting physiological characteristics [7].
The objective of this study is to differentiate and characterize the species of the genus *Chrysichthys* from the Bia river. In this work, we use morphometric and genetic analyzes to illustrate intra and inter- specific variations and to determine the validity of morphological characters and sequences of mitochondrial DNA in fish stock unit identification.

2. Materials and Methods
2.1 Morphometric analysis
A total of 71 specimens of four *Chrysichthys* species (*Chrysichthys maurus, C. auratus, C. nigrodigitatus* and *C. Johnelsi*) were collected in the lake of Ayame located on Bia River (4°8' N, 6°9'W) (Fig. 1).

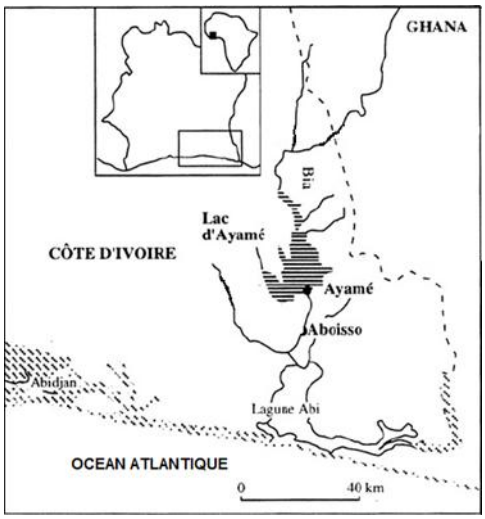

Fig 1: Geographic location of *Chrysichthys* sampling in the Bia River

On each specimen, forty-one conventional characters were measured with digital callipers to the nearest 0.05 mm (Fig. 2).

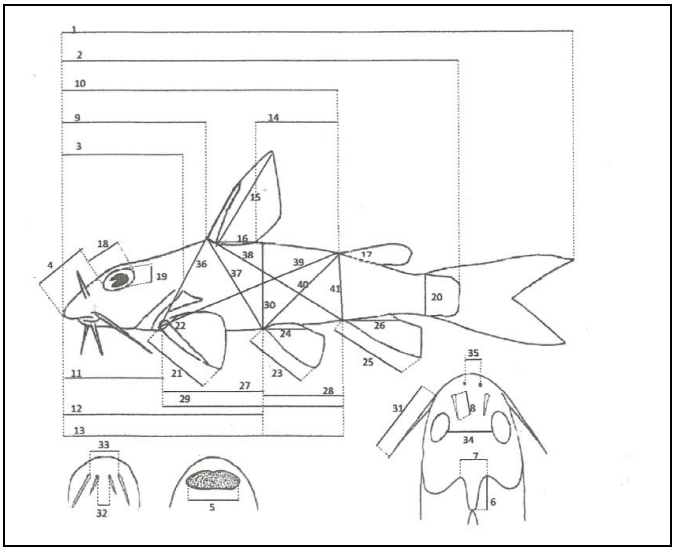

Fig 2: Metrics measurements taken from the individuals of the four species of *Chrysichthys*

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 (NBlL); 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).
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 [8].
For each morphometric variable, analysis of variance (ANOVA) and the Kruskal-wallis test were first used to evaluate significant differences among the species. The thirty-two characters which presented significant differences between the four species were submitted to the Stepwise multivariate Discriminant Analysis (SDA). DFA 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 [9; 10]. The percentage of discrimination per pair of groups was estimated as the proportion of correctly classified individuals of four groups on the total classified individuals. Hierarchical Cluster Analysis (HCA) based on Mahalanobis distance matrices determined with DFA, was used to evaluate population relationships [11]. The hierarchical clustering process was represented as a dendrogram, where a join of the tree illustrated each step in the clustering process. The minimum variance clustering method or Ward's method was used with the Euclidean distances [12]. All treatments were performed using the program STATISTICA (StatSoft, version 7.1).

2.2 Genetic analysis

Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method [13]. The segment of the mtDNA was amplified using fish primer: 5'ACCCCTAGCTCCCAAAGCTA3' (Forward) and 5'CCTGAAGTAGGACCAGATG3' (reverse**).** PCR reaction was performed in a final volume of 50 µl containing: 10 µl template DNA, 25 mM MgCl2, 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 30s 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 reverse strand was performed for each amplified fragment. DNA sequence of the D-loop region of 9 specimens was obtained: four specimens of *Chrysichthys maurus* (Ay7, Ay13, Ay17, Ay9); four of *C. nigrodigitatus* (Ay5, Ay2, Ay19, Ay24) and one of *C. johnelsi* (Ay20). The Sequences were aligned using Clustal X [14]. Due to the small number of sequences, the genetic analysis examines the two subgenus of the genus *Chrysichthys*: *Chrysichthys Chrysichthys* (*Chrysichthys maurus*) and *Chrysichthys melanodactylus* (*C. nigrodigitatus* and *C. johnelsi*).
Population genetic statistics such as number of the polymorphic sites (S), the number of haplotypes (H), the haplotype diversity (Hd) and the nucleotides diversity (π) were calculated using DnaSP v5 [15]. Tajima's D test [16] and Fu's Fs [17] were used to detect signatures of population demographic changes (bottlenecks or expansions) and deviations from the pattern of polymorphism expected from a neutral model of evolution. A genetic distance was calculated using Geneious 10.1 [18]. Phylogenetic relationships for the haplotypes were constructed using Neighbor-Joining (NJ) tree

with Tamura-Nei algorithm. Sequence of *Chrysichthys* sp downloaded in the GenBank database under the accession number APO12009.1was used as the outgroup for rooting the phylogenetic trees.

3. Results

3.1. Morphometric analysis

The stepwise discriminant analysis identified nine descriptors that discriminated the species. According to the importance of their discriminant power (table 1), there are: the distance pectoral/anal (wilk's lambda λ= 0.51), the distance anal/dorsal (λ= 0.62), the nasal barbell length (λ= 0.73), preanal length (λ= 0.76), the mandible barbell length 1(λ= 0.76), the preadipose length (λ= 0.81), the anal base (λ= 0.82), the body height (λ= 0.84), and the distance dorsal/adipose (λ= 0.87)

Table 1: Multivariate tests of Wilk's Lambda (λ) significance of the morphometric variables of the Discriminant Factorial Analysis of the *Chrysichthys* populations of the Bia River.

	λ	F	p
DPtAn	0.51	19.09	***
DAnDs	0.62	12.40	***
NBlL	0.73	7.22	***
AnL	0.76	6.19	***
MBlL1	0.76	6.14	**
AdL	0.81	4.70	**
AnB	0.82	4.45	**
BdH	0.84	3.71	*
DDsAd	0.87	2.93	*

P: probability; *: p<0,05; **: p<0,01; *** p<0,001

The discriminant analysis confirmed 95.83% of the total classification (Table 2). The predicted classification was 96% for *Chrysichthys maurus*, 100% for *C. auratus*, 96.15% for *C. johnelsi* and 93.33% for *C. nigrodigitatus*. One specimen of *C. maurus* was allocated to samples of *C. auratus*, one sample of *C. johnelsi* was reclassified in *C. nigrodigitatus* and one of *C. nigrodigitatus* was ranged in *C. maurus*. Most of the total variance (96.39%) was explained by the first two canonical variables. The first canonical variable (λ = 0.019, χ^2 =255.47, dl = 27 p<0.001) was contributed to 70.34% and the second canonical factor (λ = 0.17, χ^2 = 112.48, dl = 16, p<0.001) was contributed to 26.06% of the total variance.

Two variables, AdL and DPtAn, are positively correlated with FC1. FC2 was mainly defined by the positive values of NBlL, AnB, BdH and MBlL1while AnL and DAnDs are negatively correlated to the same axis.

Table 2: Percentage of individuals reclassified in each group, in the validation of the discriminant analyses for the morphometric data.

	Population Predicted				
Populations	***C. maurus***	***C. auratus***	***C. johnelsi***	***Chrysichthys nigrodigitatus***	**Accuracy (%)**
C. maurus	24	1	0	0	96
C. auratus	0	6	0	0	100
C. johnelsi	0	0	25	1	96.15
Chrysichthys nigrodigitatus	1	0	0	14	93.33
Total	25	7	25	15	95.83

The ordination of individuals showed that the four species were well separated. Specimens of *C. auratus* and *C. nigrodigitatus* are located on either side of axis 1 while those of species *C maurus* and *C. johnelsi* are located on either side of axis 2 (Fig. 3).

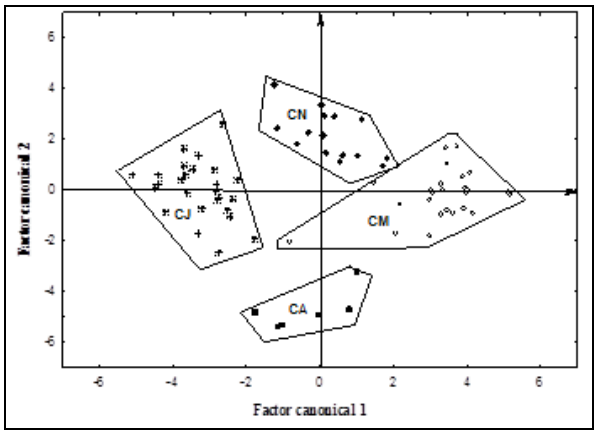

Fig 3: Plot of first two significant canonical variables from the discriminant analysis using morphometric variable for *Chrysichthys* population

NB: CA: *Chrysichthys auratus*; CJ: *Chrysichthys johnelsi*; CN: *Chrysichthys nigrodigitatus;* CM: *Chrysichthys maurus*

Fig. 4 show the Euclidean dendrogram of the four populations of Chrysichthys. Hierarchical cluster analysis revealed three distinct groups: the first group included *Chrysichthys maurus* and *C. nigrodigitatus* which have a great morphological similarity, the second group included only *C. johnelsi* and the third group included *C. auratus.*

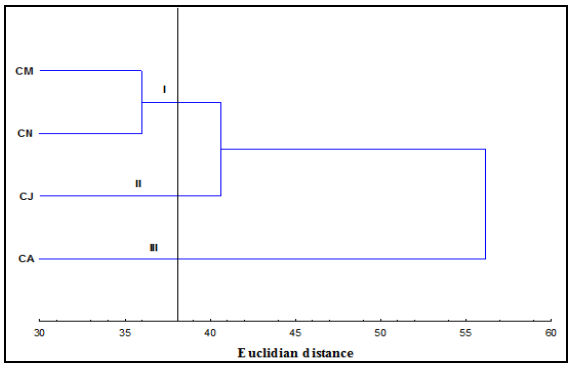

Fig 4: Dendrogram illustrating the morphometric similarity between the *Chrysichthys* species of the Bia River

NB : CA : *Chrysichthys auratus* ; CJ : *Chrysichthys johnelsi* ; CN : *Chrysichthys nigrodigitatus ;* CM : *Chrysichthys maurus*

3.2 Genetic analysis

After cutting off the primers from both ends prior to alignment, a total of 427 nucleotides sites were obtained. The distribution of haplotypes, the number of polymorphic sites, the population genetic statistics and the results of neutrality tests are shown in Table 4.

Among nine sequences of the combined populations, 47 polymorphic sites were found, 43 sites were parsimoniously informative and eight haplotypes were detected. The total nucleotide diversity was 0.067 ± 0.008 while the total haplotype diversity was 0.972 ± 0.064. Among the nine haplotypes, four were detected in *C. chrysichthys* and five were detected in *C. melanodactylus*. There was no significant difference in values of genetic diversity indices between *C. chrysichthys* (Hd, 1 ± 0.177 and π, 0.023 ± 0.01) and *C. melanodactylus* (Hd, 0.9 ± 0.161 and π, 0.022 ± 0.006). Non-significant positive Fu's Fs value and Tajima's D were obtained in combined populations and in *C. melanodactylus*. The neutrality test produced a negative Tajima's D and a positive Fu's Fs value and they were non-significative in *C. chrysichthys*.

Table 4: Haplotype and nucleotide diversities, Tajima's D values and Fu's Fs test of the fishes of the genus *Chrysichthys* in Bia River.

Paramètres génétiques	Combined population	*Chrysichthys Chrysichthys*	*C. melanodactylus*
Number of samples	9	4	5
Number of haplotypes	8	4	4
Haplotype diversity (Hd ± Sd)	0.972 ± 0.064	1 ± 0.177	0.9 ± 0.161
Nucleotide diversity (π ± Sd)	0.067 ± 0.008	0.023 ± 0.010	0.022 ± 0.006
Total number of nucléotide sites	427	427	427
Number ofpolymorphic sites	47	18	14
D de Tajima	1.530 (NS)	- 0.678 (NS)	1.388 (NS)
Fu's Fs	0.608 (NS)	0.232 (NS)	1.241 (NS)

NB: NS: not-significatif

The Hierarchical Cluster Analysis based on Neighbord-Joinning was represented in Fig. 5. The dendrogram revealed that the three species were clustered into two distincts groups: the cluster with the samples of subgenus *Chrysichthys Chrysichthys* (CC) were clearly isolated from those of subgenus *C. melanodactylus* (CM). In the first subgenus, one of the specimens (Ay9) is clearly distinguishable from the others (Ay13, Ay7, Ay17) whereas in the last subgenus, one specimen of *C. nigrodigitatus* (Ay5) is much closer to the specimen of *C. johnelsi*.

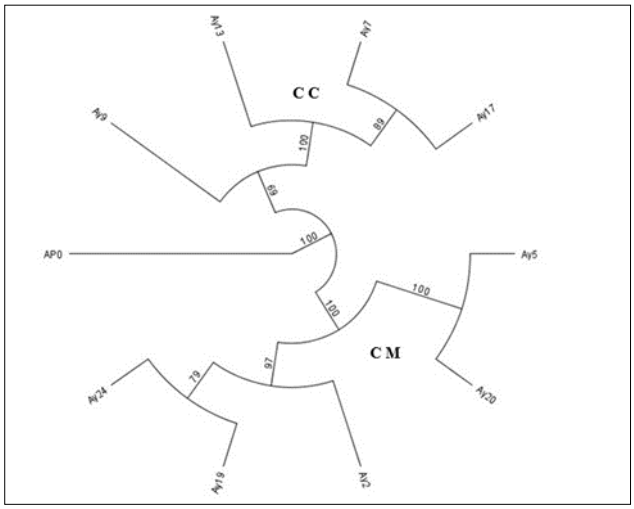

Fig 5: Dendrogram illustrating the genetic similarity between the Bia River specimens

NB : CM : *Chrysichthys melanodactylus* ; CC : *Chrysichthys chrysichthys*

4. Discussion

The biometric analysis, including morphometric characters, has been adopted by many authors to identify different fish races or populations [19; 20]. Stepwise discriminant analysis is also a method used to identify fish populations [21].

The results of the discriminant analysis revealed that only nine descriptors show significant differences between the four species of *chrysichthys*. According to Coulibaly [22], the distinction between the species of *Chrysichthys* is not always easy because, for individuals of similar size, the interspecific morphological differences are minimal, while intra-specific variability can be very large. Indeed, only six morphometric characters were selected to discriminate small sizes of *Chrysichthys nigrodigitatus* and *C. auratus* from samples collected in Aiba Reservoir (Iwo, Nigeria) [23]. Despite the low number of discriminant variables in our study, the high percentages of classification (> 90%) are obtained for all groups, indicating that the morphometric descriptors used have an important taxonomic weight. *Chrysichthys maurus* was characterized by short nose barbells, high and elongated body and large anal fins. *Chrysichthys auratus* is distinguished by moderately elongated head and body, short nose barbells and short mandible barbells. *Chrysichthys nigrodigitatus* is differentiated from other species by a high and moderately elongated body, long nasal and mandible barbells. *Chrysichthys johnelsi* is characterized by a short body and short anal fins. The results of Hierarchical Cluster Analysis confirmed the validity of the metric characters in the differentiation of fish species of the genus *Chrysichthys*. They determined three morphometrically different groups. They specify that the species *Chrysichthys maurus* and *C. nigrodigitatus* have closer morphologies than those of the two other species; *C. auratus* and *C. johnelsi*. In contrast in the study of *Chrysichthys* species, Risch [24] showed that fish belonging to the same subgenus *Chrysichthys chrysichthys* (*Chrysichthys maurus* and *C. auratus*) or *C. melanodactylus* (*Chrysichthys nigrodigitatus* and *C. johnelsi*) were morphologically closer than those belonging to different subgenera.

The study of genetic diversity focused on each subgenus because of the reduced number of sequences. High haplotype diversity and high nucleotide diversity were found in all population examined. This high genetic diversity is probably attributed to long evolutionary history in a large stable population [25]. In the study of the genetic differentiation of two species of *Chrysichthys* (*C. nigrodigitatus* and *C.*

walkeri) from the Lagos lagoon, Nwafili *et al* [26] were found high genetic diversity despite the fact that *Chrysichthys* were generally are heavily fished. This could be due to an exceptionally high initial population and possible recruitment from adjacent basins. In our study, Non-significant Tajima's D and Fu's Fs values were obtained for all populations. These non-significant neutrality tests suggest that the population is not subject to selection or geographic expansion [27]. In contrast to morphometric analysis, the distribution of specimens on the dendrogram shows that fish of each subgenus are genetically distinct. This result demonstated that morphological and genetic markers provide different but complementary information about population structure and have been widely used in population differentiation studies [28, 29].

5. Conclusion

This study was used to characterize and determine the taxonomic status and phylogenetic relationships of *Chrysichthys* species in the Bia River. Morphometric analysis revealed three different morphotypes for the four species of *Chrysichthys*. Genetic analysis was reveled high genetic diversity and absence of selection in the Bia sample. This result demonstrated that the high fishing pressure has not sufficiently reduced the size of the population to have a significant impact on genetic variability.

Although population genetics studies have greatly improved the knowledge of *Chrysichthys* species from this river, nuclear DNA analysis with more specimens should be used to improve understanding of the genetic diversity of these species.

6. Acknowledgements

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