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Genetic diversity of bonytongue fish *Heterotis niloticus* (Cuvier, 1829) in Cameroon, Central Africa

Jeanne Wikondi[a,b,*], Felix Meutchieye[b], Christopher Adenyo[c],
Richard Osei-Amponsah[d], Boniface Baboreka Kayang[d],
Minette Eyango Tabi Tomedi[e]

[a] *Department of Genetics and Biostatistics, School of Veterinary Medicine and Sciences, University of Ngaoundere, PO Box: 454, Ngaoundere, Cameroon*
[b] *Department of Animal Science, Biotechnology and Bioinformatics Research Unit, University of Dschang, FASA, PO Box. 188, Dschang, Cameroon*
[c] *Livestock and Poultry Research Centre, School of Agriculture, University of Ghana, P. O. LG38, Legon, Accra, Ghana*
[d] *Department of Animal Sciences, School of Agriculture, University of Ghana, PO Box. LG226, Legon, Accra, Ghana*
[e] *Institute of Fisheries and Aquatic Sciences of the University of Douala, Yabassi PO Box. 7236, Douala-Bassa, Cameroon*

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ABSTRACT

The African bonytongue (*Heterotis niloticus)* fish is the only species of the *Osteoglossidae* family in Africa. The characterization of this fish was carried out from January to June 2020 in Cameroon where this fish is distributed in native zones. This study aimed at contributing to the genetic characterization of *Heterotis niloticus* population in Cameroon. A total of 96 *Heterotis niloticus* were sampled from five regions namely Centre, South, Littoral, North and Far North. Caudal fins where randomly collected for genomic DNA (gDNA) extraction. Following standard procedures for simple sequence repeat markers available for this species, seven microsatellites were used for genotyping through Polymerase Chain Reaction (PCR) and electrophoresis. Genetic parameters were scored using the GenAlex 6.503 software and the population structure of *Heterotis niloticus* was assessed under STRUCTURE Version 2.3.4 software. The results showed that the local population of *Heterotis niloticus* in North-Cameroon has a higher number of allele (Na=6.00) and effective number of allele (Ne=3.66) while the highest Observed Heterozygosity (Ho) was observed for the population of *Heterotis niloticus* in the Far-North. The highest genetic distance is observed between the population of Far North and Centre, Far-North and South, Far-North and North. In general, similarity within the sub-population of *Heterotis niloticus* is higher than 65%. Genetically, the population of *Heterotis niloticus* in Cameroon was grouped into three genetic sub-populations based on microsatellites appraisal. Further investigations are suggested for analysis concerning productive traits for domestication and aquaculture purposes.

Introduction

Heterotis niloticus in Cameroon originated from the sudano-sahelian zone (Far North and North regions); this species of fish was

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* Corresponding author at: Department of Genetics and Biostatistics, School of Veterinary Medicine and Sciences, University of Ngaoundere, PO Box: 454, Ngaoundere, Cameroon.
E-mail address: wikondi2013@gmail.com (J. Wikondi).

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introduced for the first time in the Nyong River in the 1950s [1]. Because of its double respiration, *Heterotis niloticus* can survive in the oxygen-poor water environment of the Nyong River [2]. Additionally the rapid growth of *Heterotis niloticus* makes it a potential candidate for intensive fish farming and domestication [3]. *Heterotis niloticus* was subjected to various ecological investigation mainly concerning its diet [4,5], biological features [6,7], physiological characteristics [8] and the adaptive behaviour [2]. Based on the data and knowledge gathered on the targeted species, no previous genetic study has been carried out yet on *Heterotis niloticus* in Cameroon. This study aimed at contributing to the genetic characterization of African bonytongue fish species (*Heterotis niloticus*) population in Cameroon. The specific objectives were to assess the population genetic parameters using seven microsatellites, and to analyse the population structure of *Heterotis niloticus*.

Material and methods

Samples collection

The study was conducted in Cameroon from January to June 2020. The samples were collected from five regions of Cameroon: Centre (Nyong River), South (Dja and Lobo River), Littoral (Nkam River), North (Benoue River) and Far North (Logone River) regions (Fig. 1).

Sampling was done purposevily to avoid as much as possible closely related individuals, using distance and fishing points. A total of 96 *Heterotis niloticus* were caught and their caudal fins were collected: 40 samples from the Centre, 12 samples from the South, 22 samples from the Littoral, 24 samples from the North and 18 samples from the Far North regions. The fin samples (100-300 mg) were stored in 2ml cryo-tubes containing 95% ethanol (Fig. 2) (Dessauer *et al.,* 1996) [9].

DNA extraction and genotyping

DNA extraction was done using kit (Zymo Research, USA) following the manufacturer's protocol.

The samples were genotyped at seven microsatellite loci (five from Carrera et al. [10] and two from Hurtado et al. [11]) (Table 1). The choice of the seven microsatellites was based on their high specificity and high polymorphism [10,11].

PCR was conducted in two multiplexes and the loci that did not amplify were amplified in simplex reactions. The microsatellites regions were first amplified using Q5 High-Fidelity 2X Master Mix Catalogue No.M0292 (NEB), using a PCR reaction volume of 20 µl containing at least 10 ng of genomic DNA, 10mM of each primer in a primer mix, 10 µl Q5 High-Fidelity 2X Master Mix and nuclease free water Catalogue No. E476 (AMRESCO LLC, OH 44139, USA). This round of PCR added M13, PGEX5 and T7 sequences to the PCR Product.

The second round of PCR in a similar fashion as above, was done using 1 µl of round 1 PCR product as template, and the fluorescently labelled M13-FAM, PGEX3-ATTO532, PGEX5-ATTO550 and T7-ATTO565 forward primers, and corresponding reverse primers in a primer mix.

PCR was run following these conditions:

$$\left.\begin{array}{ll} -\text{Initial denaturation 3 min} & 95^{\circ}\text{C} \\ -\text{Denaturation 30 s} & 95^{\circ}\text{C} \\ -\text{Annealing 30 s} & 58^{\circ}\text{C} \\ -\text{Extension 30 s} & 72^{\circ}\text{C} \\ -\text{Final extension 5 min} & 72^{\circ}\text{C} \end{array}\right\} 35\text{X}$$

Two microliters of the fluorescently labelled PCR amplicons and 15 µl LIZ500 sizing standard and Hi-Di™ Form-amide Catalogue

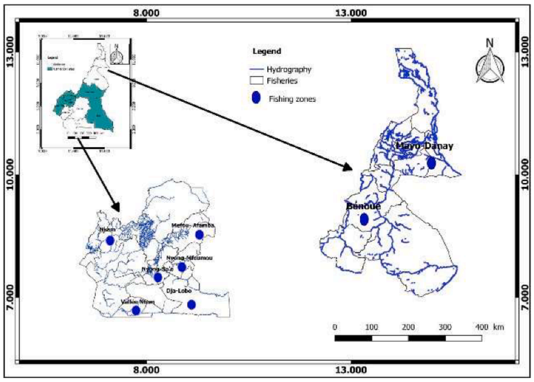

Fig. 1. Zone of study.

Fig. 2. Caudal fin of *Heterotis niloticus* stored in a cryo-tube containing 95% ethanol.

Table 1
Microsatellites used for genotyping *Heterotis niloticus.*

Name	Sequence (5′-3′)	Size Range (bp)	TA (°C)
Hni94	GCCACCGTTTGAGTGAATTT TCTGAGAACACGGTAGGATGC	166–174	55.0
Hni67	CGCCTGTAAGCAATGAATGA TGCCATGGATGTCACAAGTT	307–315	55.0
Hni61	AGCTTGCATCTGGAAGCATT GTATCCACCTGGACCCACAG	327–352	55.0
Hni5	CAGGAAGATTTGCACCACCT TGCCATTCTGGAAAAAGGAG	265-295	57.0
Hni14	CAACCACTGCCATAGGAGGT GAACCAAGTCGAGCCATCAT GATGCAACATGCACTGCTCT	125–152	55.8
Hni47	AACACATTACGCCCTTGGAG CAGCCCTGTGTACACTTCCA	270–279	55.8
Hni28	TCCCTGCAGTCTGAAACACA AGACCCACAAGATCCAGGTG	233-259	57.0

No. 4311320 (Thermo Fisher Scientific, Carlsbad, USA) mixture were denatured at 95°C for 5 min.

After the denaturation, the fragments were run on the ABI PRISM™ 3500xl Genetic Analyser (Applied Biosystems, Thermo Fisher Scientific, Carlsbad, USA), 50cm capillary array, POP-7$^{\text{TM}}$. Alleles were scored using GeneMarker V2.9.5 (SoftGenetics, State College, Pennsylvannia, USA).

Data analysis

The molecular variance analysis (AMOVA), genetic distance, number of alleles (Na), effective number of allele (Ne), observed (Ho) and expected heterozygosity (He), Hardy-Weinberg Equilibrium (HWE), Inbreeding coefficient within individuals of the same population (F_{ST}), Inbreeding coefficient of total population (F_{IT}), Inbreeding coefficient of individuals of subpopulation (F_{IS}) and Gene flow (Nm) were obtained using software GenAlex 6.4 [12]. The population structure of *Heterotis niloticus* was assessed with STRUCTURE software (Pritchard et al. [13]).

Results

Genetic parameters

Table 2 shows the different parameters of genetic diversity of *Heterotis niloticus* in Cameroon

The average number of allele, effective allele and Shannon's index were 4.5±0.35, 2.91±0.22 and 1.11±0.08 respectively. The observed heterozygosity (Ho) was 0.58 while expected heterozygosity (He) was 0.57. The average fixation index for all populations of

Table 2
Number of alleles, effective alleles and Shannon's index of *Heterotis niloticus* in Cameroon.

Region	N	Na	Ne	I
Centre	20	3.57±0.52	2.66±0.57	0.99±0.1
South	12	3.42±0.42	2.31±0.39	0.84±0.19
Littoral	22	3.71±0.64	2.69±0.47	0.98±0.20
North	24	6.00±0.92	3.66±0.62	1.42±0.13
Far-North	18	5.857±0.85	3.20±0.51	1.30±0.16
Mean	19.20	4.51±0.35	2.91±0.22	1.11±0.08

N=Number of samples, Na = Number of different alleles, Ne=Number of effective alleles. I: Shannon's index

Heterotis niloticus was -0.01 which indicates excess of heterozygosity.

The population of *Heterotis niloticus* in the North-Cameroon had the highest number of alleles (6.00) and effective number of alleles (3.66) while the highest observed Heterozygosity (Ho) was observed for the population of *Heterotis niloticus* from the Far-North region. The lowest fixation index was observed in the Centre (-0.07) and the Far-North (-0.13) regions, suggesting that excess heterozygotes exist in those populations of *Heterotis niloticus* (Table 3).

African bonytongue of the South, Littoral and North Cameroon have respectively 0.06, 0.02 and 0.03 value of fixation index and these suggest an excess of homozygotes for most of the allele size classes.

Table 4 shows the index flow above the diagonal and the coefficient of differentiation under the diagonal. The F_{ST} for pairwise comparisons between the Centre and the Littoral, the Centre and the South, the South and the Littoral were respectively 0.04, 0.04 and 0.05. These values suggest that no genetic differentiation exists among the fish of those regions. The high value of index flow (5.26, 5.14, 3.45) from these 3 regions confirmed the low genetic diversity of *Heterotis niloticus* populations. Whereas F_{ST} for pairwise comparisons between the Centre and Far-North, the Centre and North, the Far-North and North, the Far-North and Littoral, the North and Littoral, and the North and South were 0.14, 0.06, 0.08, 0.11, 0.07, 0.11 respectively. The genetic differentiation was moderate and large (0.19) between the population from Far-North and South. Those regions have the lowest index flow (1.06).

Table 5 shows Wright's F statistic of African bonytongue fish. All the values related to the coefficient of differentiation (F_{ST}) are positive and these values vary from 0.05 to 0.16. According to Wright [14], the rate of differentiation is moderated when the F_{ST} runs from 0.05 to 0.15 and is large for its value superior to 0.15. The average inbreeding coefficient of the individual of the sub-population (F_{IS}) is -0.02 and is lower than the average inbreeding coefficient of the population while some loci have positive value 0.07 and 0.04 respectively for loci Hni19 and Hni28. The gene flow varies from 1.23 to 4.23. The highest value of Nm (4.23) was observed at locus Hni28.

Equilibrium (HWE) test

The Hardy-Weinberg equilibrium test was not significant for the majority (96.87%) of microsatellites, except three loci which were significant, namely Hni19 (for the Littoral populations), Hni47 (for the Southern and Northern populations) and Hni62 (for Northern populations). The level of significance at the 5% and 0.1% threshold reflects a deviation of equilibrium in the total population. Locus Hni94 was monomorphic for the Littoral population (Table 6).

Analysis of molecular variance

Fig. 3 shows the variation of *Heterotis niloticus* population in Cameroon.

9% of the total variation is due to the individuals regrouped in populations and 89% comes from the individuals taken as a whole population.

Genetic distance within the population of Heterotis niloticus

The genetic distance varies from 0.9 to 0.44 (Table 7). The genetic distance of Nei varies from 9% to 44% below diagonal. The population of the Centre, South and Littoral have the same distance (0.9) but the genetic distance of the population of the Littoral and South is 0.11.

The highest genetic distance was observed between the population of the Far-North and Centre, Far-North and South, Far-North and North. Generally, similarity within the population of *Heterotis niloticus* was more than 65%. The Nei genetic identity was higher (91%) for the Centre, South and Littoral population.

Structure of Heterotis niloticus population in Cameroon

Fig. 4 illustrates the different genetic groups of *Heterotis niloticus* population among five regions (Centre, South, Littoral, North and Far-North).

This Fig. 4 shows that the population of *Heterotis niloticus* partitioned into K colours corresponding to the number of the estimated population. The black vertical lines separate the different populations studied. Indeed, the five regions where samples of *Heterotis*

Table 3
Observed and Expected Heterozygosity and Fixation index.

Region	Ho	He	F
Centre	0.62±0.10	0.56±0.07	-0.07±0.08
South	0.42±0.1	0.46±0.10	0.06±0.06
Littoral	0.50±0.1	0.53±0.10	0.02±0.08
North	0.65±0.02	0.69±0.03	0.03±0.05
Far-North	0.71±0.04	0.63±0.05	-0.13±0.04
Mean	0.58±0.03	0.57±0.03	-0.01±0.03

Ho= Observed Heterozygosity, He= Expected Heterozygosity, F= Fixation Index.

Table 4
Index flow (Nm) and coefficient of differentiation (F_{ST}) of *Heterotis niloticus* populations.

	Centre	Far-North	Littoral	North	South
Centre		**1.50**	**5.26**	**3.45**	**5.14**
Far-North	0.14		**1.94**	**2.59**	**1.06**
Littoral	0.04	0.11		**3.11**	**4.27**
North	0.06	0.08	0.07		**1.93**
South	0.04	0.19	0.05	0.11	

Table 5
Wright's F statistic for *Heterotis niloticus* populations in Cameroon.

Loci	F_{ST}	F_{IS}	F_{IT}	Nm
Hni19	0.16	0.07	0.23	1.23
Hni94	0.16	-0.06	0.11	1.27
Hni67	0.06	-0.02	0.03	3.87
Hni47	0.11	0.08	0.19	1.87
Hni5	0.06	-0.10	-0.02	3.41
Hni62	0.13	-0.16	-0.01	1.66
Hni28	0.05	0.04	0.09	4.23
Mean	0.10	-0.02	0.09	2.51

F_{ST}: Inbreeding coefficient within individual of the same population, **F_{IT}**: Inbreeding coefficient of the total population **F_{IS}:** Inbreeding coefficient of individual of subpopulation, **Nm**= Gene flow

Table 6
Hardy Weinberg Equilibrum of *Heterotis niloticus* populations.

Loci	Centre	South	Littoral	North	Far-North
Hni19	ns	ns	*	ns	ns
Hni52	ns	ns	ns	ns	ns
Hni94	ns	ns	mono	ns	ns
Hni67	ns	ns	ns	ns	ns
Hni47	ns	***	ns	*	ns
Hni5	ns	ns	ns	ns	ns
Hni62	ns	ns	ns	*	ns
Hni28	ns	ns	ns	ns	ns

ns=not significant, * P<0.05, *** P<0.001, mono= monomorphic

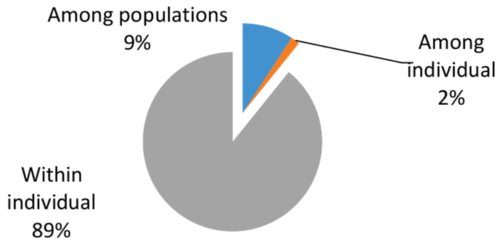

Fig. 3. Analysis of molecular variance of populations of *Heterotis niloticus* in Cameroon.

Table 7
Nei's genetic distance and genetic identity.

	Centre	South	Littoral	North	Far-North
Centre		0.91	0.91	0.85	0.71
South	0.09		0.90	0.78	0.65
Littoral	0.09	0.11		0.85	0.79
North	0.17	0.25	0.16		0.76
Far-North	0.34	0.44	0.24	0.28	

niloticus were collected, are made up of three distinct genetic types (red, blue and green) but equivalent in terms of population. However, in the North and Far-North regions, although it is made of two genetic types, the groups represented by the red and green colours are the most dominant. The best structuring was obtained at K=3. This result was confirmed by the Evanno curve indicating the putative number of sub-populations (Fig. 5). In this figure, the optimal value of sub-population was 3.

Discussion

In this study, the highest numbers of alleles, number of effective alleles, expected heterozygosity, Shannon's index indicated that the population from the North had lower values than those from the Kainji Lake in Nigeria (Na=8.33, Ne=4.56, He=0.70, I=1.60) Oladimeji et al. [15] and in Benin (Ouemé-So River) where Hurtado et al. [11] found that Na=9.25 and He=0.60. The low level of genetic diversity in our study may be due to the fewer number of samples (n=24) in the North region compared to the number of samples (n=40) used by Carrera et al. [10] and the number of samples (n=184) used by Hurtado et al. [11]. On the other hand, those genetic parameters were higher than the number of allele (2.44), He (0.38) and fixation index (0.30) which Shafiqur et al. [16] found on *Sclerofagus formosus* in Malaysia and higher than the same parameters on *Arapaima gigas* (number of allele (2.22) and He (0.24)) Vitorino et al. [17].

The lowest F observed in the population of *Heterotis niloticus* from the Centre and Far-North regions showed a lower inbreeding while in the Littoral, North and South region, the population of *Heterotis niloticus* have positive values of fixation index, reflecting an excess of homozygotes. This excess of homozygotes results from inbreeding in these regions. The inbreeding may be due to the limited gene flow of *Heterotis niloticus* due to the Lagdo hydroelectic dam on the Benoué River.

Similar cases are found in the population of the South region in the Lobo River (Mekin) and in the population of the Littoral region in the Nkam River (Yabassi). The case is different for the Far-North and Centre regions, where *Heterotis niloticus* were found everywhere in different rivers like Nyong River, Mefou, River, So'o River in the Centre; Logone river, Guere Lake and Maga Lake in the Far-North. This could explain the low inbreeding in *Heterotis niloticus* populations for the two regions. This result is similar to the work of Torati et al. [18] who found that the population of Pirarucu in Amazon and Araguaia-Toncantin river basins have low inbreeding.

Deviation from HWE means that for *Heterotis niloticus* of Far North and Centre regions, excess of heterozygotes could lead to a heterogamy. This result is similar to those of Hrbek et al. [19] where population of *Arapaima gigas* showed excess heterozygosity. Our results showed that most of the loci did not deviate from HWE. In Malaysia, Shafiqur et al. [16] observed that most of the loci deviated from HWE in the population of *Scleropages formosus.* The population of *Heterotis niloticus* from the Central, South and Littoral were similar genetically at 91% and inbreeding is very low because of the high rate of migration in these localities. The genetic distance among the population of *Heterotis niloticus* and within individuals of sub-population are very low. Moreover, in the population of *Arapaima gigas* AMOVA showed that the variation within the populations is 52.63% Vitorino *et al* [17] compared to the study of Stewart [20] and Watson et al. [21] who found that genetic diversity within *Arapaima gigas* population in the large Amazon River basin was higher than the other river systems due to minimal gene flow and high genetic differentiation between the populations.

Then STRUCTURE software allowed us to distinguish three genetic groups of *Heterotis niloticus* population confirmed by Evano curve. These three genetic groups were the group of the North and Far-North and group composed by the Central, South and Littoral populations. These results confirmed the hypothesis of Moreau in 1982 [6] that *Heterotis niloticus* in the South Cameroon comes from the Soudano-Sahelian zone (Far-North region). Our results contradict the study of Hurtado et al. [11] who did not find genetic differentiation among populations of *Heterotis niloticus* in Benin while Oladimeji *et al.* [15] found that all the populations are differentiated. Compared to *Araipaima gigas* and *Sclerofagus formosus*, our result is similar to Watson et al. [21] and Yue et al. [22] who found respectively that the population of *Arapaima gigas* in Guyana have three clusters and in Singapore, the population of *Sclerofagus*

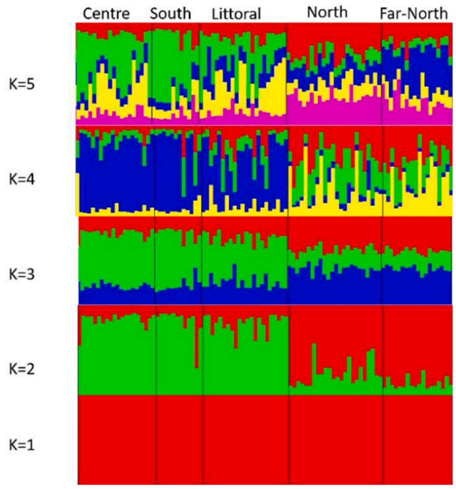

Fig. 4. Population structure of *Heterotis niloticus* in Cameroon.

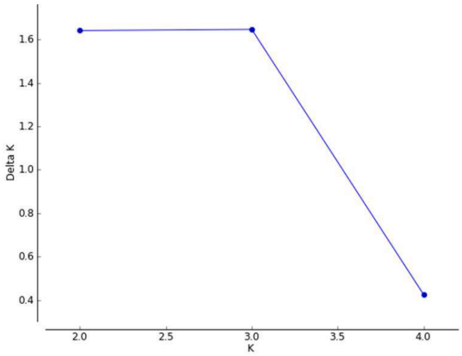

Fig. 5. Evanno curve indicating the putative number of population of *Heterotis niloticus* in Cameroon.

formosus have three clusters while in Amazon and Araguaia-Toncantin river basins, Torati et al. [18] distinguished two clusters of *Arapaima gigas* like Vitorino et al. [17].

Conclusion

The genetic diversity of *Heterotis niloticus* in Cameroon was determined. The highest number of alleles and effective number of alleles were found in the population of the North region. In the Far-North region, the observed heterozygosity (Ho) and expected heterozygosity (He) were higher than those of other regions. The average fixation index for all the populations of *Heterotis niloticus* was negative indicating excess heterozygosity. The lowest Fst value between the Centre and Littoral, the Centre and South, the South and Littoral means that very little genetic differentiation exist among African bonytongue fish of those three regions. Three genetic groups of *Heterotis niloticus* in Cameroon were found. From Northern to Southern part of Cameroon, *Heterotis niloticus* is caught and raised, an indication of the importance of this fish in the lives of the inhabitants of Cameroon and neighbouring countries. It is recommended that this study should be extended to other countries in Central Africa in order to conserve this fish species which has a high potential for aquaculture in Africa.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2023.e01975.

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