

PHENOTYPIC POLYMORPHISM OF AFRICAN BONY TONGUE FISH *Heterotis niloticus* (CUVIER, 1829) IN CAMEROON RAINFOREST REGION.

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ABSTRACT

The study on the phenotypic polymorphism of *Heterotis niloticus* was undertaken from February to May 2020 in the rain forest region of Cameroon. The main objective was to contribute to a better understanding of the genetic diversity of this adapted species for adequate breeding and preservation strategies. More specifically, the investigation aimed at evaluating the variability of phenotypic, morphometric and meristic features according to the study sites. Further, it was also in order to analyse the phenotypic variability, the structure, as well as the phylogenetic relationships among the *Heterotis niloticus* subpopulations. A total of 125 adult individuals were collected in three localities and characterized. Two (2) phenotypic traits were observed, while 16 morphometric traits were measured and 5 meristic characters were counted. The results showed that there is a colour variability of eyes and body in *Heterotis niloticus* from the study area with a predominance of golden eyes (97.60%) and grey body (96.80%). The number of scales on the lateral line was higher in the individuals collected in Mbalmayo (38.67 ± 1.46) than those in Ayos (37.70 ± 0.95). Discriminant factor analyses and phylogenetic analyses revealed that the overall population studied is made up of 3 morphotypes cohabiting in the three localities; thus it was possible to establish the phylogenetic relationships that exist between them. Morphotypes 1 and 2 are closer while morphotypes 2 and 3 are far apart, showing a tendency of splitting genetic make up. The recorded diversity suggests that *Heterotis niloticus* from the study area constitutes a natural genetic resource having the interesting variability for further improvement and sustainable management.

Keywords : *Heterotis niloticus*, phenotype, traits, forest area, Cameroon.

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RESUME

L'étude sur le polymorphisme phénotypique de *Heterotis niloticus* a été réalisée de février à mai 2020 dans la zone forestière du Cameroun. L'objectif était de contribuer à une meilleure connaissance de la diversité phénotypique de cette espèce adaptée afin de développer des stratégies idoines pour son élevage et sa préservation. Plus spécifiquement, l'étude visait à évaluer la variabilité des caractéristiques phénotypiques, morphométriques et méristiques en fonction des localités identifiées. Il s'agissait plus loin d'analyser la variabilité phénotypique, la structure, ainsi que les relations phylogénétiques entre les sous-populations de *Heterotis niloticus*. Un total de 125 individus adultes a été collecté dans trois localités et caractérisé. Deux (2) caractères phénotypiques ont été observés, tandis que 16 caractères morphométriques ont été mesurés et 5 caractères méristiques comptés. Les résultats ont montré qu'il existe une variabilité colorée des yeux et du corps chez *Heterotis niloticus* de la zone d'étude, avec une prédominance des yeux dorés (97,60%) et du corps gris (96,80%). Le nombre d'écaillés sur la ligne latérale était plus élevé chez les individus collectés à Mbalmayo ($38,67 \pm 1,46$) par rapport à ceux d'Ayos ($37,70 \pm 0,95$). Les analyses des facteurs discriminants et phylogénétiques ont révélé que l'ensemble de la population étudiée est composée de 3 morphotypes cohabitant dans les trois localités ; il a donc été possible d'établir les relations phylogénétiques qui existent entre eux. Les morphotypes 1 et 2 sont plus proches l'un de l'autre tandis que les morphotypes 2 et 3 sont éloignés l'un de l'autre, montrant une tendance à la scission de la structure génétique. La diversité évaluée suggère que *Heterotis niloticus* de la zone d'étude constitue une ressource génétique naturelle ayant un niveau de variabilité intéressante pour des perspectives d'amélioration ultérieure et d'une gestion durable.

Mots-clés : *Heterotis niloticus*, phénotype, caractère, zone forestière, Cameroun.

Introduction

Biodiversity is an important contributor to nutritional diversification, for instance, the availability of essential food for human health and welfare (FAO, 2018). Despite this, biodiversity and related genetic resources are being depleted at an alarming rate due to several factors such as habitat fragmentation, overexploitation of wild species, climate change, introduction of invasive species and pollution (IUCN, 2011). Concerning freshwater and sea products, it has been noted that one third of fisheries resources are overexploited (FAO, 2002). Nowadays, the conservation of diversity remains a major issue for most countries. However, the lack of knowledge about the diversity of genetic resources makes the process of its conservation difficult. It is therefore in line with the recommendations of the Agenda 21 Program of the Convention of Biological Diversity (CBD), that the identification and characterization of genetic resources are the basis for the

conservation of genetic diversity of domestic animals and related species for food (FAO, 2013). *Heterotis niloticus* is cited among the African native promising species which are suspected to suffer from decreasing population, density as well as catch in rivers and reservoirs. Considered as cuisine delicacy, the species is prone to overexploitation. This popularity is due to the good taste of its flesh and its relatively high commercial value (Oswald *et al.*, 2003 ; Monentcham, 2009). Although it has long been caught in wild or recently experimentally reared in Cameroon, very little work has been done on the genetic characterization of this species despite its wide distribution in rivers and lakes in Cameroon ecosystems where it is named *kanga* (Wikondi *et al.*, 2022).

Thus, the objective of this study was to contribute to a better knowledge of the phenotypic diversity of *Heterotis niloticus* in the rainforest zone of Cameroon . More specifically, this study aimed at, describing the phenotypic variability of *Heterotis*

niloticus according to localities, then, evaluating the biometric and meristic characteristics of *Heterotis niloticus* according to localities, and finally, describing the phenotypic variability, structure and relationships between the different morphotypes of the population of *Heterotis niloticus* in the study area.

Material and methods

Study period and area

The investigation was done between February and May 2020 in rainforest region of Cameroon

(Figure 1) located between 2°47' and 6°5' Northern and between 11°40' and 14° Eastern. The hydrological regime of the Nyong is of the transitional equatorial type ; the rainfall of the basin (29,000 km³) varies between 1,500 and 1,800 mm (Dépierre and Vivien, 1977), with temperature ranging between 20 and 24 °C at one-meter depth (Nguenga and Brummett, 2010).

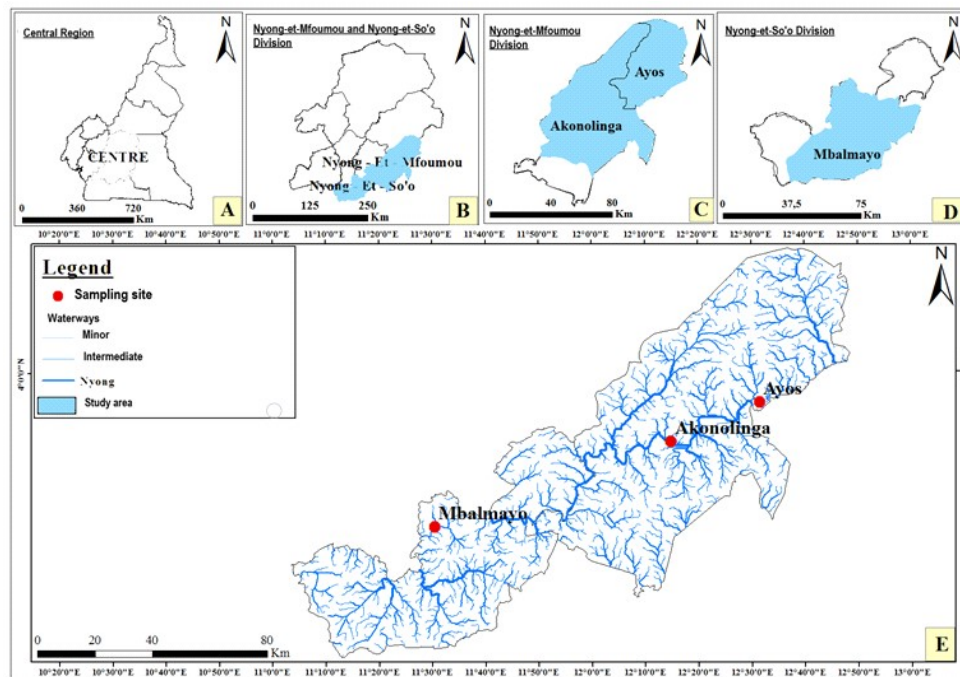


Figure 1 : Hydrological map of study area

Methodology

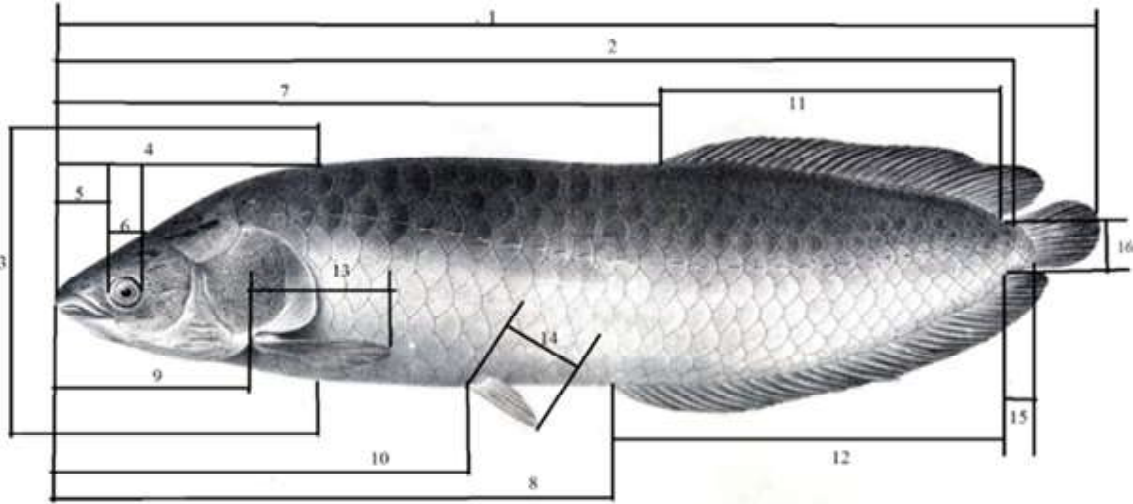
Samples of *Heterotis niloticus* were purposely collected mainly from three localities Akonolinga (n=52), Mbalmayo (n=43) and Ayos (n=30) in Cameroon rainforest. A total of 125 adults with an average weight of 4000g were collected from fishermen and volunteer dealers with the help and collaboration of the Regional, Divisional and Subdivisional Delegations of MINEPIA.

The waterways of these three localities are the dismemberments of the Nyong River basin. The description of the phenotypic characteristics (eye and body colour) was done by direct observation immediately after fishing.

Body measurements (Figure 2) : Total length (TL); Standard length (SL); Body depth (BD); Head length (HL); Snout length (SL): Eye diameter (ED); Pre-dorsal distance (PDD); Pre-anal distance (PAD); Pre-pectoral distance (PPD); Pre-pelvic distance (PPeD); Dorsal fin base length (DFL); Anal fin base length (AFL); Pectoral fin length (PFL); Pelvic fin length (PeFL); Caudal peduncle depth (CPD) and Caudal peduncle length (CPL) were carried out using the Vernier Calliper (0.1mm accuracy), a tape measure and an ichthyometer. All individuals were weighed to the

nearest gram using a weighing portable electronic scale.

Five meristic characters were also considered: the number of rays on the dorsal fin; the number of rays on the anal fin; the number of rays on the pectoral fin; the number of rays on the pelvic fin; and the number of scales on the lateral line.



1: total length; 2: standard length; 3: body depth 4: head length; 5: snout length; 6: eye diameter; 7: pre-dorsal distance; 8: pre-anal distance; 9: pre-pectoral distance; 10: pre-pelvic distance; 11: dorsal fin base length; 12: anal fin base length; 13: pectoral fin length; 14: pelvic fin length, 15: Caudal peduncle length; 16: Caudal peduncle depth

Figure 2 : Body measurements of *Heterotis niloticus*

Statistical analysis

Descriptive statistics were used to describe the distribution of qualitative traits (eye and body colour). The contingency test was performed to test the association or independence between the locality factor and the qualitative traits. All measurements were expressed as a percentage of standard length. The analysis of variance (ANOVA) was used to evaluate the influence of the locality factor on the different measurements considered

Principal Component Analysis (PCA) was performed to evaluate the phenotypic clustering of the *Heterotis niloticus* subpopulations. Discriminant Factor Analysis (DFA) was used to determine the population structure (FAO, 2013). The relationships between the different morphotypes of the population were established using the construction of the phylogenetic tree following the Hierarchical Ascending

Classification (HAC) protocol (Roux, 2006; Carpentier, 2007).

All these different statistical analyses were performed using SPSS 21.0 and XLSTAT 2015 software.

Results

Variability of phenotypic features

The phenotypic features of *Heterotis niloticus* according to localities are summarized in Table 1.

Table 1: Distribution of eye and body colour of *Heterotis niloticus* according to localities in Cameroon rainforest region

Parts of the fish	Colour	Localities			Combined localities	P
		Akonolinga (52)	Mbalmyo (43)	Ayos (30)		
Eyes	<i>Red</i>	3.85% (2)	0.00%	0.00%	1.60% (2)	0.36: ns
	<i>Black</i>	1.92% (1)	0.00%	0.00%	0.80% (1)	
	<i>Golden</i>	94.23% (49)	100.00% (43)	100.00% (30)	97.60% (122)	
Body	<i>Brown</i>	1.92% (1)	4.65% (2)	3.33% (1)	3.20% (4)	0.75: ns
	<i>Grey</i>	98.08% (51)	95.35% (41)	96.67% (29)	96.80% (121)	

From the table, it can be seen that whatever the locality, golden eyes predominate (97.60%). As for the colour variability of the body, there is a dominance of the grey colour (96.80%) compared to the brown colour (3.20%). However, the contingency test shows that there is no significant difference ($p > 0.01$) between localities.

Figures 3 and 4 illustrate the colour variability of the eyes and body of *Heterotis niloticus* in the bimodal rainforest zone of central Cameroon.

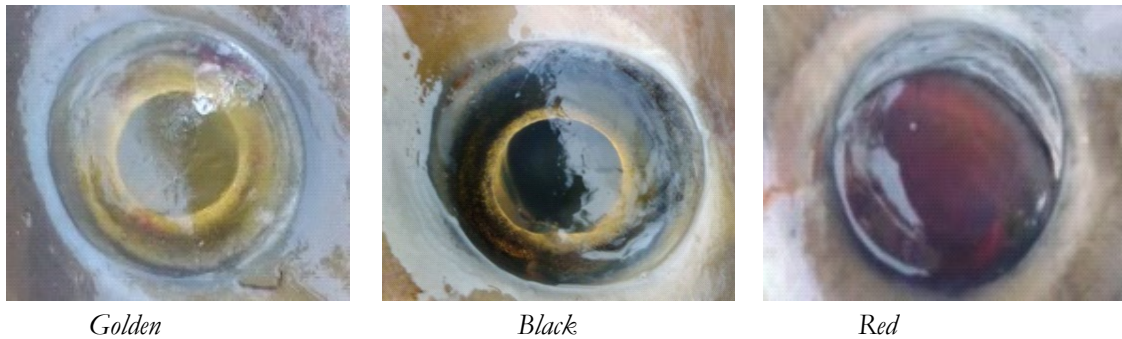


Figure 3: Eye colours of *Heterotis niloticus* in Cameroon rainforest zone



Figure 4: Body colour patterns of *Heterotis niloticus* in Cameroon rainforest zone

Body measurements and total weight

Results of the measurement of biometric characters (expressed in the percentage of standard length) and total weight of *Heterotis niloticus* in Cameroon rainforest region were illustrated in Table 2.

Table 2 : Means(m) and coefficients of variation (vc) of morphometric characters of *Heterotis niloticus* according to localities in Cameroon rainforest region

Traits	Values	Localities			Combined localities
		Akonolinga	Mbalmayo	Ayos	
W[kg]	m	4.49± 1.13 ^a (52)	4.04 ±1.22 ^{ab} (43)	3.42 ± 1.11 ^a (30)	4.08 ± 1.22(125)
	vc	25.16	30.20	32.45	29.90
HL	m	25.00±2.00 ^a (52)	23.00±1.00 ^a (43)	26.00±6.00 ^a (30)	24.66±3.00 (125)
	vc	8.00	4.34	23.07	11.80
SL	m	2.00 ± 0.00 ^a (52)	5.00±1.00 ^a (43)	6.00±1.00 ^a (30)	4.33±0.66 (125)
	vc	0.00	2.00	16.66	6.22
ED	m	3.00± 0.00 ^a (52)	3.00 ± 0.00 ^a (43)	3.00± 0.00 ^a (30)	3.00±0.00 (125)
	vc	0.00	0.00	0.00	0.00
TL	m	112.00 ±8.00 ^a (52)	111.00 ±3.00 ^a (43)	115.00±7.00 ^a (30)	113.00±6.00(125)
	vc	7.14	4.34	2.70	5.30
BD	m	26.00±2.60 ^a (52)	26.00±2.08 ^a (43)	26.00±1.82 ^a (30)	26.00±2.34(125)
	vc	10.00	8.00	7.00	9.67
DFL	m	39.00± 6.00 ^a (52)	38.00± 5.00 ^a (43)	50.00±6.00 ^a (30)	41.00±5.67(125)
	vc	15.38	13.15	12.00	12.19
AFL	m	42.00 ± 4.20 ^a (52)	41.00± 4.10 ^a (43)	42.00±3.78 ^a (30)	41.67±3.9(125)
	vc	10.00	10.00	9.00	9.67
PFL	m	22.00 ± 2.00 ^a (52)	22.00±5.00 ^a (43)	23.00±1.00 ^a (30)	22.00±3.00(125)
	vc	9.09	9.09	13.04	13.63
PDD	m	64.00± 9.00 ^a (52)	63.00±10.00 ^a (43)	61.00±14.00 ^a (30)	63.00±11.00(125)
	vc	14.06	15.87	22.95	17.46
PAD	m	63.00±7.00 ^a (52)	62.00±6.00 ^a (43)	65.00±4.00 ^a (30)	63.00±6.00(125)
	vc	11.11	9.67	6.15	9.52
PPeD	m	48.00 ± 5.28 ^a (52)	48.00 ± 5.76 ^a (43)	49.00 ± 4.90 ^a (30)	48.00 ± 5.00(125)
	vc	11.00	12.00	10.00	10.41
PeFL	m	13.00±1.00 ^a (52)	14.00±5.00 ^a (43)	15.00±1.00 ^a (30)	14.00±3.00(125)
	vc	7.69	35.71	8.33	21.42
PFL	m	8.00± 1.00 ^a (52)	7.00±0.00 ^a (43)	11.00±1.00 ^a (30)	9.00±1.00 (125)
	vc	12.50	0.00	9.09	11.11
CPL	m	4.00 ±1.00 ^a (52)	5.00 ± 1.00 ^a (43)	4.00 ±0.00 ^a (30)	4.50 ± 0.65(125)
	vc	25.00	20.00	0.00	14.45
CPD	m	7.00±2.00(52)	7.00±1.00 ^a (43)	6.00±0.00 ^a (30)	6.68±1.00(125)
	vc	28.57	14.28	0.00	15.03

a: Means with the same letter in the same row indicate that there are no significant differences between localities ($p < 0.01$); m: mean; vc: variation coefficient; W: weight; HL: head length; SL: snout length; ED: eye diameter; TL: total length; BD: body depth; DFL: dorsal fin length; AFL: anal fin length; PFL: pectoral fin length; PDD: pre-dorsal distance; PAD: pre-anal distance; PPD: pre-pectoral distance; PPeD: pre-pelvic distance; PeFL: pelvic fin length; PFL: pectoral fin length; CPL: Caudal peduncle length; CPD: Caudal peduncle depth.

The analysis of variance shows that these parameters are not significantly influenced ($p > 0.01$) by the localities except for the total weight which was significantly influenced by the locality factor. However,

it should be noted that fishes from the locality of Akonolinga was heavier ($W=4.49\text{kg}$) and less variable ($CV=25.16\%$) while those from the district of Ayos were less heavy (3.42kg) and more variable (32.45).

Table 3 : Variation coefficient and means of meristic characters of *Heterotis niloticus* according to localities in Cameroon rainforest region

Traits	Values	Localities			Combined localities
		Akonolinga	Mbalmayo	Ayos	
P	m	11.32±1.6 ^a (52)	11.27±1.58 ^a (43)	11.13±0.50 ^a (30)	11.24±1.23(125)
	vc	14.22	14.01	4.49	10.94
D	m	33.00±1.44 ^a (52)	32.30±1.58 ^a (43)	32.50±2.06 ^a (30)	32.60±1.67(125)
	vc	4.36	4.89	6.33	5.12
V	M	6.00±0.00 ^a (52)	6.00±0.00 ^a (43)	6.00±0.00 ^a (30)	6.00±0.00 (125)
	vc	0.00	0.00	0.00	0.00
A	M	34.53±1.05 ^a (52)	34.74±1.32 ^a (43)	34.60±1.07 ^a (30)	34.62±1.15(125)
	vc	3.04	3.79	3.09	3.32
LL	M	37.81±1.46 ^{ab} (52)	38.67±1.99 ^a (43)	37.70±0.95 ^b (30)	34.62±1.62(125)
	vc	3.86		2.51	4.67

a, b: means assigned the same letter in the same row to indicate that there are no significant differences between localities (p<0.01); m: mean; vc: variation coefficient; P: number of rays on the pectoral fin; D: number of rays on the dorsal fin; V: number of rays on the ventral fin; A: number of rays on the anal fin; LL: number of scales on the lateral line.

It appears from the findings that all the meristic characteristics as well as their coefficients of variation are comparable between localities, with the exception of the coefficient of variation of the number of scales on the lateral line which varied from 2.51 (Ayos) to 5.14 (Mbalmayo).

Population structure

Figure 5 shows the population structure and Table 4 shows the biometric and meristic characteristics of the different morphotypes. The Discriminant Factor Analysis (DFA) allowed us to detect that the study population consists of 3 morphometric groups.

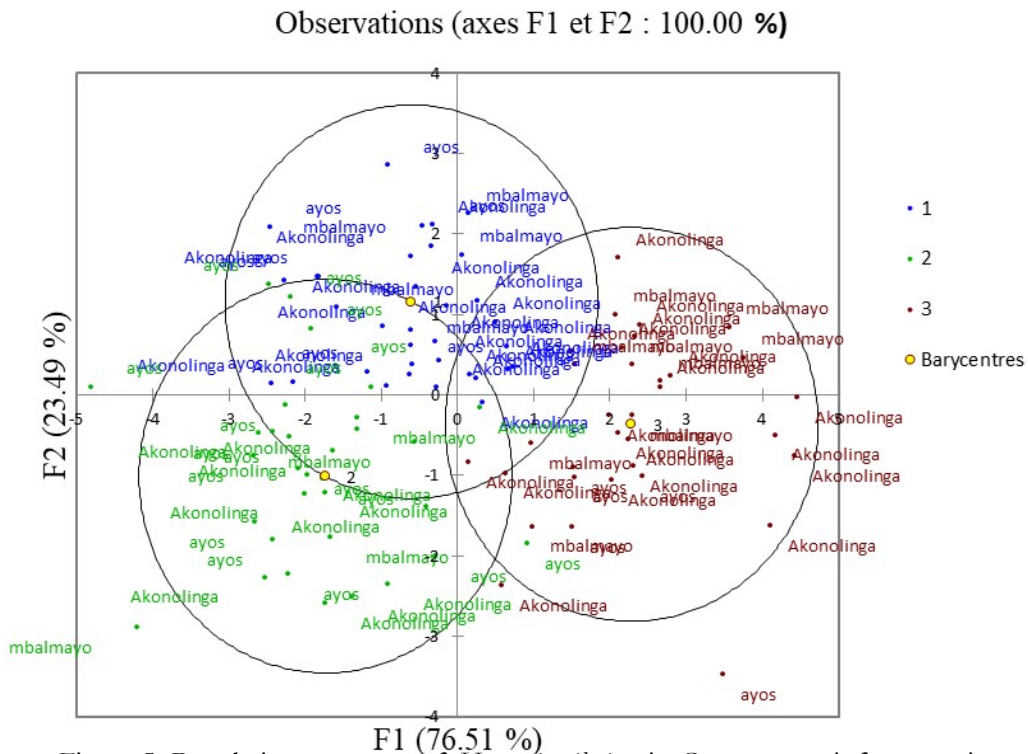


Figure 5: Population structure of *Heterotis niloticus* in Cameroon rainforest region

Table 4: Biometric and weight traits of the different morphotypes of *Heterotis niloticus* in Cameroon rainforest region

Morphotypes	W	TL	SL	BD	HL	SL	OD	PFL	PAD	PPD	PPeD	DFL	AFL	PDD	PeFL	CPL	CPD
1	3.60	58.25	51.83	13.45	13.18	3.18	1.84	7.25	32.61	11.46	25.13	23.90	21.34	33.23	4.06	2.75	3.42
2	3.31	53.76	47.22	12.97	12.41	3.22	2.71	7.71	30.42	11.24	23.44	18.80	20.60	29.64	5.00	2.59	3.36
3	5.33	67.03	60.03	15.39	14.63	3.66	1.84	8.02	37.48	12.89	27.37	22.44	24.69	37.12	4.46	3.15	3.89

W: weight; HL: head length; SL: snout length; ED: eye diameter; TL: total length; BD: body depth; DFL: dorsal fin length; AFL: anal fin length; PFL: pectoral fin length; PDD: pre-dorsal distance; PAD: pre-anal distance; PPD: pre-pectoral distance; PPeD: pre-pelvic distance; PeFL: pelvic fin length, PFL: pectoral fin length; CPL: Caudal peduncle length; CPD: Caudal peduncle depth

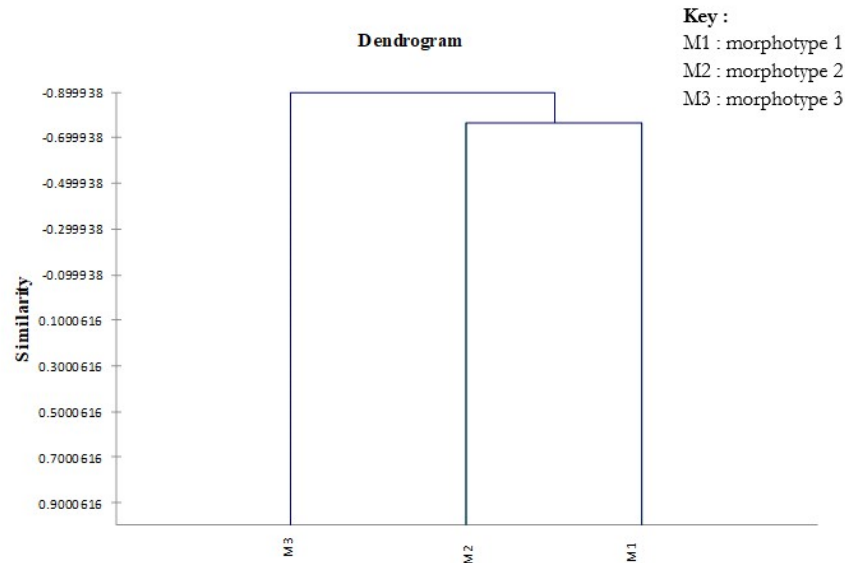


Figure 6: Dendrogram of *Heterotis niloticus* populations in Cameroon rainforest region.

The distance between the barycenters of the three morphotypes is summarized in Table 8.

Table 5: Distance between the barycenters of the different morphotypes of *Heterotis niloticus* in Cameroon rainforest region

Morphotypes	Morphotypes		
	1	2	3
1	0	9.79	14.73
2	9.79	0	22.71
3	14.73	22.71	0

The phylogenetic tree (Figure 6) and the distance between the barycenters of the morphotypes (Table 5) allowed us to establish the relationships that exist between the morphotypes. Thus, morphotypes 1 and 2 are closer together while morphotypes 2 and 3 are far apart. This closeness would probably be related to the genetic distance that would exist between the morphotypes. Thus, morphotypes 1 and 2 would share a more important genetic heritage.

Discussion

Phenotypic characteristics of *Heterotis niloticus* population

The main results of this study show that there is colour variability concerning eyes and body features of *Heterotis niloticus* in the study area. Thus colour variability would be due to genetic effects, as well as the action of the environment or simply physiological variations as mentioned by Daget (1957). It should be noted, however, that body coloration could be associated with social hierarchy in the population, it has been shown that social stress alters the internal state of the fish so that certain physiological processes are affected (Volpato *et al.*, 2003). Our results for eye colouration in *Heterotis niloticus* differ from those of Blanche *et al.*, (1964), Daget (1957) and Moreau (1982) who indicate that the eyes of

Heterotis niloticus would have a uniformly golden colouration. This difference would probably be due to the action of the environment or to a mutation having affected certain genes. Regarding the colour variability of the body, these findings are similar to those of Daget (1957); Blanche *et al.* (1964) and Moreau (1982) which indicate that *Heterotis niloticus* can have a uniformly greyish or brownish colouration. Thus, the different colourations encountered would probably be due to the action of the environment or to the interaction between genotype and environment.

Biometric and weight traits of *Heterotis niloticus* populations

Many authors (Adite *et al.*, 2017; Jimoh *et al.*, 2012; Lawson, 2010; Moreau, 1982) have used morphological measurements in the characterisation of fish species and measurements expressed as percentage of standard length, total length, body height, etc. They have established that it was an appropriate approach for fish species characterisation. In the current study, results indicated that there was no significant difference ($p > 0.01$) between the various calculated ratios and localities; this is similar to observations made by Adite *et al.* (2017) in which the calculated ratios showed no significant difference except for the ratios, eye diameter to head height and eye diameter to head length (which showed significant differences). Indeed, in fish, morphometric measurements, when expressed as the ratio of standard length, total length or body height, are very effective in describing and identifying fish species (Adepo-Gourène and Gourene, 2008).

Regarding total weight, individuals from Akonolinga district are heavier (PT=4.49kg) and less variable (CV=25.16%) while those from Ayos district are less heavy (3.42kg) and more variable (32.45%).

Adite *et al.*, (2017) in southern Benin found a total weight that varied from 1.1-4.55kg. This result

although comparable to this observation (TP=1.47-7.57kg) is still relatively lower. It is however lower than the total weight (TP=10kg) recorded by Paugy (1990). Feed considerations (variations or availability depending on the season) as well as fishing pressure would explain the large weight fluctuations. In general, the coefficients of variation of the total weight found in this study are important and are 25.16% (Akonolinga); 30.20% (Mbalmayo) and 32.45% (Ayos).

Meristic traits of *Heterotis niloticus*

Five meristic counts related to scales and fin rays were also considered to describe and characterise *Heterotis niloticus*. In this study, the results showed that there was no significant spatial variation ($p > 0.01$) for most of the meristic counts except for the number of scales at the lateral line which showed a significant difference ($p < 0.01$). The significant difference observed for this trait reveals some heterogeneity within the study population. Blanche (1964) in earlier studies noted: 33-37 rays on the dorsal fin, 34-38 rays on the anal fin, 11-12 rays on the pectoral fin and 5-6 rays on the ventral fin in the Lake Chad basin. Daget (1954) also described the number of soft rays on the dorsal fin (D=32-37), anal fin (34-39) and the number of scales on the lateral line (LL=34-40). Overall, these results are consistent with the observations from this study (D=26-36 ; A=31-38 ; P=9-12; V=6) although there are slight fluctuations. Indeed, the meristic characters are not variable within the species. Straus (1985) stated that meristic traits are fixed early in development and do not change during growth.

Conclusion

From the findings of this research, it appears that there is a colour variability of eyes and body in *Heterotis niloticus* in the rainforest region of Cameroon with a predominance of golden eyes and grey body. The locality had no significant effect

on most biometric and meristic characters except for the number of scales on the lateral line which was significantly influenced by different localities. Principal component analysis revealed phenotypic variability within the population of *Heterotis niloticus* in the study area. The hierarchical ascending classification showed that the bonytongue African fish population is composed of 3 morphotypes in the zone. The diversity observed in the *Heterotis niloticus* population in the rainforest region of Cameroon is due to its probable adaptation to the environmental conditions and suggests that it constitutes a natural genetic resource with the necessary variability for its exploitation. Variability observed could be used in breeding schemes, either for selection or crossbreeding, given further appropriate ongoing investigation.

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