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Morphometric and Meristic Identification of Wild Populations of *Clarias sp* and Their Hydro-Geographical Structuring in Burkina Faso

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Abstract
The species *Clarias gariepinus* plays an increasingly important role in the aquaculture sector in Sub-Saharan Africa. As there are two species morphologically very similar *(with C. anguillaris)*, the use of this species in aquaculture requires first of all a proper identification. Both species exist in Burkina Faso but no studies have confirmed their presence, outside the Volta basin. The objective of this study is to characterize morphological both species on the one hand, and to investigate about their structuring in the hydrographic system of country on the other hand. The study was carried out with eleven populations spread over the three main basins (Niger, Volta and Comoé) across the country and a domesticated population of *C. gariepinus* from Benin. Each sampled population is constituted at minimum of 30 individuals. Sixteen meristic and metric variables were measured on each individual sampled. PCA and correlations performed on these variables allowed to identify two groups based of the number of gill rakers on the first branchial arch. The first group identified as *C. anguillaris* has a smaller number of gill rakers ranging from (13-39) for individuals whose standard length varies from 98 up to 610 mm. The number of gill rakers of the second group, identified as *C. gariepinus,* is higher and is varied (28-120) for individuals with standard length between 109 up to 583 mm. About geographical distribution, the results show that *C. gariepinus* is present only in Volta's basin. However, *C. anguillaris* is present in all the three basins. Moreover, both species are sympatric in Bama, Bala, Sourou and Kompienga. Thus, our results show the presence of two species in the country hydrographic network and also highlight

the high prevalence of *C. anguillaris* compared to *C. gariepinus*.

1. Introduction

The subgenus *Clarias (Clarias)* (Scopoli, 1777), from Clariidae family, include only two species, *Clarias anguillaris* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822). These two species play a very important economic role in various fisheries of their distribution area in Africa and Southeast part of Asia. Of the two species, *C. gariepinus* is now most commonly used in aquaculture for its better growth performance [1]. Moreover, its well growth coupled with his tolerance to diseases and its ability to hybridize with other species to provide superior hybrids has expanded its introduction in South East Asia, outside its natural range [2-5]. Thailand produced more than 116,900 tons of hybrid catfish (*Clarias gariepinus* x *C. macrocephalus*) in 2010 [6]. In sub-Saharan Africa, this species (*Clarias gariepinus*) replaced the tilapia (*Oreochromis niloticus*), as most fish produced in fish farms since 2004 [6]. The gradual dominance of catfish species in aquaculture in Africa is particularly remarkable in Nigeria and Uganda [6]. All this production is supposed to be only the species *C. gariepinus*. Referring to fry supply sources and given that some farmers are supplied in the natural environment, there is the question of whether individuals actually used all belong to the species *C. gariepinus*. Indeed, the two are often sympatric species in rivers and lakes in Africa and distinction on the basis of morphological characteristics poses enormous difficulties. Moreover, one cannot exclude the possibility of hybrids between the two species. Thus several studies have been devoted to their morphological characterization [4,7-9]. Almost all of these studies have concluded that the most distinctive criterion of both species is the number of gill rakers located on the first gill arch. This criterion constitutes the only valid one which discriminates these species. According to [4], this number varies from (16-50) for *C. anguillaris* and (24-110) for *C. gariepinus*. For [10] this number is (14-40) for the first species and (20-100) for the second. Considering these data, it appears an important overlap which does not allow precise identification, and thus a separation of the two species on the basis of this criterion. Studies using microsatellite markers and enzyme coupled with morphological data showed an ability to identify more efficient [11,12]. It remains, however, that the identification of these two species in the inventory work on the field continues to set a real problem and leads most of the time to a false naming of the species. Very often, this situation leads to a mixture farming of two species or breeding a species instead of the other particularly in Africa. This situation is the same in Burkina Faso since no work of precise characterization of these two species and their distribution in different watercourses and reservoirs was not realized so far. Almost all of the documents most often mention the *Clarias gariepinus* species without reference to the possibility of the presence of *Clarias anguillaris*. The current study aims to identify wild populations of *Clarias* based on the morphological characters and to design the map of their distribution in the hydrographic basins of the country for their better use in intensive aquaculture in Burkina Faso.

2. Material and Methods

2.1. Study Area

Samplings were realized in the three international basins namely Comoé, Niger and Volta which cover the country. The Comoé Basin, with an area of 1800 km², covers the southwestern part of the country. It included the upstream part of the Comoé River and its tributary the Leraba. The Niger Basin covers 72 000 km² in the Northeastern part. It includes the upstream part of many tributaries (Gourouol, Goudebo and Sirba) of the right bank of the Niger River. The Volta Basin, which is the largest one, covers an area of 172,968 km^2, or about 63% of the national territory. It is drained by rivers Mouhoun, Nakambé and Nazinon. These three international basins are subdivided into four national sub-basins namely Comoé, Niger, Mouhoun and Nakanbé.

2.2. Biological Samples

Eleven populations of *Clarias sp* were collected from all the four national basins (fig1). Among these eleven populations, two belong to the Comoé Basin, two to the Niger Basin and the other seven in the Volta Basin (fig1). The characteristics of these sampling sites (geographic co-ordinates, climatic zone) are summarized in Table 1. Each sampled population is constituted of 30 individuals selected randomly and including all development stages of the species. These individuals were caught with different types of engines (gillnets, hawk net, trap) with the help of local fishermen.

A domesticated population of *C. gariepinus* currently reared in our lab and brought from Benin was also added as referential. This population belongs to Netherlands strain and was imported to Benin.

2.3. Methodology

Two types of variables were collected, morphometric and meristic variables. On each individual, 16 metric variables were measured and one meristic variable. Metric variables used are those proposed by [13] for identifying a siluriforme fish (fig 2). For meristic data, the number of gill rakers located on the first gill arch was counted.

Table 1. *Characteristics of sampling sites based on the hydrographic basins of Burkina Faso.*

Internationals basins	Nationals basins	Sampling sites	Nature of stretch of water	Type of water	X co-ordinates	Y co-ordinates	Climatic zone	Vegetation cover of the basin	Aquatic vegetation
Comoé	Comoé	Tengrela	Natural	Tributary of Comoé river	300130	1178465	South-sudanese	Sudan savanna woodlands	Abundant and diverse
		Moussodougou	Artificial	Comoé river	287989	1198222	South-sudanese	Sudan savanna woodlands	Scarce
Niger	Niger	Oursi	Natural	Tributary of Niger river	773518	1624157	Strict-sahelian	Grassy savannas and shrublands	Abundant
		Tapoa	Artificial	Tributary of Niger river	1022796	1340633	North-sudanese	Sudanese savannah woodlands and tree	Abundant
Volta	Nakambé	Kompienga	Artificial	Nakambé River	891972	1244800	South-sudanese	Sudanese savannah woodlands and tree	Scarce
		Bagré	Artificial	Pendjari River	774998	1276401	North-sudanese	Sudanese savannah woodlands and tree	Scarce
	Mouhoun	Bala	Natural	Tributary of Mouhoun River	382182	1271451	South-sudanese	forest Galilean	Abundant and diverse
		Bama	Natural	Tributary of Mouhoun River	345042	1258545	South-sudanese	Sudan savanna woodlands	Abundant and diverse
		Bapla	Artificial	Tributary of Mouhoun River	471194	1203365	South-sudanese	Sudanese savannah woodlands and tree	Scarce
		Sourou	Natural	Tributary of Mouhoun River	476364	1417621	North-sudanese	Savanna and tree	Abundant and diverse
		Boromo	Natural	Mouhoun River	507798	1298875	South-sudanese	rypicoles forests	Scarce

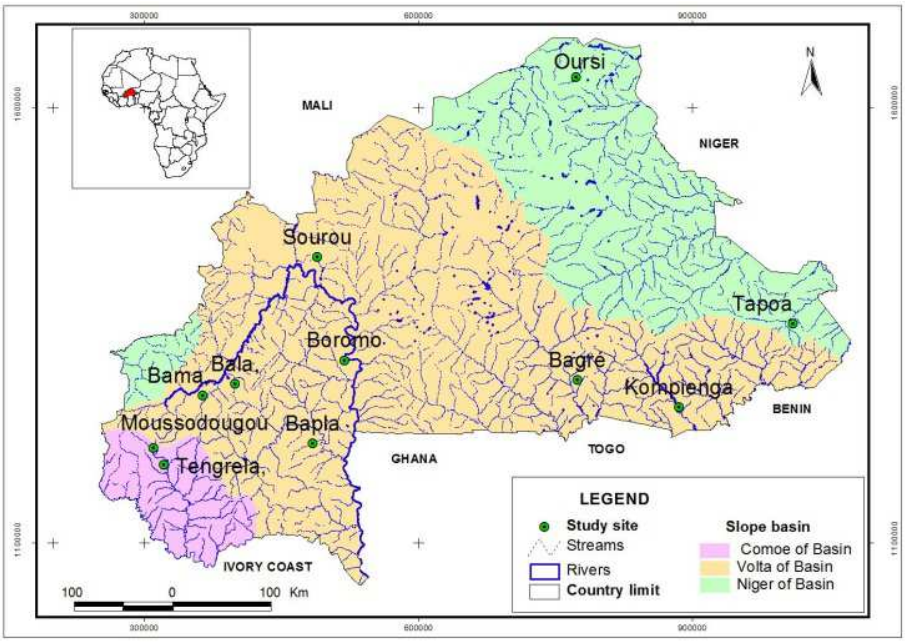

Figure 1. *Sampling sites in the different hydrographic basins of Burkina Faso.*

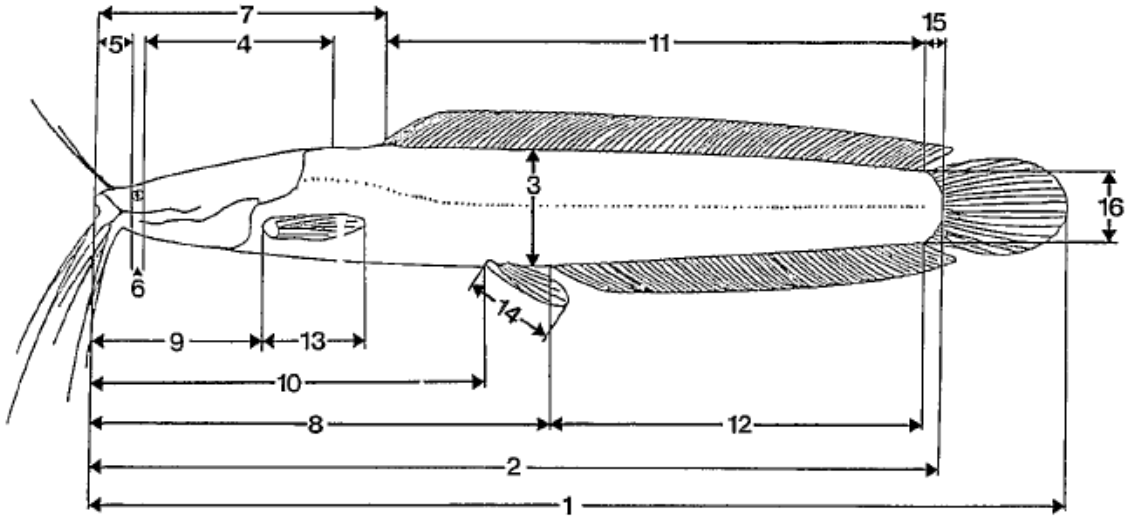

Figure 2. *Schematic illustration of the measurements realized on each individual according to [4].*

1. Total length (TL); 2. Standard length (SL); 3. Body depth (BD); 4. Head length (HL); 5. Snout length (SNL); 6. Eye diameter (ED); 7. Predorsal length (PDL); 8. Preanal length (PAL); 9. Prepectoral length (PPEL); 10. Prepelvic length (PPL); 11. Length of dorsal-fin base (DFL); 12. Length of anal-fin base (AFL); 13. Pectoral-fin length (PCFL); 14. Pelvic-fin length (PLFL); 15. Caudal-peduncle length (CPL); 16. Depth of caudal peduncle (DCP)

Table 2. *List of principal measurements in accordance with number of the figure 2.*

N°	Parts	Abbreviation	Full name	Definition
1	Body	TL	Total length	Horizontal distance from front tip of snout to hind tip of caudal fin
2	Body	SL	Standard length	Horizontal distance from front tip of snout to base (or articulation) of caudal fin
3	Body	BD	Body depth	Maximum vertical depth of fish, excluding fins
4	Head	HL	Head length	Horizontal distance from front tip of snout to hind margin of gill cover, or the horizontal distance from front tip of snout to hind tip of occiput or to bony rim of the notch formed by the scapular girdle behind the head.
5	Head	SNL	Snout length	Horizontal distance from front tip of upper jaw to anterior margin of eye.
6	Head	Ed	Eye diameter	Horizontal diameter of eye.
7	Fin	PDL	Predorsal length	Horizontal distance from front tip of snout to the articulation of first dorsal-fin ray.
8	Fin	PAL	Preanal length	Horizontal distance from front tip of snout to the articulation of the first anal-fin ray.
9	Fin	PECL	Prepectoral length	Horizontal distance from front tip of snout to the articulation of the first pectoral-fin ray.
10	Fin	PELL	Prepelvic length	Horizontal distance from front tip of snout to the articulation of the first pelvic-fin ray.
11	Fin	LDF	Length of dorsal-fin base	Maximal horizontal distance measured between both ends
12	Fin	LAF	Length of anal-fin base	See dorsal-fin base
13	Fin	PECF	Pectoral-fin length	Length from articulation of the first ray to tip of longest ray
14	Fin	PELF	Pelvic-fin length	See pectoral-fin length
15	Stalk	CPL	Caudal-peduncle length	Horizontal distance from hind margin of anal fin (or from that of dorsal fin if this extends further backwards than anal) to base of caudal fin.
16	Stalk	DCP	Depth of caudal-peduncle	Minimum vertical depth of caudal peduncle

2.4. Data Analysis

First of all, multivariate analysis (Principal Component Analysis and Ascending Hierarchical Classification) were performed on matrix data to identify variables that discriminated taxonomic groups. Then a linear regression with these discriminating variables was realized to better characterize these groups. Finally, an assessment of the representativeness of these groups was illustrated using a histogram. All these analyzes and the input of collected data were performed using excel 2010 software

3. Results

3.1. Principal Component Analysis on Metric and Meristic Variables

Principal Component Analysis (PCA) realized with all of metric and meristic variables shows that the bidimensional space formed by the first two axis (Fact 1- Fact 2) cumulative 93.17% of the total inertia (fig 3a). This shows that it is the best representation space for viewing clearly all the

individuals. The first axis (Fact 1) that contains 87.92% of inertia is highly correlated by the metric variables (TL, SL, BD, HL, SNL, ED, PDL, PAL, PPEL, PPL, DFL, AFL, PCFL, PLFL, CPL and DCP). The second axis (Fact 2), which represents 5.25% of inertia is highly correlated with the meristic variables i.e. the number of gill rakers. The representation of individuals on this space (Fact 1- Fact 2) shows two groups of individuals (fig 3b). Individuals within group 1, located above of factor 1 and left side, were strongly correlated with the metric variables. Individuals within group 2 are highly correlated with the meristic variable and were located below the factor 2. Individuals within this group are opposed to those of group 1 by their higher number of gill rakers (NGR).

The Hierarchical Classification Ascending (HCA) performed with these data splits the individuals in two groups (fig 4). In the fig 4b, we distinguished two groups of individuals based on to the two groups of variables of the fig 4a. The group of individuals in red color is characterized by the meristic variable and the group in black is characterized by the metric variables.

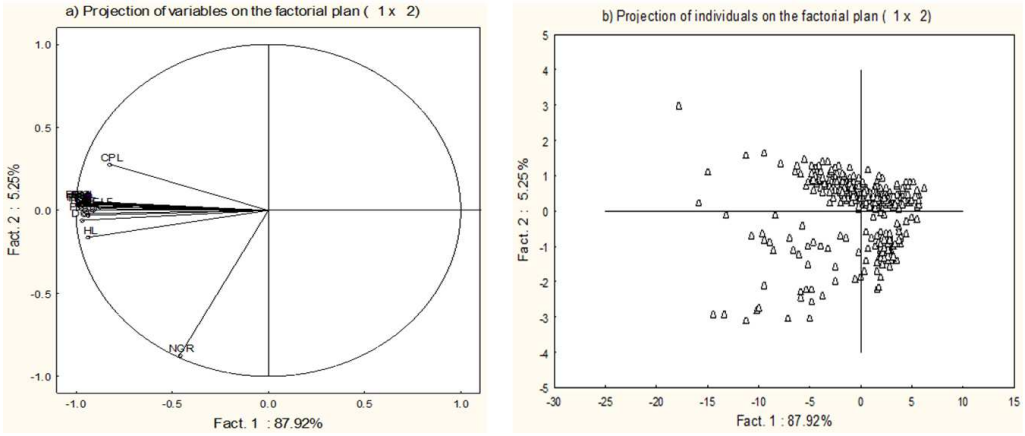

Figure 3. *Projection of variables (a) and individuals (b) on the factorial plan (Fact 1- Fact 2) with all metric and meristic variables.*

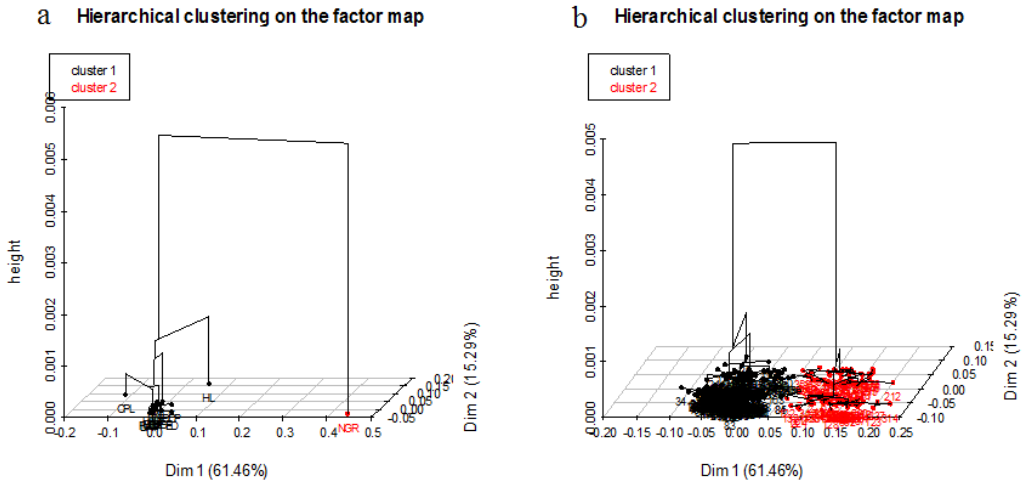

Figure 4. *Grouping of variables (a) and individuals (b) from Hierarchical Classification Ascending.*

3.2. Relation Between the Number of Gill Rakers and the Standard Length

The relationship between standard length and the number of gill rakers allows a distinct differentiation between the two groups (fig.). These two groups correspond to those identified during the Principal Component Analysis (PCA) and the Hierarchical Classification Ascending (HCA). Individuals group 1 are especially marked by a fewer gill rakers than those in group 2. The number of gill rakers ranges from 13 (in a specimen of 98.52 mm LS) to 39 (in a specimen of 610 mm SL) for group 1 and 28 (in a specimen of 109. 12 mm LS) to 120 (in a specimen of 583 mm SL) for the group 2. Individuals of group 2 contain the wild populations and the domesticated population from Benin used as control in this study. The observation of the curves of individuals supposed to be *C. gariepinus* show that for a same standard length, wild individuals possess an average number of gill rakers more higher (41-120) than those derived from domesticated population (28-79) (fig 6). The graphics shows that the number of gill rakers increases with the increase of standard length in both species. According to the descriptions realized by [4] on the two species of the subgenus *Clarias (Clarias),* the individuals of group 1 could be identified as being *C. anguillaris* and those in group 2 as *C. gariepinus.*

Table 3. *Contribution in percentage of the variables to the creation the Fact 1 and Fact 2 of PCA.*

Variables	Fact 1 *100	Fact 2*100
Total length (TL)	6.59	0.09
Standard length (SL)	6.59	0.26
Body depth (BD)	6.21	0.24
Head length (HL)	5.86	2.94
Snout length (SL)	6.34	0.34
Eyes diameter (EY)	5.88	0.09
Predorsal fin length (PDL)	6.57	0.15
Preanal fin length (PAL)	6.40	0.36
Prepectoral fin length (PPFL)	6.49	0.32
Prepelvic fin length (PPL)	6.50	0.16
Dorsal fin length (DFL)	6.45	0.06
Anal fin length (AFL)	6.46	0.20
Pectoral fin length (PFL)	6.16	0.04
Pelvic fin length (PFL)	5.26	0.01
Caudal peduncle length (CFL)	4.54	8.68
Depth of caudal peduncle (DCP)	6.29	0.40
Number of gill rakers (NGR)	1.43	85.66

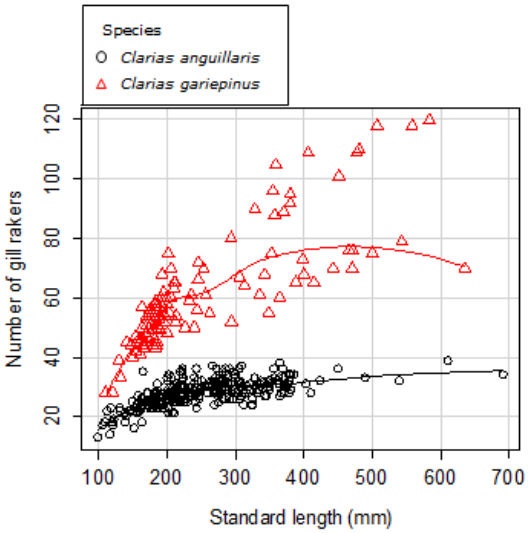

Figure 5. *Number of gill rakers on first gill arch in relation to standard length (SL) in C. gariepinus and C. anguillaris.*

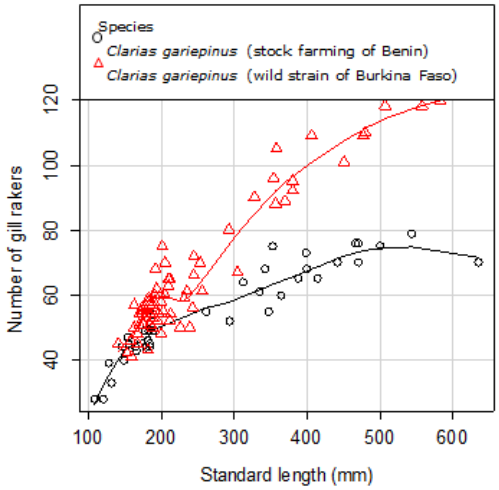

Figure 6. *Number of gill rakers on first gill arch in relation to standard length (SL) in C. gariepinus.*

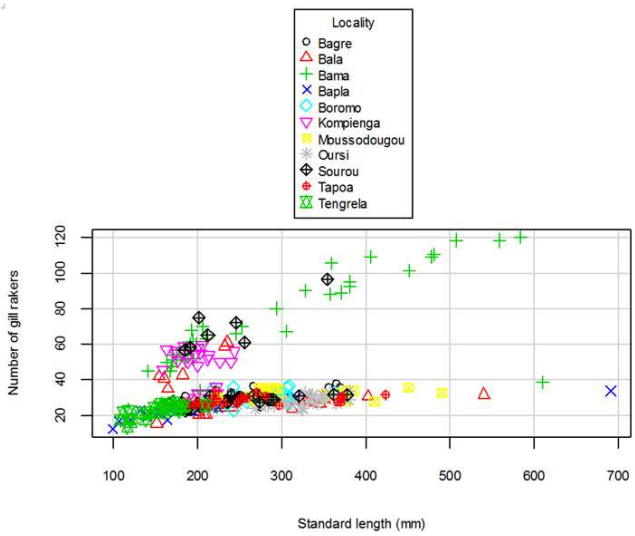

Figure 7. *Distribution of C. anguillaris and C. gariepinus in the sampled sites.*

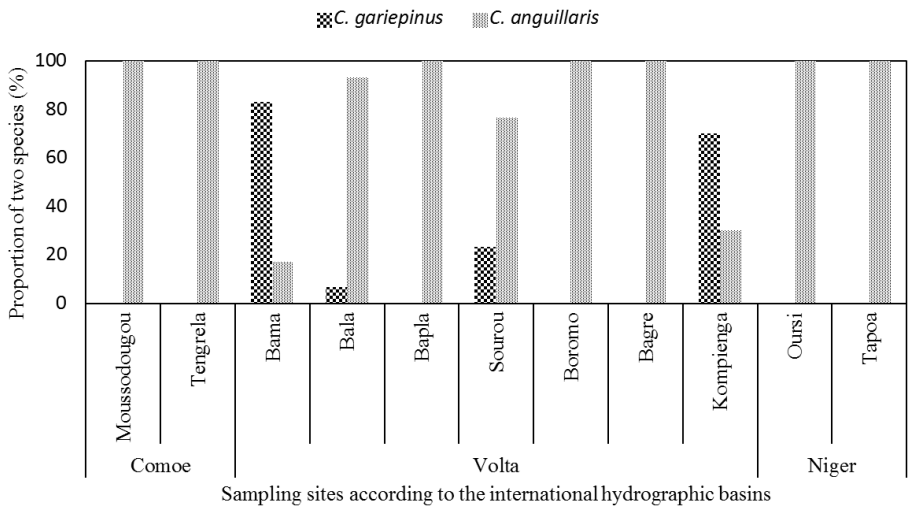

Figure 8. *Proportion of C. gariepinus and C. anguillaris in the sampled sites according to the international hydrographic basins.*

3.3. Hydro-Geographic Structure of the Two Species in Burkina Faso

The linear regression on wild populations highlights the distribution of the two species throughout the hydrographic network of Burkina Faso (fig 7). Individuals of *C. anguillaris,* characterized by reduced gill rakers number (13-39) are distributed in all the 11 waterbodies. Wild individuals of *C. gariepinus* from Burkina Faso, which have a high number of gill rakers (39-120) are only present over 4 waterbodies (Bama, Bala, Sourou and lake dam Kompienga), all located in the Volta Basin.

3.4. Proportion of Populations of *C. gariepinus and C. anguillaris* in the Sampled Sites

The figure 8 shows the proportion of both species on all of the sites sampled. *C. anguillaris* represents 79.2% and 20.8% for *C. gariepinus* of all the wild individuals collected. The sites located in the basins of Comoé (Moussodougou, Tengrela) and of Niger (Oursi, Tapoa) and also 3 Volta basin (Bapla, Boromo and Bagré) contains only individuals of *C. anguillaris*. Individuals of *C. gariepinus* are present in 4 waterbodies of the Volta basin where, they are sympatric with *C. anguillaris*. However, in the waterbodies, where the two species are sympatric, it is observed a predominance of *C. gariepinus* at Bama and Kompienga with a percentage respectively of 82.39 and 70% of individuals sampled. In the waterbodies of the Sourou and Bala, their proportions are less important, 24 and 7% respectively.

3.5. Morphological Differentiation of Gill Rakers Between the Two Species

On the gill arches, the gill rakers looked like combs slender, longs and very close to each other (fig 9A) for the individuals of *C. gariepinus*. However, for *C. anguillaris*, they looked like also as combs but short, thick and more spaced (fig 9B). The existence of an arched form (fig 10A) of the gill rakers was observed in some individuals belonging to the two species. On the gill arches where the arched form was identified, their number did not exceed one or two and they are inserted as the others (fig.10B).

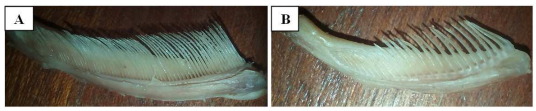

Figure 9. *Form of gill rakers in C. gariepinus 320 mm SL (A) and in C. anguillaris 310 mm SL (B).*

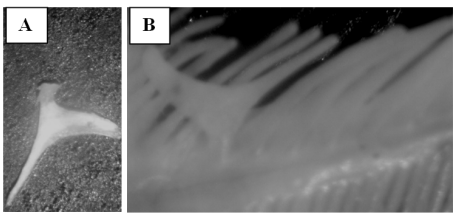

Figure 10. *Illustration of arched form and their inserting on the gill arch in both species.*

4. Discussion

Data analysis using Principal Component Analysis (PCA), Hierarchical Classification Ascending (HCA) and linear regression reveal all existence of two distinct groups based on the variability of the number of gill rakers on the first gill arch. Compared to data available in the literature [4,14], we concluded that the first group with a smaller number of gill rakers (13-39) is *C. anguillaris* and the second group with a greater number of gill rakers (28-120) is *C. gariepinus*. The two groups belong to the subgenus *Clarias (Clarias)* (Scopolli, 1777) defined by [4,14]. These data clearly show for the first time the existence of two species in the Burkina Faso waterways. Moreover, during the taxonomic revision of species of the subgenus by [14], they mentioned that in *C. anguillaris*, the number of gill rakers were between 14 and 40 when it was between 20 and 100 in *C. gariepinus*. Later, [4] published that this number in *C. anguillaris* varies from 16 (in a specimen of 31.5 mm SL) to 50 (in a specimen of 650 mm SL) while in *C. gariepinus*, it is 24 (for a specimen of 27.7 mm SL) to 110 (in a specimen of 600 mm SL). Our results are in the same ranges as those previous studies, with however, higher values in *C. gariepinus*. The maximum number of gill rakers reached 120 in this species, which had never been mentioned in the literature. We also found that the number of gill rakers increases regularly with the standard length and maybe therefore with age. This increase is done within defined limits according to each species. If for the subgenus *Clarias (Clarias)*, this character evolves continuously with age, this is not the case in some species of the Clupeidae family as *Sardinella eba* and *S. aurita*. Indeed, [15] notes that the increase of number of gill rakers with age is not continuous; it is through bearings, rest periods succeeding periods of organogenic activity where appear new rakers. Furthermore, our study also shows that the average number of gill rakers of *C. gariepinus* from domesticated strain from Benin is lower than that of wild populations for individuals with the same standard length. Most of the works on this subject [15,16], have sought to show that the average number of gill rakers, in fish of the same size varies from one region to another. These studies consider that the variations of ambient conditions, particularly temperature and salinity, are responsible for this variability. These variations could be linked to farming conditions which would affect genes governing this character. Among all the variables we analyzed, the meristic variable was the only one allowed to differentiate the two species. This is consistent with the literature for which the number of gill rakers is the only valid criterion for distinguishing *C. gariepinus* and *C. anguillaris* [4,12,14]. Some authors, [16-18] got the same results, arguing that this character is a good criterion for separation of species, and populations within the same species. In contrast to the meristic variable, metrics variable globally following a continuum of increasing size, thereby limiting their discriminatory power. Our work has revealed the existence of these two species in the river system of Burkina Faso but with a specific allocation for each. *C. anguillaris* is distributed in all sampling sites, meaning in the three watersheds (Niger, Volta

and Comoé). Moreover, *C. gariepinus* is found in four waterbodies (Bama, Bapla, Sourou and Kompienga) in the Volta basin's where it lives in sympatry with *C. anguillaris*. The first three localities are located upstream of the Volta Basin and the last is in the far east of the Basin. His presence in this body of water is probably due to an introduction conducted as part of the policy of poisoning of waterbodies by the fisheries office. The absence is surprising and raises questions because it was reported in the catches of fishermen settled in the basins of the Comoé and Niger by [14]. This absence could mean that the species is under threat for disappearance of its residential areas. Given its ubiquitous nature, it was expected a wider distribution such as *C. anguillaris* given that it is a freshwater species living in primary rivers and woodland lakes across Africa [19]. In addition, it survives well in low oxygen and disperses easily as it adapts to the ambient air, tolerates extreme desiccation, swims well and moves on earth [20]. However, other studies [21, 22] indicated the presence of *C. gariepinus* in Comoé but this can be justified by a bias in their sample as they didn't took into account the existence of *C. anguillaris*. On the other hand, its strong presence in the waterbodies of Bama and Sourou could indicate an ecological preference. Indeed, [23] state that the distribution of species locally and the factors that influence the most are mostly abiotic factors (physical, chemical, climatic) and biotic (competition, predation, disease). Furthermore, diet or trophic guild is the most determining factor in the ecological packaging of fish species [24]. Indeed, the upstream part of the Volta is characterized by the presence of natural ponds with abundant and diverse aquatic vegetation, which could explain the presence of *C. gariepinus* in these places. Regarding the morphology of rakers, we have identified two types during this study. A tapered form that we have described as the normal form because it is the predominant form and is regularly inserted along the gill arch. The second form is arched, qualified as abnormal form is very rare. This latter is inserted between normal and gill hardly exceeds one or two in a gill arch. This abnormal shape was observed on wild strains of both species and absent at the strain level domesticated from Benin was never reported in the literature. The presence of the arched shape in wild strains could be explained by a gene mutation occurred when water or nutritional stress where the presence of heavy metals.

5. Conclusion

This study reveals the existence of two species of the subgenus *Clarias (Clarias)* of family Clariidae namely *C. anguillaris* and *C. gariepinus* in the hydrographic network of Burkina Faso. Analyzes on the geographical structure have shown that *C. gariepinus* was localized in the Volta basin unlike *C. anguillaris* which is ubiquitous in all water bodies of the country. Our results also highlight the high prevalence of *C. anguillaris* compared to *C. gariepinus*. It should however be noted that the diversity of fish species obtained on the basis of morphological characters biometric is generally complex and often misinterpreted because these can be affected by environmental factors. It is therefore important to refine these results by genetic characterization using molecular and enzymatic markers.

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