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Meristic, morphological and genetic identification of the fish *Brycinus nurse* (Alestidae) from Sinnar, Blue Nile

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Abstract
A sample of 20 fishes of *B. nurse* was collected in very high level of freshness from Sinnar fish market for meristic, morphometric measurements and DNA extraction. Statistical analysis indicated significant correlation ($p< 0.05$-$p< 0.001$) between the total length and any of the measurements except with upper jaw length ($p>0.05$) and lachrymal depth ($p>0.05$). The DNA analysis resulted in a DNA fragment of 730 pb long. This can be used for comparison between the *B. nurse* of the main Nile and its tributaries. The resultant DNA fragment can also be used for DNA sequencing and verification of phylogenetic relations between *Brycinus* spp. and other members of Family Alestidae. Precise fish species identification is vital for fishery management.

Keywords: Morphometric, DNA, brycinus nurse, Sudan

Introduction
Brycinus nurse (Ruppell, 1832) is widely distributed in Chad Basin and Nile River up to Lake Albert (Fishbase.org) [1]. The species was adequately described by Abu Gideiri [2]. The genus contains 36 spp., but Murray and Stewart [3] claimed that several species should be removed out of this genus. *Brycinus* spp. are abundant and of commercial value in African inland fisheries (Reynolds; Iyaboo) [4, 5]. Neumann *et al.* [6] in his annotated Checklist for fishes of the Main Nile Basin elaborated on *B. nurse* conservation status and distribution. Guma'a and Yassin [7] studied its food and feeding habits. Saliu [8] related size and sex to seasonal dynamics in the dietary composition of Brycinus nurse from Asa reservoir, Nigeria.
The molecular biology of *B. nurse* from Kreima samples was studied by Mohammed *et al.* [9]. Quantification of morphometric measurements and meristic counts of fish became a powerful tool in the description of fish species (Mahmoud and Hassan) [10] especially when coined with genetic studies.
Length-weight relationship and growth pattern were studied in a number of *Alestess* pp and *Brycinus* spp. An examples are the work of Ikomi and Sikoki [11] on *B*rycinus *longipinnis* in River Jamieson, Nigeria; Abobi and Ekau [12] study on *B. nurse* in the lower reaches of White Volta River, Ghana, and Adeosun *et al.* [13] study on *Brycinus macrolepidotus* from lower Ogun.
The study aimed to quantify meirstic counts, morphometric measurements and amplify COI DNA fragments of *B. nurse* from Sinnar.

Materials and Methods
Sampling
Twenty highly fresh specimens of *B. nurse* were randomly collected from the commercial landing site at Sinnar, in December 2017. From each left side of a fish specimen the pectoral fin and gills were collected and fixed in 100% ethanol for molecular studies.

Meristic and Morphometric measurements
After identification of the specimen and collection of tissues for molecular work specimens were kept wet. The rays of dorsal, pectoral, pelvic, anal and caudal fins were counted from the left side of each fish, and 18 morphometric measurements were recorded using a measuring

board, a tape and a verneir caliper as appropriate. The measurements followed Ebraheem [14]. These were: total length (TL); standard length (SL); body depth (BD); body width (BW) head length (HL); head width (HW); snout length (SNL); lower jaw length (LJL); upper jaw length (UJL); eye diameter (ED); lachrymal depth (LD); dorsal fin base (DFB); anal fin base (AFB); pre-anal length (PAL); pre-pectoral length (PL); pre-pelvic length (PPL); caudal peduncle length (CPL) and caudal peduncle depth (CPD).
In meristic counts, the mode was used as a more appropriate over the mean (which involves
fractions) as well as the median when describing distinct clear-cut data and hence it was preferred as a measurements of central tendency.

DNA extraction using potassium acetate (KAC)
DNA extraction procedures and detection followed Ghossein *et al*. [15]. Stored 0.1gm pectoral fin of each individual samples were processed for DNA extraction by soaking in 100µl standard extraction buffer and homogenized gently with a glass rod in 1.5 ml eppendorf tube. After adding 100µl extraction buffer, the tube was immediately kept in a 68 °C water bath for 15 min. After that 100µl of KAC 0.099M was added and the mixture was transferred to an ice box for 45 min with occasional inverting of the tubes. The tubes were spun in a microfuge at 14000 rpm for 10 min to collect the cellular debris and precipitated proteins. The supernatant was then transferred to a fresh tube. The last step was repeated twice, and then 600µL of absolute ethanol was added to the solution. The solution was left at -20°C for at least 2 hours or overnight to precipitate the DNA. The tubes were spin in a microfuge for 15 min at 14000 rpm and the supernatant was discarded. Nucleic acid were washed by adding100µl of 70% ethanol and spun at 14000 rpm for 10 min and the ethanol was decanted. This step was repeated twice. The tubes were then inverted on a tissue paper and left to dry for two hrs.

Polymerase chain reaction (PCR)
The details of PCR followed Ebraheem [14]. PCR reaction contained 10 pmol of each primer (F2_t1, FishR2_t1and FR1d_t1), 2µl of extracted DNA was adjusted to 25 µl by double distilled water. The PCR used was initial denaturation at 94°C for 1 min; followed by 35cycles of denaturation at 45°C for 30 sec, annealing at 94°C for 30 seconds and extension at 72°C for 45 sec and the final extension was at 72°C for 5 min. The machine was programmed to store the reaction at 4°C until collected.
PCR products were separated by electrophoresis using 2% agarose gel dissolved in 20 ml 1-XTBE. The PCR products were stained with ethidum promide and visualized under the UV light using gel documentation system. The product size was estimated using a 100 pb DNA ladder**.**

Data analysis
The data was analyzed using the SPSS programme (Version 24.0. Armonk) as appropriate.

Results
Meristic and Morphometric characters
Table 1 showed the number of rays and ranges in dorsal fin, pelvic and caudal fin. Kreima data was after Mohammed *et al.* [9].

Table 1: Mode of meristic counts of *B*rycinus *nurse* from Sinnar and Kreima

Meristic	Sinnar 13°34'N 33°34'E		Kreima 18°33′N 31°51′E	
	Median	Range	Median	Range
DFR	9	8-9	11	9-12
AFR	15	14-15	16	15-18
PFR	13	12-14	13	13-15
PVFR	9	9	9	9

Morphmetric measurements in % of SL were generally higher in Sinnar samples, while in % of HL it was lower in HW and ED (Table 2).

Table 2: Morphmetric measurements of *B*rycinus *nurse* from Sinnar and Kreima (NR= not recorded; NA= not applicable).

Parameter in cm	Mean ± SD	Range	in % SL		In %HL	
			Sinnar	Kreima	Sinnar	Kreima
TL	11.04±0.78	10 -13.2	1.232	0.218	NA	NA
SL	8.96±0.67	8.0-10.9	1.000	NR	NA	NA
BD	2.76±0.25	2.3-3.1	0.308	0.291	NA	NA
BW	15.75±4.74	9.1-30.7	1.758	0.132	NA	NA
HL	2.14±0.29	1.7-2.6	0.234	NR	1.000	NA
HW	1.02±0.13	0.8-1.3	0.121	NR	0.477	0.545
SNL	0.68±0.11	0.5-0.9	0.076	NR	0.318	0.317
LJL	0.73±0.112	0.5-0.9	0.081	NR	0.314	NR
UJL	0.57±0.07	0.5-0.7	0.064	NR	0.124	NR
ED	0.63±0.06	0.6-0.8	0.070	NR	0.294	0.339
LD	1.73±0.32	0.6-2.1	0.193	NR	0.794	NR
DFB	0.99±0.12	0.8-1.3	0.110	NR	NA	NA
AFB	1.30±0.17	1.0-1.8	0.145	NR	NA	NA
PAL	6.75±0.54	6.1-8.1	0.753	NR	NA	NA
PL	1.70±0.13	1.5-2.1	0.190	NR	NA	NA
PPL	2.24±0.16	1.9-2.6	0.250	0.229	NA	NA
CPL	1.37±0.17	1.1-1.6	0.153	0.153	NA	NA
CPD	0.91±0.08	0.8-1.2	0.102	0.093	NA	NA

Regression between standard length and the other morphometric measurements (cm) and the standard length and the body weight (gm), Table 3 showed that all correlations between the standard length and the measured parameters were significant and varied from $p< 0.05$ to $p< 0.001$. It also showed negative highly significant correlation ($p< 0.001$) between the SL and UJL, and significant correlation ($p< 0.05$) between SL and ED. The standard length body weight relationship was negatively allometric (b<3.0).

Table 3: Regression between total length and the other morphometric measurements (cm) and the total length and the body weight (gm).

Parameters	Regression equation	R	Significance
SL vs TL	Y= 1.567 + 1.054X	0.927	$p< 0.001$
SL vs BD	Y= 0.308X - 0.003	0.817	$p< 0.001$
SL vs HL	Y= 0.214 + 0.226X	0.655	$p< 0.01$
SL vs HW	Y= 0.161X - 0.226	0.844	$p< 0.001$
SL vs SNL	Y= 0.133X - 0.511	0.806	$p< 0.001$
SL vs LJL	Y= 0.097X - 0.147	0.583	$p< 0.05$
SL vs UJL	Y= 9.803 – 0.997X	-0.919	$p< 0.001$
SL vs ED	Y= 0.792 - 0.599X	-0.515	$p< 0.05$
SL vs LD	Y= 0.226X - 0.730	0.512	$p< 0.05$
SL vs DFB	Y= 0.112X - 0.010	0.668	$p< 0.01$
SL vs AFB	Y= 0.209X - 0.569	0.810	$p< 0.001$
SL vs PAL	Y= 0.178 + 0.737X	0.854	$p< 0.001$
SL vs PL	Y= 0.177 + 0.170X	0.867	$p< 0.001$
SL vs PPL	Y= 0.9784 + 0.141X	0.590	$p< 0.05$
SL vs CPL	Y= 0.199X - 0.437	0.744	$p< 0.01$
SL vs CPD	Y= 0. 104 + 0.091X	0.746	$p< 0.01$

3.2. The gel electrophoresis showed bands of approximately 730 pb fragment resulting from the amplification (Fig. 1) that can be used for identifying the species. Results of agarose gel electrophoresis analysis showing PCR amplicons related to six distinct samples had chosen randomly. Lane 1: DNA marker, lane 2-5&7 COI barcode fragment.

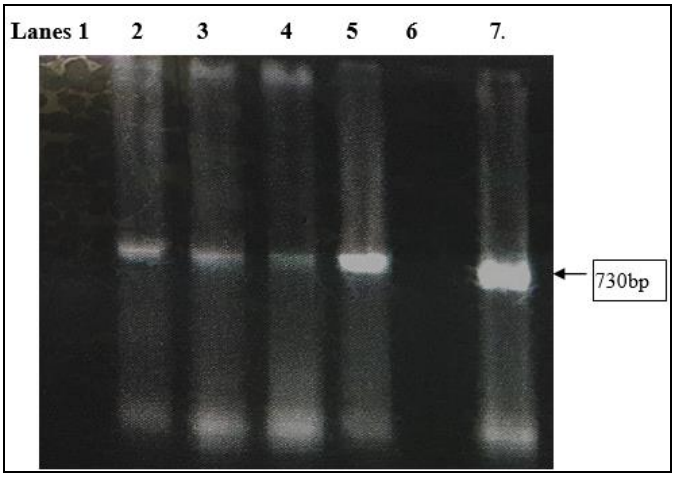

Fig 1: PCR amplicons showing five COI barcode fragment.

Discussion

Mode of dorsal fin rays can be used to discriminate between *B. nurse* from Sinnar (9) and the findings of Mohammed *et al*. [9] from Kerima in (11) and *B*rycinus *macrolepidotus* (10). The wide range of anal fin rays of 14-19 given by Abu Gideiri [2] was not assigned to a definite location rendering it unsuitable for comparison. Morphometric measurement in % of SL was higher in Sinnar samples when compared with the calculated ratios from Mohammed *et al*. [9] from Kerima samples except for PAL. In % of HL it was lower in HW and ED and slightly higher in SNL when compared with Mohammed *et al*. [9] findings. The study made by Awad [16] on seven morphometric characters of *Alestes* (=*Brycinus*) *nurse*, and his data was not comparable with the present findings. He used morphometric ratios between LD/BD, IOW/ED, PL/PD and neglected meristic counts. In the present study morphometric characters showed a proportional positive increase with increase in length of *B. nurse* and revealed an exponent b<3.0 which is indicative of negative allometric growth. *B*rycinus *longipinnis* in River Jamieson, Nigeria expressed allometric positive growth pattern (b>3) for males and isometric (b=3) growth for immature and females (Ikomi and Sikoki [11]). Abobi and Ekau [12] reported nearly isometric growth of 3.07 for *Brycinus nurse* in the lower reaches of White Volta River, Ghana. Adeosun *et al*. [13] studied *Brycinus macrolepidotus* from lower Ogun River and reported negative allometry for all sizes (b>3.0) and highly correlated, r (0.825-0.9963) length-weight relationship. They related the negative growth to diet. Mohammed *et al*. [9] found positive allometric growth in *B. nurse* and *B. macrolepidotus* from the Nile in Kerima.

Phylogenetic relationships of the African genera *Alestes* and *Brycinus* was documented by Murray and Stewart [3]. Later Zanata and Vari [17] performed a comprehensive morphological analysis including 19 Alestid genera and provided anatomical information and synapomorphies for intrafamilial relationships. Calcagnotto *et al*. [18] in a study of suprafamilial relationships within Characiformes, included 14 Alestid genera in a phylogenetic analysis using a molecular dataset with nuclear and mitochondrial genes.

Arroyave and Stiassny [19] presented a comprehensive phylogenetic study of the family including 19 genera and 53 taxa with a molecular dataset of two nuclear (SH3PX3 and myh6) and two mitochondrial (COI and cyt-b) genes. They found that Alestidae as non-monophyletic and discussed the possibility of this result as an artifact of missing data of the genus Lepidarchus. Although the family Alestidae has been considered monophyletic in most analysis (Arroyave and Stiassny, 2011). Arroyave *et al*. [20] employed DNA barcoding and morphometric to investigate the presence of cryptic diversity in *Bryconalestes longipinnis* and documenting for the first time genetic and body shape variation in the species.

Conclusion

The mode of dorsal fin rays can be used to discriminate

between *B. nurse* from Sinnar and Kerima Morphometric measurement in % of SL was higher in Sinnar samples when compared with Kerima samples. These need to be tested in populations from other tributaries of the Nile.

The primers used in this study resulted in a very clear DNA fragment around 730 pb which can be used as a tool of identification after sequencing and comparing in GenBanks

Further studies to probe the relations between *Brycinu* spp. and Alestidae are recommended.

Ethical Matters

Ethical issues pertaining to approval and consent to participate, human and animal rights, consent for publication, availability of data and materials are Not Applicable.

Competing interests

The authors declare no competing interests

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