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Morphometrical characterization of the Atlantic mudskipper species (*Periophthalmus barbarus*) (Linnaeus, 1766) (Perciformes; Gobiiae) from Abonema in Port Harcourt, Rivers State, Nigeria

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Abstract
Mudskippers are borderline organisms whose biology, systematics and evolution has attracted research attention for so long. There are controversies in their systematics especially because the morphological classifies are plastic and can be dependent on life stage, living conditions and the expertise of the scientist. This paper sort to characterize the Nigerian mudskippers using firstly morphometry, then DNA barcoding and the complete mitogenome sequences. Ten parameters were measured on One hundred specimens and the result was subjected to exploratory data analysis. The Nigerian mudskipper is in size and shape not different from other species found around the world. The standard deviation of all the measurements range from low (0.24-055) for six of the parameters, medium for two (0.56-0.86) and high for the remaining two (0.87-1.17). Low and medium standard deviation is an indication of very low variability in the parameters with the data clustering around the mean closely. Scatter plotting indicated five of the specimen that can be classified as outliers. Principal component analysis indicated two namely PC1 and PC2 as being responsible for 68% of the variance observed. Principal component plots 1 and 2 indicated head width, head depth and snout length loaded positively while only body depth loaded positively for the PC2. The Nigerian species were also amphidromous. The essence of this initial analysis was to see if body measurements will indicate any significant grouping as we plan to select samples for molecular analysis.

Keywords: Mudskippers, morphometry, amphidromy, principal component analysis, border line organism

Introduction
Mudskippers are distributed in the seven, disjunct mangrove divisions of the world, namely West Africa, East Africa, the Arabian Gulf, Indo-Malaya, Australia/New Guinea, the China sea and the Oceania (Murdy, 1989) [11]. Oxudercine gobies are a common sight in the several markets around the coastal areas of Nigeria. Even though their origin is established as being the Indo-West Pacific and the tropical western parts of Africa, they are naturally occurring in several muddy flood plains from Lagos to Port Harcourt in Rivers State, Nigeria. Mudskippers are often one of the dominant ecological components on tidal flats and they play an important role as carnivores and the preferred prey for avian predators (Clayton 1993) [1]. They are also major contributor to the maintenance of the integrity and health of mudflat ecosystems, which are usually vulnerable to the negative anthropocentric activities of man. They are frequently become part of the menu of riverine areas providing the needed animal protein. Their distribution is largely along that of modern mangrove habitats (Irvine, 1947; FAO, 1990) [7, 6]. Several revisions and classifications of the group based on updated distributional and taxonomic data has left out the Nigerian group.
One major controversy about the Nigerian species of Mudskippers is its systematics. The superficial resemblance of species has caused many cases of misidentification. Severally, they are generally called Atlantic, blue spotted and silver line mudskippers with the basis of these name being passed on across generations. In some other works that documents their morphometry and meristic characteristics, for instance Lawson and Aguda (2010) [9], he referred to them as *Periphthalmus papilio*. The basis of this nomenclature is not stated.

Most of the research works have focused aspects of Mudskipper's biology (Udo, 2002) [14], patterns of consumption in selected communities (Edun, *et al*., 2010) [3], associated parasite (Elele and Aziaka, 2019) [4] and the biology of *Periphthalmus barbarus* in Jaja Creek of the Nigerian Niger Delta (Nsikak, *et al*. 2021) [12]. Of the thirty-one species that the Food and Agricultural Organization of the United Nations (FAO) mentioned, only one has been principally featuring in researches in Nigeria.

Several other studies from different parts of the world and by researchers have reported mudskippers as being generally cryptic, exhibiting morphological plasticity and convergent evolution (Thacker 2003, Vanhove *et al*. 2012, Winterbottom *et al*. 2014) [13, 15, 16]. Cryptic species are usually so similar that they are difficult or impossible to be distinguished based on morphological characters alone. This suggests that clear identification within these group is poorly known. Therefore, the aim of this study is to take advantage of molecular genetic data using the cytochrome c oxidase subunit 1 (CO1) mitochondrial gene which is a DNA barcoding tool to identify the mudskippers collected at the central market in Port Harcourt, Nigeria due to the unclear nature of the basis of the species into which experts and non-experts classify then into and also see if there is any congruence in this classification.

Materials and Methods

Field sampling and samples preservation for molecular analysis

Sampling was carried out in conjunction with Dr Akinrotimi of the Institute of Oceanography and Marine Research, Port Harcourt outstation in Port Harcourt, Nigeria. These mudskippers were purchased at the open market and were identified at the species level as the Atlantic mudskipper, blue spotted and the silverline mudskippers. The market women noted that they also catch them at Buguma in the sub-urban areas of Port Harcourt. The Creek system consists of the main creek channel (Amayanabo Okolo) and associated interconnecting creeks such as Ido canal and Jordan Creek (local name) which interconnect and surround Buguma and Ido community (Dublin-Green and Ojanuga, 1988) [2]. The climate of the area is characterized by the dry season and rainy season. Red mangrove (*Rhizophora* sp.), White mangrove (*Laguncularia* sp.), black mangrove (*Avicennia* SP.) and Nypa palm (*Nypa fruticans*) dominates the vegetation. They noted that these were the names that these species have been called from time immemorial and that the morphological basis was unclear. Five specimen each group as specified by the expert and market women were purchased. They are the Atlantic (AM), the blue spotted (BM) and the silver line (SM) mudskippers and another five from a seller who got her from Abonema Waterside, also in Port Harcourt were simply labelled as GM. These specimens were quickly taken to the laboratory in ice where tissue samples were collected. 50 mg to 100mg of muscle tissue was dissected with a sterilized surgical scissors, placed into 1.5 ML screw top cryogenic vials and preserved in 95% Ethanol. Fish tissue samples were then shipped to the Institute of Marine Biotechnology, Universiti Malaysia Terengganu for laboratory processes.

Field sampling and morphometric analysis

A total of 100 samples of *P. barbarus* were collected from Abonnema, Nigeria (4.73075, 6.77565). From these samples, metric and meristic measurements were taken according to Lawson *et al.* (2010) [8] and subjected to exploratory statistical analysis to see if these measurements can be used as a statistical classifier for separating into distinct groupings. These morphometrics are total length (TL), standard length (SL), head length (HL), head width (HW), head depth (HD), snout length (SNL), predorsal length (PDL), eye diameter (ED), body depth (BD), first dorsal fin length (D1L), second dorsal fin length (D2L), pectoral fin length (PFL), a nal fin length AFL), caudal fin length (CFL), and caudal peduncle length (CFL).

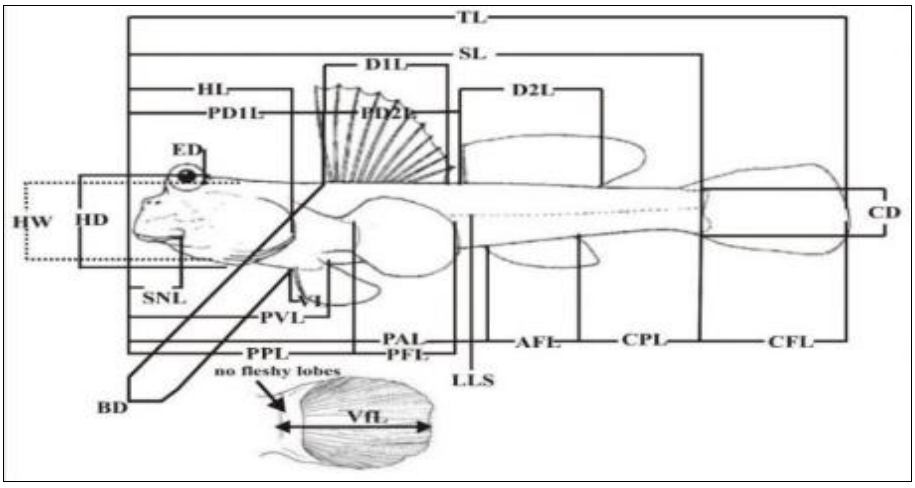

Fig 1: Morphometric and meristic measurements of Mudskipper and their abbreviations.

Data Analysis

The quantitative descriptive data analysis was carried out to describe the results of the morphometric and meristic characters of the mudskipper from Abonema waterside in River State, Nigeria. Further, evidences of the statistical classifiers being able to show any evidences of sub-speciation in the samples measured was looked out for as the local inhabitants identified our samples by different names based only on colour.

Results and Discussions

Morphometric and meristics of mudskipper

The results of the morphometric and meristic data is shown in Table 2. One major limitation is that the Mudskippers were identified as being of different species. But the result from molecular identification simply showed that they are of the

same species, Periopthalmus barbarus. As such comparison was limited. It is important to report that standard deviation for.

0.24 For eye diameter to 1.15 for total length. The morphometric measurements were classified into three with the ranges being 0.24-0.55 for head width (HW), head depth (HD), snout length (SNL), pre-dorsal length (PDL), eye diameter (ED), body depth (BD), caudal peduncle length (CPL), second dorsal fin length (D2L), first dorsal fin length ((DIL), anal fin length (AFL) and caudal fin length (CFL). The mid-ranged (0.56-0.86) have measurements of the pectoral fin length (PFL) and head length (HL). The third and widest range of standard deviation (0.87-1.17) had the total length (TL) and standard length (SL). Since Low standard deviation means data are clustered around the mean, and high standard deviation indicates data are more spread out, the observed results indicates that most measurements for the mudskipper are not very wide ranged. Tables 2 and 3 below shows a summary of these measurements.

Table 1: Range (cm), mean and standard deviation of morphometric characters of *Periophthalmus barbarus* (n=100).

Character	Minimum (cm)	Maximum (cm)	Mean (cm)	Standard deviation
TL	9.28	14.86	11.41	1.15
SL	3.9	12.19	9.48	1.11
HL	1.47	7.5	3.01	0.69
HW	0.39	2.29	1.43	0.31
HD	0.91	2.66	1.74	0.34
SNL	0.1	1.9	0.95	0.31
PDL	0.71	4.64	3.29	0.53
ED	0.03	1.42	0.71	0.24
BD	0.57	11.58	1.59	1.13
D1L	0.36	2.8	1.46	0.50
D2L	1.08	3.13	1.90	0.38
PFL	0.57	3.35	1.96	0.68
AFL	0.13	1.83	1.03	0.25
CFL	0.7	3.1	1.80	0.45
CPL	0.84	2.83	1.95	0.41

TL = Total Length, SL = Standard Length, HL = Head Length, HW = Head Width, HD = Head Depth, SNL = Snout Length, PDL = Predorsal Length, ED = Eye Diameter, BD = Body Depth, D1L = First Dorsal Fin Length, D2L = Second dorsal fin length, PFL = Pectoral Fin Length, AFL = Anal Fin Length, CFL - Caudal Fin Length, CPL = Caudal Peduncle Length.

Table 2: Range, mean and standard deviation of the ratios of each morphometric character to total length (TL) or head length (HL) of *Periophthalmus barbarus* (n=100).

Table 2: Minimum, maximum, means and standard deviation of Morphometrical measurements.

Character	Minimum	Maximum	Mean	Standard deviation
SL/TL	0.39	0.96	0.83	0.05
HL/TL	0.13	0.70	0.26	0.06
HW/HL	0.17	1.29	0.49	0.13
HD/HL	0.20	1.05	0.59	0.12
SNL/HL	0.03	0.65	0.32	0.10
ED/HL	0.01	0.50	0.24	0.07
PDL/TL	0.07	0.36	0.29	0.04
BD/TL	0.06	1.03	0.14	0.10
D1L/TL	0.03	0.19	0.13	0.04
D2L/TL	0.09	0.23	0.17	0.03
PFL/TL	0.06	0.27	0.17	0.05
AFL/TL	0.01	0.14	0.09	0.02
CFL/TL	0.07	0.24	0.16	0.03
CPL/TL	0.09	0.25	0.17	0.03

The scatter diagram also indicates only a few (five) outliers in terms of placement in this population. Majority clustered around the center. It is noteworthy that all the measurements were factored in the scatter plot.

Also the scree plot indicates that two factors are important for further consideration in the principal component analysis. The scree graph shows a sharp decline for only 2 factors after which the steepness drops. Hence the use of PC1 and PC 2 in further analysis. These two factors account for 69% of the variance found in the morphometric measurements.

Table 3: The linear measurements, Eigenvalue and the percentage variance.

PC	Eigenvalue	% variance
1	0.0402861	55.892
2	0.00907681	12.593
3	0.00525164	7.286
4	0.0044251	6.1393
5	0.00371241	5.1505
6	0.00292212	4.0541
7	0.00193359	2.6826
8	0.000979485	1.3589
9	0.000803069	1.1142
10	0.000724781	1.0055
11	0.000611192	0.84796
12	0.000581671	0.807
13	0.000536577	0.74444
14	0.000233605	0.3241

When the measurements were subjected analysis using the PC1 and PC 2, Head Width, Head Depth and Snout Length were the positive and most important morphological characters for PC1 while Head Length was negative. On the PC 2 plot, Body Depth was positive accounting for about 90% while Eye Diameter was negative.

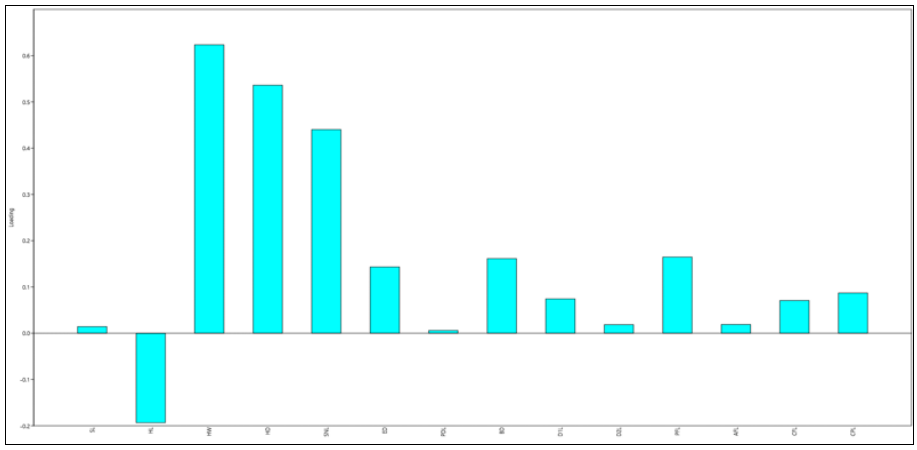

Fig 2: Principal component plot 1 of the measurements.

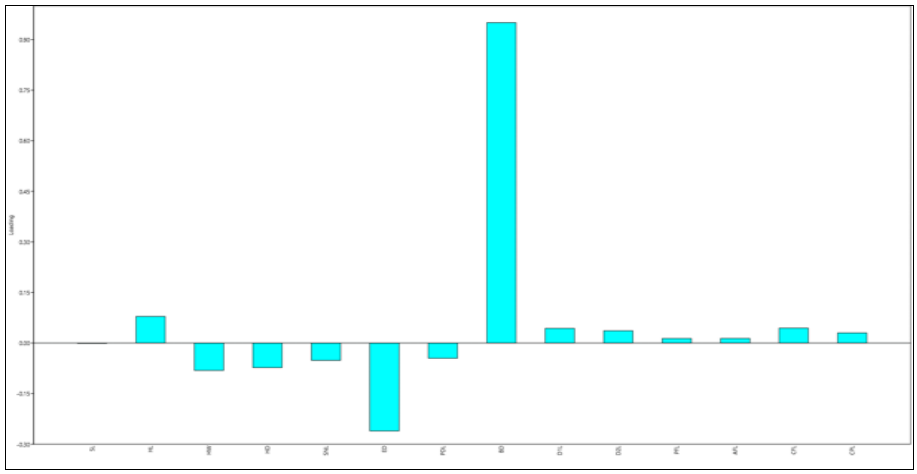

Fig 3: Principal component plot 1 of the measurements.

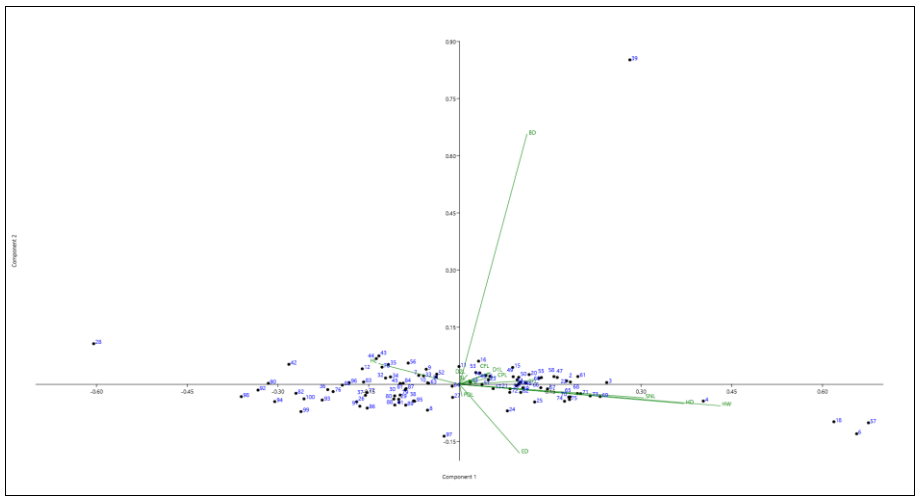

Fig 4: Scatter plot showing the positions of the measured specimens on a scatter plot. Note the few outliers in the plot.

Discussion

Species that are aquatic are usually more diverse than terrestrial ones. The main reason being the almost unlimited range that water and several other activities of man in water like shipping can move species to. In the tropics where there are megadiversity, the problem is further compounded by a lack of expertise and depth amongst experts in animal systematics. Severally, despite reports that around Africa, there is only one species of Mudskippers, various authors have called what are morphotypes different names because of the plasticity of the morphological classifiers and a general separation of same species by barriers leading to adaptions that are seen in morphology which are easily confused in nomenclature of species. Another major limitation is the dearth of research works on the species to affirm or debunk the age long conclusion by Irvine (1974) [7] and FAO (1990) [6] that only *Periophthalmus papilio* is the only indigenous species in brackish and estuarine waters of Africa. Systematic synonyms like Boleophthalmus boddarti, Periophthalmus chrysospilos, Periopthalmus grasilos and Periophthalmus novemoradiatus have been used in several other authors (Etim *et al*., (2002) [5], King and Udo (1996) [17], Etim *et al*, (2002) [5] without any strong scientific basis. The statistical classifiers didn't show any clear sub speciation and separation

of our samples into further groupings. Like the data from three substations as sampled by Lawson (2010) [9] did not show any sub-division, so does our data. As such for our further research using molecular methods, we took samples from our whole collections. Diversity within the population can therefore be said to be limited, hence our proceeding to further use CO1 barcoding to determine exactly what species we have and also its phylogenetic relationship with other species around the world.

Further it should be noted that Parenti *et al*. (2017) [10] had inferred from their worldwide survey of mudskippers that from the western African coast to Morocco and Angola, ust one species of mudskippers is found in this zone. This greater implication of this is that there should a review of the morphological basis of classification.

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