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Resolving the taxonomic confusion in the Nigeria Snakeheads (Teleostei, Channidae): DNA barcoding of the Lagos lagoon populations

Olusola Sokefun

Abstract
This current study assessed the population of *Parachanna obscura* from the Lagos lagoon. It is the first study that is examining the usefulness of DNA barcoding in identifying the Lagos Lagoon in Nigeria populations and also shedding more light on their taxonomic status. The CO1 gene yielded consistently 654 base pairs after alignment. Intraspecific divergence is low ranging from 0.00 to 0.05, while interspecific divergence with other available gene sequences is between 0.00 to 0.2. Average nucleotide composition were in the order T > C > A > G (T: 29.1, C: 28.7, A: 22.1 and G: 20.0). There were 648 conserved sites and 4 variable sites. One hundred and five (105) of the sites were polymorphic, with One Hundred and twenty One (121) mutations and a nucleotide diversity of 0.0937, Tajima's diversity of 2.52 at a statistical significance of $p<0.05$ was also observed. There were fourteen (14) unique haplotypes. The Lagos lagoon population all mapped together with a bootstrap value of 99% indicating some level of uniqueness, even though their genetic diversity is very low. Further research will be required to add more sampling sites around the Lagos lagoon and also more genetic markers with deeper analysis to see if there are population cryptics.

Keywords: Taxonomic confusion, Snakeheads, DNA barcoding, Lagos lagoon populations

Introduction
The family Channidae are a group of teleosts that are found through-out Asia and Africa. The freshwater dwelling group of predatory fishes is represented by two major species, the *Parachanna obscura* and Parachanna africana (Serrao 2014) [1]. Though there are a few reports of Parachanna insignis (Nwani, 2011) [11]. There are indications that this may be a case of misidentification. Several authors have described them as being of elongated body, with fusiform or sub-cylindrical body usually covered with cycloid scales of medium sizes. In Nigeria, they are known to occur in varying numbers in the rivers Anambra, Imo, Ibbi, Kaduna, Katsina-Ala, Hadejia, Ogun, Sokoto, the great Kwa, Niger, Ovia, Lake Chad and their tributaries. Essentially, they are almost ubiquitous in the Nigerian river drainage system. There are however undocumented evidences of a decline in numbers amongst artisanal fishermen. The International Union for Conservation of Nature (2017) classifies them as being endangered. The culturing of the species is not a known yet, although they are being considered as an emerging aquaculture candidate in Nigeria. The species is very palatable and has a good fillet quality. Several aspects of the biology of the species have been documented in Nigeria. Ola-Oladimei *et al*. 2020) [3] its cytogenetics, Ama-Abasi and Anthony (2013) [2] proximate analysis, helminth infections, length and weight relationship and their food (Olasunkanmi & Ipinmoroti, 2014) [5], in literature there is a very sparse information and details about aspects of their morphological and molecular systematics, population structure, DNA barcoding and a lot of other aspects. The proper desalination of species may must be the most important step in our quest to truly develop the species into the next candidate for aquaculture. This study seeks to document aspects of their genetic diversity and barcode them, especially in the very large water body that has never been any report about, the Epe lagoon which an important part of the fishing system of the south western part of Nigeria using the cytochrome oxidase gene, the gene of choice for fauna barcoding.

Materials and Methods
Study Site and Experimental layout
The Lagos lagoon is a large expanse of water bordering the fringes of Lagos, Ikorodu, Epe,

Victoria Island, Ikoyi and extending further to Ogun and Ondo states in the south western part of Nigeria. For decades it has been an established fishing grounds with large numbers of artisanal fishermen using boats of various sizes to fish. The Ebute Chief in Epe where the fishes used for this research was purchased, is an established fish landing jetty where wholesalers and retailers come to purchase many species of fish and other aquatic animals. Figure 1 below shows the map of Nigeria and the collection point.

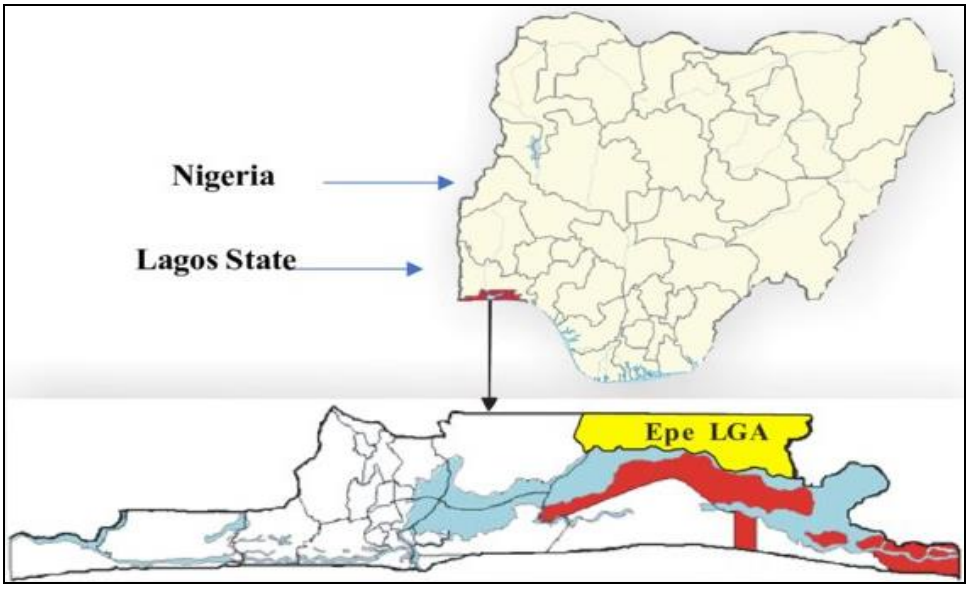

Fig 1: Map of Nigeria showing Lagos State and the location of Epe LGA Source: Adapted from Ojuri and Bankole (2013)

Sample collection

Live fishes identified as *Parachanna obscura* according to Bolaji *et al*. (2011) [14], were purchased from artisanal fish farmers at the Ebute Chief in Epe local government area (6.57480, 3.96272). These specimens were immediately frozen and brought to the laboratory of the organismal genetics group of the Department of Zoology and Environmental Biology, Lagos State University, Ojo in Lagos, Nigeria. Fin clippings were cut following standard laboratory procedure and stored in cryotubes with 98% Ethanol from where they were shipped to the Paul Hebert Centre for DNA Barcoding & Biodiversity Studies, BAMU in Aurangabad, India where further processes were done.

Morphometrical analysis

Morphometrical studies was also done taking measurements of metric and meristic traits of various body parts according to Osho *et al*. 2022 and Talwar 1991 [7, 8]. The details of this is reported in another research paper.

DNA extraction and amplification

DNA was extracted using the Qiagen DNA extraction kit according to manufacturer's instructions. The CO1 gene was successfully amplified using the universal fish primer named FishF1 and FishR1. The sequences of the primers are: FishF1 (5′TCAACCAACCACAAAGACATTGG CAC3′) and FishR1 (5′TAGACTTCTGGGTGGCCAAAGAA TCA3′). Each master mix contained 2µl extracted DNA template, 12.5µl of Taq, 1µl of forward primers ad 1µl of reverse primer, 8.5µl of nuclease free water. The PCR thermal cycling conditions involved an initial denaturation of 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30s, annealing temperatures of 54 °C for 40seconds and extension at 72 °C for 1 minute, with a final extension for 72 °C for 7 minutes.

Sequencing, phylogenetic and statistical analysis

The purified PCR products were sequenced in both directions and the chromatograms were curated and converted into FASTA format. A blast of the CO1 sequence was done on the National Centre for Biotechnology Information (NCBI) database using sequences generated from this experiment. Evolutionary analyses of the aligned sequences followed in the program MEGA 7.0 with the drawing of the phylogenetic tree rooted using *Parachanna insignis* as the out group. A total of seventy seven (77) sequences of the genus Channa were included in the study a reference. The evolutionary history was inferred using all the three common methods (maximum likelihood ML, minimum evolution and neighbour joining. Nei and Kumar 2000 [9]) to guarantee robustness of the phylogenetic relationship obtained. Pairwise genetic distance, intraspecific and interspecific distances and codon usage were calculated using the Kimura 2-parameter distance model (Kimura 1980) [10].

Results

The sequence yielded consistently 654 base pairs for all the Nigerian species of Parachanna. They were all checked for stop codon and none had. Average nucleotide composition were in the order T > C > A > G (T: 29.1, C: 28.7, A: 22.1 and G: 20.0). Similar compositions have been reported by previous authors. There were 648 conserved sites and 4 variable sites. Intraspecific pairwise genetic distance ranged from 0.00 to 0.05 for the Nigerian *Parachanna obscura* species indicating a very low genetic diversity in our species. There are a few instances when a 0.00 intraspecific distance was observed between our samples from the Epe lagoon and others from river systems that are not contiguous and very far away. Our first sample (*Parachanna obscura* 1 {PO1}-ascension number pending) was a hundred percent similar with *P. obscura* with ascension numbers K937346.1, HM882955.1, HM882956.1, HM882957.1, HM882958.1 and

HM882959.1 all documented by Nwani *et al*., 2010 [15]. The rivers sampled were Ebonyi, Anambra and Afikpo, all in the south eastern part of Nigeria. There are also case of an intraspecific distance of 0.00 between PO1 and Parachanna insignis (MG824633.1 and MG824634.1) from the rivers Oyun, Asa, Awon, ebba and Niger, all in the north central part of Nigeria. Further, blast analysis was done and thirty- seven other highly similar sequences were obtained. A total of seventy-seven sequences were analysed using DAMBE. Of these nine (9) were found to have stop codons, hence unsuitable for any further analysis. Of the fourty sequences for the Nigerian Parachanna species, the software found redundancies and collapsed all in eight (8) unique sequences. In all, twenty-eight (28) sequences were then subjected to further analysis to determine the relative phylogenetic position of the Nigerian species. Intra and interspecific pairwise distances ranged from 0.00 to 0.2 indicating the discriminatory power of DNA barcodes for the species. 105 of the sites were polymorphic, 121 mutations with a nucleotide diversity of 0.0937 and Taima's diversity of 2.52 at a statistical significance of $p<0.05$ was observed using DNAsp (Rozas 2017) [13]. 14 haplotypes were also found in the sequences. A high haplotype diversity is an indication that the population as not experienced any genetic bottleneck in recent times.

Figure 2: Intra and inter specific pairwise distances between the species assessed in this research and others.

The aligned sequences were then used to generate K2P-distances and infer the phylogenetic relationship with bootstrap values of 500 replications to be sure that the inferred phylogenetic relationships are not due to chance. Further a plot of the number of transitions and transversions versus the Kimura's two-parameter distance was done. Figure 1below is a graphical representation of this. Phylogenetic analysis clustered the Nigerian *Parachanna obscura* into one clade with a 100% bootstrap value. *Parachanna obscura* with ascension numbers HM882955.1, HM882956.1, HM 882957.1 and P. insignis MG824633.1 were clustered as sister clades with a 68% bootstrap support. These three (HM882955.1, HM882956.1, HM 882957.1) are all *Parachanna obscura* from the rivers Afikpo, Anambra and Ebonyi reported by Nwani *et al*. 2011 [11]. The clustering of *P. insignis* MG824633.1 submitted by Iyiola (2018) [16] in the sister clade as our P. obscura cast some doubts on the proper identification of that specimen. A review of its systematics is herein being suggested. The reason for this being a clustering of other P. insignis into another clade with a 100% bootstrap support. The last of the three major clades is an admixture of *P obscura* and *P. africana*. As an indication of the very complex nature of the systematics of the group, the authors of the submission with ascension KT193349.1 noted the taxonomic challenges in freshwater fishes, especially the mismatch between morphology and DNA barcoding in fish of the north-eastern part of the Congo basin. As such taking the reported taxonomy with caution may not be unideal. All of the other submission in that clade were by Serrao *et al*. (2014) [1] who basically harvested sequences from the Genbank for her analysis. That the Parachanna species are a poorly inventoried fauna is further established by the fact apart from this research work, only one other author has attempted its taxonomic analysis. To check the robustness of our results, we had used three major phylogenetic inference methods (maximum like hood, neighbor joining and minimum evolution). In all of these, the classification of *Parachanna obscura* (K937346.1) and the P. insignis with the Nigerian species have been consistent. The separation of the P. insignis into a different clade with a 100% bootstrap support is also consistent.

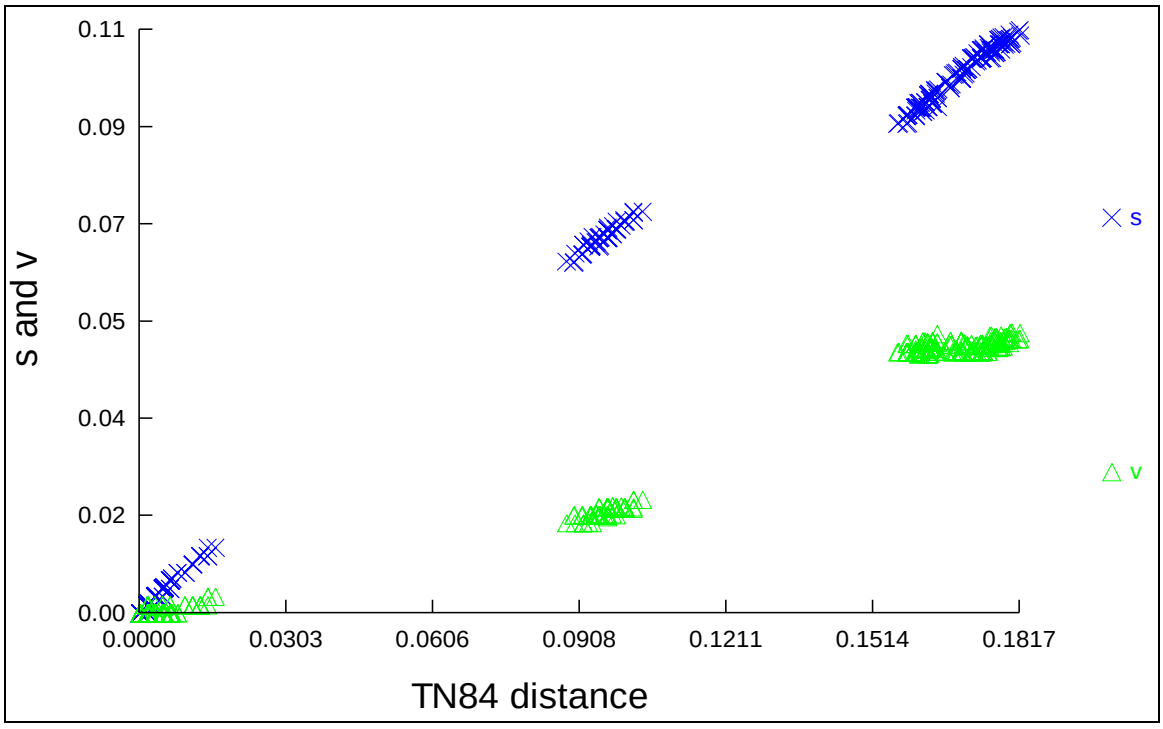

Fig 1: Plot of transition/transversion ratio using the Kimura's two parameter distance

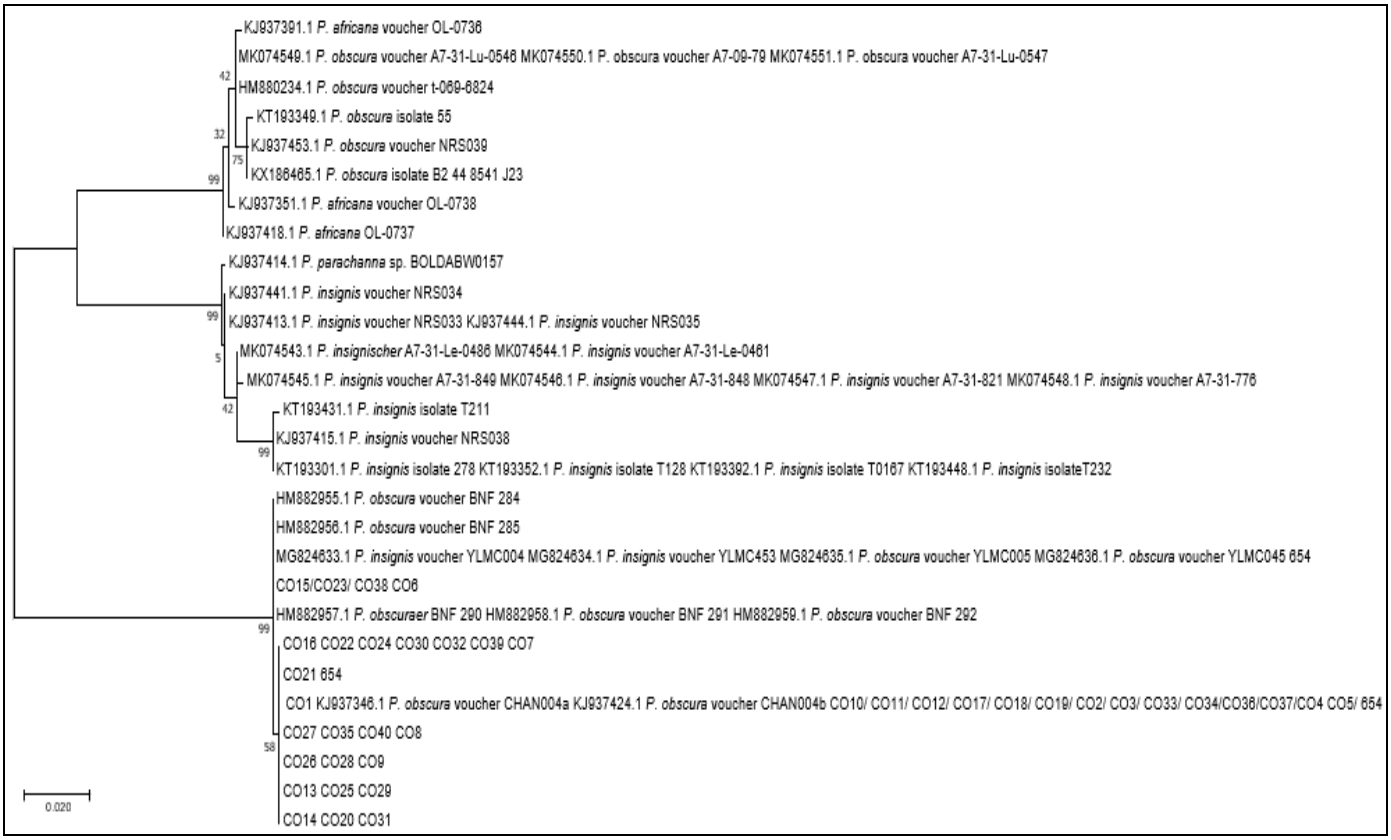

Fig 2: Phylogenetic relationship of the Parachanna species. Note that CO 1 to CO 37 are *Parachanna obscura* from this research

Table 1: Estimates of evolutionary intraspecific distances between our sampled *Parachanna obscura*

	CO1	CO10	CO11	CO12	CO15	CO17	CO18	CO19	CO2	CO21	CO23	CO27	CO3	CO33	CO34	CO35	CO36	CO37	CO38	CO4	CO40	CO5	CO6	CO8	CO16	CO22	CO24	CO30	CO32	CO39	CO7	CO26	CO28	CO9	CO13	CO25	CO29	CO14	CO20	CO31
CO1																																								
CO10	0.000																																							
CO11	0.000	0.000																																						
CO12	0.000	0.000	0.000																																					
CO15	0.003	0.003	0.003	0.003																																				
CO17	0.000	0.000	0.000	0.000	0.003																																			
CO18	0.000	0.000	0.000	0.000	0.003	0.000																																		
CO19	0.000	0.000	0.000	0.000	0.003	0.000	0.000																																	
CO2	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000																																
CO21	0.002	0.002	0.002	0.002	0.005	0.002	0.002	0.002	0.002																															
CO23	0.003	0.003	0.003	0.003	0.000	0.003	0.003	0.003	0.003	0.005																														
CO27	0.002	0.002	0.002	0.002	0.005	0.002	0.002	0.002	0.002	0.003	0.005																													
CO3	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.002																												
CO33	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.002	0.000																											
CO34	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.002	0.000	0.000																										
CO35	0.002	0.002	0.002	0.002	0.005	0.002	0.002	0.002	0.002	0.003	0.005	0.000	0.002	0.002	0.002																									
CO36	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.002	0.000	0.000	0.000	0.002																								
CO37	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.002	0.000	0.000	0.000	0.002	0.000																							
CO38	0.003	0.003	0.003	0.003	0.000	0.003	0.003	0.003	0.003	0.005	0.000	0.005	0.003	0.003	0.003	0.005	0.003	0.003																						
CO4	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.003																					
CO40	0.002	0.002	0.002	0.002	0.005	0.002	0.002	0.002	0.002	0.003	0.005	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.005	0.002																				
CO5	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.003	0.000	0.002																			
CO6	0.003	0.003	0.003	0.003	0.000	0.003	0.003	0.003	0.003	0.005	0.000	0.005	0.003	0.003	0.003	0.005	0.003	0.003	0.000	0.003	0.005	0.003																		
CO8	0.002	0.002	0.002	0.002	0.005	0.002	0.002	0.002	0.002	0.003	0.005	0.000	0.002	0.002	0.002	0.000	0.002	0.002	0.005	0.002	0.000	0.002	0.005																	
CO16	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000																
CO22	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000															
CO24	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000														
CO30	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000													
CO32	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000												
CO39	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000											
CO7	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000										
CO26	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
CO28	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000								
CO9	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000							
CO13	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
CO25	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
CO29	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
CO14	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
CO20	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
CO31	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Table 2: Estimates of Evolutionary distances between our experimental species and other gene sequences harvested in the GenBank

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
[1]																											
[2]	0.01																										
[3]	0.10	0.10																									
[4]	0.11	0.10	0.01																								
[5]	0.00	0.01	0.09	0.10																							
[6]	0.10	0.10	0.00	0.01	0.09																						
[7]	0.10	0.10	0.00	0.01	0.09	0.00																					
[8]	0.01	0.01	0.10	0.11	0.01	0.10	0.10																				
[9]	0.10	0.10	0.01	0.01	0.10	0.00	0.00	0.10																			
[10]	0.10	0.10	0.01	0.01	0.10	0.01	0.01	0.10	0.00																		
[11]	0.00	0.00	0.10	0.11	0.00	0.10	0.10	0.00	0.10	0.10																	
[12]	0.22	0.22	0.21	0.23	0.21	0.21	0.21	0 22	0.22	0.22	0.22																
[13]	0.22	0.22	0.21	0.23	0.21	0.21	0.21	0 22	0.22	0.22	0.22	0.00															
[14]	0.22	0.22	0.21	0.23	0.21	0.21	0.21	0 22	0.22	0.22	0.22	0.00	0.00														
[15]	0.01	0.01	0.10	0.11	0.01	0.10	0.10	0.00	0.10	0.11	0.01	0.21	0.21	0.21													
[16]	0.11	0.11	0.01	0.00	0.11	0.02	0.02	0.11	0.01	0.01	0.11	0.23	0.23	0.23	0.11												
[17]	0.11	0.10	0.01	0.00	0.10	0.01	0.01	0.11	0.01	0.01	0.11	0.23	0.23	0.23	0.11	0.00											
[18]	0.00	0.00	0.10	0.11	0.00	0.10	0.10	0.00	0.10	0.10	0.00	0.22	0.22	0.22	0.01	0.11	0.11										
[19]	0.01	0.01	0.10	0.11	0.01	0.10	0.10	0.00	0.10	0.10	0.00	0.22	0.22	0.22	0.00	0.11	0.11	0.00									
[20]	0.22	0.22	0.21	0.23	0.21	0.21	0.21	022	0.22	0.22	0.22	0.00	0.00	0.00	0.21	0.23	0.23	0.22	0.22								
[21]	0.22	0.22	0.22	0.22	0.21	0.22	0.22	022	0.22	0.21	0.22	0.00	0.00	0.00	0.21	0.23	0.22	0.22	0.22	0.00							
[22]	0.22	0.22	0.22	0.22	0.21	0.22	0.22	022	0.22	0.21	0.22	0.00	0.00	0.00	0.21	0.23	0.22	0.22	0.22	0.00	0.00						
[23]	0.22	0.22	0.21	0.23	0.21	0.21	0.21	022	0.22	0.22	0.22	0.00	0.00	0.00	0.21	0.23	0.23	0.22	0.22	0.00	0.00	0.00					
[24]	0.22	0.22	0.22	0.22	0.21	0.22	0.22	022	0.22	0.21	0.22	0.00	0.00	0.00	0.21	0.23	0.22	0.22	0.22	0.00	0.00	0.00	0.00				
[25]	0.22	0.22	0.22	0.22	0.21	0.22	0.22	022	0.22	0.21	0.22	0.00	0.00	0.00	0.21	0.23	0.22	0.22	0.22	0.00	0.00	0.00	0.00	0.00			
[26]	0.22	0.22	0.22	0.22	0.21	0.22	0.22	022	0.22	0.21	0.22	0.00	0.00	0.00	0.21	0.23	0.22	0.22	0.22	0.00	0.00	0.00	0.00	0.00	0.00		
[27]	0.22	0.22	0.22	0.22	0.21	0.22	0.22	022	0.22	0.21	0.22	0.00	0.00	0.00	0.21	0.23	0.22	0.22	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
[28]	0.22	0.22	0.22	0.22	0.21	0.22	022	022	0.22	0.21	0.22	0.00	0.00	0.00	0.21	0.23	0.22	0.22	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00

Discussion

With advancing science, it has become fairly easy to distinguish members of the same genius but different species that are morphologically similar. Meristics, morphometry and the more advanced geometric morphometry have remained the principal methodologies before now for inferring species relationships and understanding the role(s) that speciation across time can do. However several fish species have shown varying levels of difficulties in their systematics. The Parachanna species are one of such. The efficacy of DNA barcoding in resolving the ambiguities is herein established as the bootstrap support are quiet high and evidences of misclassification are minimal. Apart from adding more information to the genetic database on the Parachanna species in Nigeria, we have also established for the first time that the Parachanna species of the Lagos lagoon are largely P. obscura. Our submitted sequences are also useful for further studies on Parachanna species of Nigeria and a comparative study with those found in other parts of the world. The genetic diversity is very low thereby supporting the assertion by the IUCN that the species are endangered. The use of genetic information in species management of wild species is an addition output of our data, as it puts in public space some reference data that is useful for planning the conservation of the species. Educating the artisanal fishermen on the status of the species is also essential as they have also reported a reduced abundance of the species in their catches. In the only other specific publication on the species P. obscura, the sampling was done in the rivers Anambra, Imo, lower Niger, Upper Nigeria, Benin-Owena, Chad, Cross, Hadeia-ama' are, lower Benue, Niger Delta, Ogun-Osun, Upper Benue and Sokoto-Rima. The Lagos lagoon which is an important part of the fisheries sector in Nigeria is not included. The addition of further information from this important part is necessary and cannot be overemphasized.

Conclusion

The Parachanna species have surmounted the several ecological barriers to colonize almost all water bodies in Nigeria. There occurrence in the Lake Chad which is the northern extremity of Nigeria is an important and noteworthy for scientists interested in species distribution and diversity. However, despite their being widely distributed, the minimal number of species is a reason to be worried about. Speciation and increased diversity should normally come with the conquering of niches. A need to investigate this is needed. The mapping of Parachanna insignis in the same clade and P. obscura is also worrisome. If P. insignis is a species in all senses of what a species is, especially because in my analysis using the principal phylogenetic analysis methods, it occupies a separate clade, then a review of single species that maps in the same clade as our P. obscura is needed. A case of misidentification is suspected. The genetic diversity in the species is very low, with intraspecific diversity being between 0.00 to 0.05 for species analysed in this research and between 0.00 and 0.2 between species in this research work and others that have been reported earlier despite the locations being hundreds of kilometers apart. The noted decrease in catch by artisanal fishermen during our interaction with them is another worrisome finding. It corroborates the observation by the IUCN (2017) [4] that the species in endangered. The breeding behaviour of the group should be keenly looked into. I am hoping that having submitted the sequences generated from this research, would be approved and hoisted in good time that so deeper analysis will be conducted with these sequences combined. Finally, further sampling in term of spread and number of samples cum deeper and more refined species partitioning methods may be required to check for cryptics.

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