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Molecular resolution of some West African Birds using DNA Barcoding

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Abstract

DNA barcoding is widely used for specie cataloging for unambiguous identification and conservation. In the present study, baseline information on the DNA barcoding of mitochondrial CO1 gene of some randomly caught West African birds were provided with five newly reported sequences for *Tringa* sp, *Streptopelia decipens, Francolinus bicalcaratus, Tyto alba* and *Francolinus squamatus.* Species identifications were made by comparing unknown CO1sequences against the DNA barcodes of known species through distance-based tree construction and alignment searching. The newly generated sequences are deposited in GenBank under accession numbers JX160000 - JX160017 and JX220905. Current study may contribute towards the development of a reference DNA barcoding database of West African birds.

Keywords: Conservation, COI sequence, DNA reference library, mtDNA, West African birds

Introduction

Although, certain birds are abundant in certain continent (Keith et. al., 1993); within countries and localities certain species are much more abundant or occur than others. Therefore, a total of nineteen birds that are ubiquitous in Eastern Nigeria, West Africa were used to attempt the possibility of identifying West African birds' species using DNA Barcoding. Africa has rich biodiversity with many endemics and forms one of the richest tropical fauna and flora of the world. Ergo, the biology of many species have not been known, they are conventionally regarded as data deficient species and are not catalogued yet (Echi and Ezenwaji, 2010). The aim of the present study was to determine whether DNA barcoding can be used as an effective tool to perform unambiguous identification of some West African bird species with a view towards establishing a DNA barcode reference library for biodiversity assessment in future. We performed DNA barcoding of nineteen avian species from West Africa using CO1 gene and compared with the Barcode of Life Data Systems (BOLD; www.barcodinglife.org) and GenBank/National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov) databases. A phylogenetic relationship with some closely related species was constructed using the DNA barcode sequence.

In recent times, DNA barcoding has risen as a speedy and consistent molecular tool for species identification. It consists of using a small portion of mitochondrial DNA from a standard agreed-upon position in the genome that can be searched and compared with sequences deposited in databases such as NCBI GenBank and BOLD. Approximately 655-bp-long fragment from the 5′ region of mitochondrial cytochrome c oxidase subunit I (COI) gene was proposed as the standard region for DNA barcoding of animal species (Hebert *et. al.,* 2003a,b; Hajibabaei *et. al.*, 2007). One of the most important features that DNA barcoding offers is the use of same primer pairs for species from a wide taxonomic range (Roe and Sperling, 2007). A good barcode is expected to exhibit a greater interspecific distance than intraspecific variation (Hebert *et. al*., 2003a, b; Hajibabaei *et. al*., 2007) and a pattern generally known as 'barcode gap' (Meyer and Paulay, 2005). The concept of a universally recoverable segment of DNA that can be applied as an identification marker across species has been most successfully applied to animal groups (Hebert *et al* 2004). It consists of a standardized short

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sequence of DNA (CO1 for animals) that can easily be generated and characterized for all species on earth (Savolainen *et. al.,* 2005; Dawnay *et.al.,* 2007).

Materials and Methods

A total of nineteen bird species (Table 1) were collected by chance of traps from various parts of West Africa and were selected for the study after confirming their taxonomy following Serle *et al* (1977). DNA was extracted from alcohol preserved muscle tissue (~25 mg) by using Qiagen DNeasy Blood and Tissue kit. Universal (VF1d & VR1d) primers were used in the present study for amplifying CO1 gene (Ivanova *et. al*., 2007). PCRs were performed in 25 µl reactions consisting of 2.5 µl each of 10x PCR buffer, $MgCl_2$ (25 mM) and 0.5 µl dNTPs (2 mM), 0.25 µl of each primer (10 µM), 1 unit of AmpliTaq Gold DNA polymerase, 14 µl of dH_2O and 4 µl of template DNA (10-20 ng) in a Thermocycler (ABI 9700). The following thermo cycling conditions were used for amplifications: 95^0C 5 minutes, 95^{0C} 30 seconds, 54^{0C} 45 seconds, 72^{0C} 45 seconds for 40 cycles, 72^{0C} 7 minutes, 4^0C. The PCR products were visualized on 1% agarose gels and the most intense products were purified using Exo Sap IT (USB). Bidirectional sequencing was performed using the PCR primers and products were labelled with BigDye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems, Inc.) and sequenced in an ABI 3730 capillary sequencer following manufacturer's instructions.

West African Birds Species Studied	Collection Locality	GenBank Accession No. for CO1
Phasianid ae ***Meleagris gallopavo*** *Numididae* *Numida meleagris*	 Eastern Nigeria Eastern Nigeria	 JX160013 JX160000
Scolopacidae *Tringa sp*	 Eastern Nigeria	 JX160001
Accipitridae *Milvus migrans*	 Eastern Nigeria	 JX160002
Cuculidae *Centropus senegalensis*	 Eastern Nigeria	 JX160003
Pynconotidae *Pynconotus barbatus*	 Eastern Nigeria	 JX160004
Apodidae *Apus afinis*	 Eastern Nigeria	 JX160005
Ardeidae *Bubulcus ibis*	 Eastern Nigeria	 JX160006
Phasianidae *Gallus gallus* *(Iyaya) Gallus* *gallus* *Gallus gallus (Auke)* *Francolinus bicalcaratus*	 Eastern Nigeria Eastern Nigeria Eastern Nigeria Eastern Nigeria Eastern Nigeria	 JX160008 JX160009 JX160014 JX160015 JX220905
Columbidae *Columba livia* *Streptopelia decipens*	 Eastern Nigeria Eastern Nigeria	 JX160011 JX160007
Nectariniidae *Anthreptes collaris*	 Eastern Nigeria	 JX160012
Tytonidae *Tyto alba*	 Eastern Nigeria	 JX160016
Turdidae *Turdus pelios*	 Eastern Nigeria	 JX160017
Anatidae *Cairina moschata*	 Eastern Nigeria	 JX160010

Table 1. List of Bird species used in the present study

The sequences were aligned using ClustalW and potentially misaligned sequences were excluded. The extent of sequence differences between species was calculated averaging pairwise comparisons of sequence differences across all individuals. Pairwise evolutionary distance was determined by the Kimura-2-parameter method using the software programme Mega 5 (Tamura *et al* 2011). The number of polymorphic sites and nucleotide diversity (pi), nucleotide composition and number of transition and transversion between species were determined. Gaps were considered as missing data on the phylogenetic reconstructions. Neighbour Joining (NJ) tree was constructed. The new sequences are deposited in GenBank under accession numbers JX160000 - JX160017 and JX220905.

Results and Discussion

All 19 bird species had different COI sequences (Hye *et. al.*, 2006). 6 had highest frequency of capture and belong to a common family Phasianidae, *Gallus gallus, Gallus gallus (Avuke), Gallus gallus (Iyaya), Meleagris gallopavo, Francolinus bicalcaratus and Francolinus squamatus*. This is partly due to their relatively high consumption rate of domestic Phasianids and pathological consumption habits of wild Phasianids as bush-meat in West African due its very tasty meat (Onyenekwe, 1988). The COI sequence differences between closely related species for instance in the family Phasianidae in most cases the neighbor-joining (NJ) tree showed shallow intraspecific and deep interspecific divergence (Hye *et. al.*, 2006). Example, genetic distance between members of this family is insignificant 0.00. Similarly, in the family Columbidae with 2 members *Columbia livia* and *Streptopelia_decipens* the genetic distance is also insignificant 0.00. However, the genetic distances of species in different families are wide. For instance, Tyto alba (Tytonidae) and *Francolinus bicalcaratus* (Phasianidae) is 0.209 whereas Carina moschasta (Anatidae) and Turdus pelios (Turdidae) 0.187 (Table 1).All samples were successfully sequenced for CO1 using the forward and reverse primers to obtain robust forward and reverse sequences approximately of 618 bp. No insertions, deletions or stop codons were observed in any sequence of CO1. After alignment there were 380 common sites in the partial sequence of CO1 genes including inserted gaps used for this analysis. No areas of uncertain alignment were identified. After filtering there were 238 (38.5 %) variable sites and 207 (33.5%) of them were phylogenetically informative, the nucleotide composition was extremely guanine poor T = 24,064 (24.5%), A= 24,986 (25.5%), C = 32,381 (33.0%) and G = 16,658 (17.0%). The Guanine–Cytosine composition is GC: 49,039 (50.0%). The overall mean divergence (d) was 0.1915% (Table 2). The sequence comparison with CO1 sequence data of West African birds revealed that five among the nineteen species included in the present study are not represented earlier in public data bases (BOLD and GenBank). They are *Tringa* sp, *Streptopelia decipens, Francolinus bicalcaratus, Tyto alba* and *Francolinus squamatus* (see below) Neighbor joining (NJ) tree based on CO1 sequence generated in the present study as well as the sequences of all the West African birds available with the GenBank are represented in Fig 1.

Mt COI gene sequences of domestic fowls with ruffled feathers; *Gallus_gallus*_(Iyaya)-BD11 without tail and *Gallus_gallus*_(Avuke)-BD17 with tail were the same, ergo, they differed from the domestic fowls with unruffled feather *Gallus_gallus*-BD12 (Table 2). *Gallus gallus* (*Avuke*) and *Gallus gallus* (*Iyaya*) are clustered together since they are subspecies of *Gallus gallus* that interbreed in nature and cannot be resolved by DNA barcoding. *Tringa* sp Linnaeus, (1758) is a genus of waders, containing the shanks and tattlers. They are mainly freshwater birds, often with brightly coloured legs. However no data is yet available for this specie; this specie is considered data deficient. Mourning Collared Dove *Streptopelia decipens* Hartlaub and Finsch, 1870 is a widespread resident breeding bird in Africa south of the Sahara. Unlike several other species in this genus, they are quite gregarious and often feed in groups. It builds a stick nest in trees, often mangroves (IUCN, 2012). Like most francolins, *Francolinus bicalratus* Linnaeus, 1766 and *Francolinus squamatus* Cassin, 1857 are restricted to Africa. They are resident breeder in tropical West Africa, but there is a small and declining isolated population in Morocco. They are found in open habitats with trees. In the tropics, very little study has been made of birds of prey of

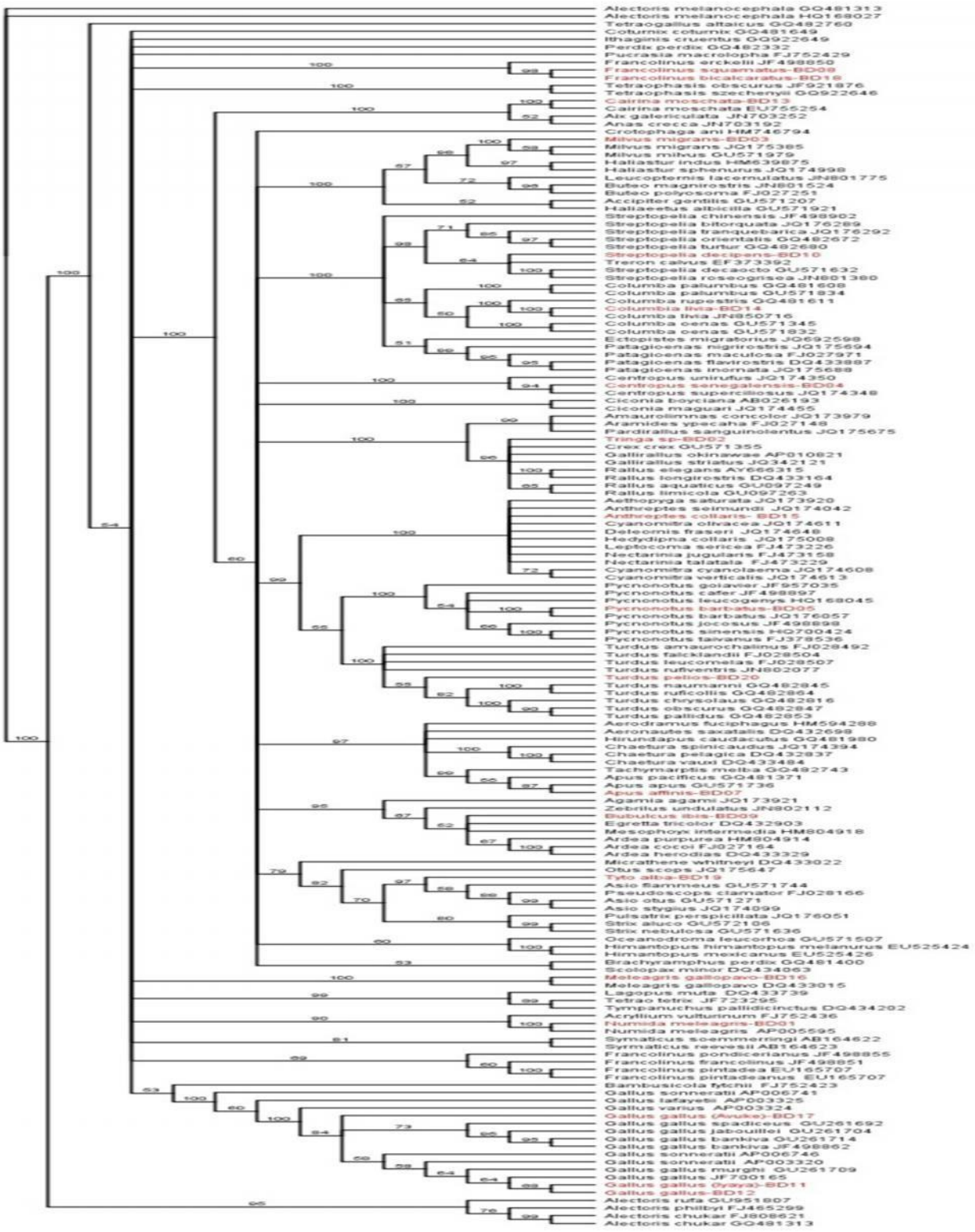

Fig. 1: Neighbour Joining Tree based on CO1 Gene (500 bootstrap replicates)

Table 2. Estimates of Evolutionary Divergence between Sequences. The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Kimura 2-parameter model [Kimura, 1980]. The analysis involved 19 nucleotide sequences. All positions with less than 100% site coverage were eliminated. That is, less than 0% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 516 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [Tamura *et. al*., 2011].

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
1	*Numida meleagris*-BD01	0.000																		
2	*Tringa*_sp-BD02	0.199	0.000																	
3	*Milvus migrans*-BD03	0.223	0.176	0.000																
4	*Centropus_senegalensis*-BD04	0.201	0.177	0.192	0.000															
5	*Pycnonotus_barbatus*-BD05	0.215	0.195	0.184	0.201	0.000														
6	*Apus_affinis*-BD07	0.199	0.157	0.189	0.206	0.169	0.000													
7	*Francolinus_squamatus*-BD08	0.144	0.239	0.235	0.236	0.197	0.208	0.000												
8	*Bubulcus_ibis*-BD09	0.194	0.170	0.162	0.185	0.167	0.164	0.190	0.000											
9	*Streptopelia_decipens*-BD10	0.195	0.203	0.210	0.178	0.195	0.203	0.232	0.177	0.000										
10	*Gallus_gallus_*(Iyaya)-BD11	0.161	0.209	0.177	0.212	0.187	0.185	0.155	0.187	0.215	0.000									
11	*Gallus_gallus*-BD12	0.161	0.209	0.177	0.212	0.187	0.185	0.155	0.187	0.215	0.000	0.000								
12	*Cairina_moschata*-BD13	0.195	0.194	0.204	0.215	0.190	0.174	0.200	0.167	0.200	0.192	0.192	0.000							
13	*Columbia_livia*-BD14	0.203	0.182	0.200	0.197	0.177	0.192	0.212	0.167	0.134	0.212	0.212	0.190	0.000						
14	*Anthreptes_collaris*-_BD15	0.202	0.208	0.210	0.205	0.171	0.187	0.212	0.182	0.190	0.221	0.221	0.200	0.187	0.000					
15	*Meleagris_gallopavo*-BD16	0.170	0.209	0.211	0.233	0.217	0.208	0.179	0.185	0.213	0.149	0.149	0.221	0.207	0.229	0.000				
16	*Gallus_gallus_*(Avuke)-BD17	0.161	0.209	0.177	0.212	0.187	0.185	0.155	0.187	0.215	0.000	0.000	0.192	0.212	0.221	0.149	0.000			
17	*Francolinus bicalcaratus*-BD18	0.152	0.241	0.241	0.225	0.222	0.216	0.068	0.208	0.213	0.192	0.192	0.203	0.210	0.223	0.209	0.192	0.000		
18	*Tyto_alba*-BD19	0.213	0.216	0.224	0.197	0.194	0.179	0.215	0.165	0.213	0.221	0.221	0.192	0.199	0.202	0.227	0.221	0.205	0.000	
19	*Turdus_pelios*-BD20	0.196	0.182	0.202	0.184	0.148	0.177	0.207	0.179	0.187	0.212	0.212	0.187	0.162	0.163	0.244	0.212	0.202	0.207	0.000

which the Barn Owl *Tyto alba*, Scopoli, 1769, being a cosmopolitan species, is generally well known, but with scanty history in the tropics, except for very few studies (IUCN, 2012). Mitochondrial DNA is so widely used in molecular systematics of birds (Galtier *et. al.,* 2009) because it is common in cells and it evolves rapidly (Bates *et al* 2004). Although, utility of a reference DNA barcode is well established (Kerr *et. al.*, 2007), data on many West African birds are lacking in the database, the present work may contribute much towards establishing a DNA barcode reference library for utilization in biodiversity assessment and conservation in Africa.

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