DNA BARCODING OF ENDANGERED AVIAN FAUNA IN CAPTIVITY: THE AFRICAN PARROTS

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BACKGROUND

- More than one third of the world's avian fauna (about 23,000 sequences from 3,800 species) were barcoded through the All Birds Barcoding Initiative (ABBI) and the initial North American avian species barcoding project (Lijtmaer *et. al*., 2014). Most avian DNA barcodes records deposited in BOLD are derived from archived museum tissue collections - *© Barcode of Life Initiative, 2018*
- Little work has been done in Africa, particularly in Nigeria with respect to DNA barcoding of her avian species, regardless of the fact that many of the species of birds are threatened to extinction
- Barcoding endangered species in the wild or from orphanages, as against museum tissue collections (which are hard to come by), is laborious as researchers are careful not to sacrifice the animals

LEVEL OF GLOBAL ENDANGEREMENT

- Four hundred and sixteen (416) avian species were listed as endangered, representing 4.0% of the total number of avian species evaluated.
- -- IUCN (2016)
- Nigeria possesses more than 889 species of birds (FEPA, 1992), about 25 of them are either threatened or endangered and 20 of them are listed in CITES as Appendices 1 and 11 from nine Orders of birds

THREAT TO SPECIES SURVIVAL

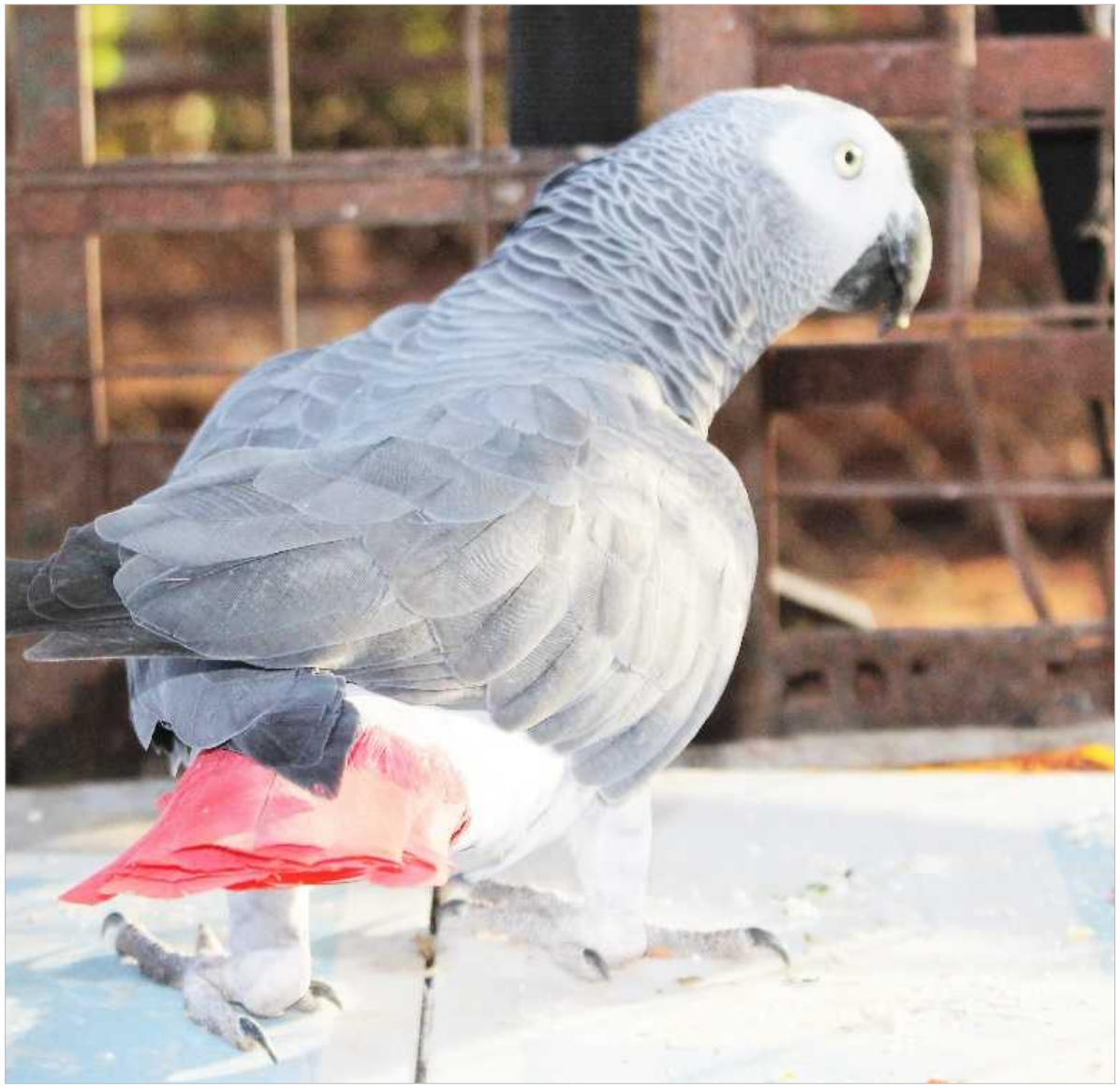
- Humans are the largest threat to survival of species of birds
- From records, between 1994 and 2003, close to three hundred and sixty grey parrots were traded on the international market
- Mortality rates are alarming (60-65%) from wild to the market
- There is high mortality of imported birds before acclimatization
- Birds are hunted for meat and use of their parts for traditional medicines
- Habitat loss, due to climate change, industrialization and changes in land use further threaten the their survival

THE PARROTS AND INTERNATIONAL TRADE

- Parrots are good companions to humans; they are kept as pets
- They have the ability to mimic human speech and noises around their environment
- They are very intelligent but very sensitive

and can be easily

GREY PARROT



INTERNATIONAL TRADE

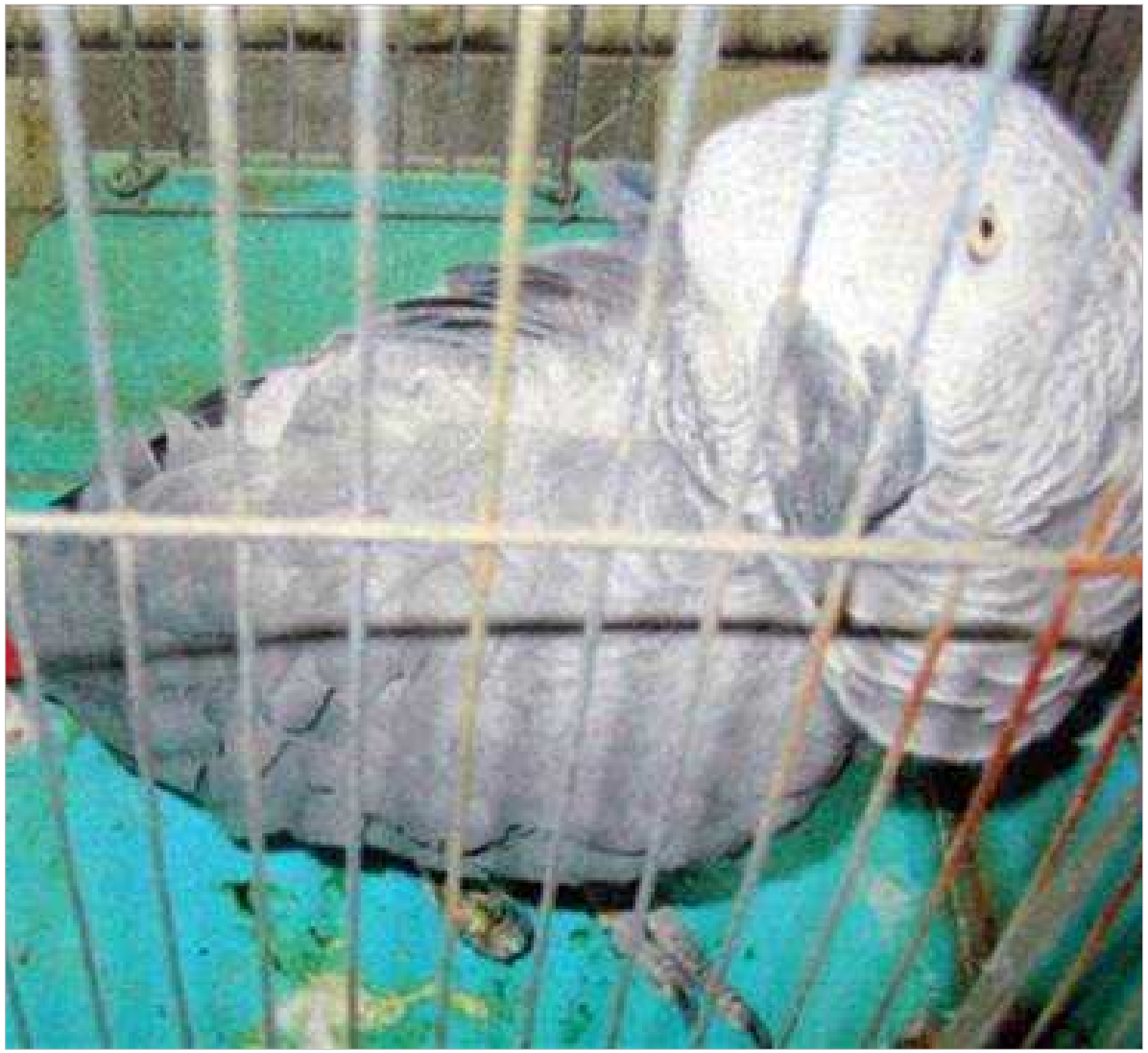
African grey parrots have been traded as far as to Japan

When “Yosuke” the parrot flew out of his cage and got lost, he did exactly what he had been taught — recite his name and address to a stranger willing to help.

Police rescued the **African** grey parrot from a neighbor's roof in the city of Nagareyama, near Tokyo. After spending a night at the station, he was transferred to a nearby veterinary hospital...

The parrot kept mum with the cops, but began chatting after a few days with the vet.

“I'm Mr. Yosuke Nakamura," the bird told the veterinarian, according to Uemura. The parrot also provided his full home address, down to the street number and was eventually returned to the owner



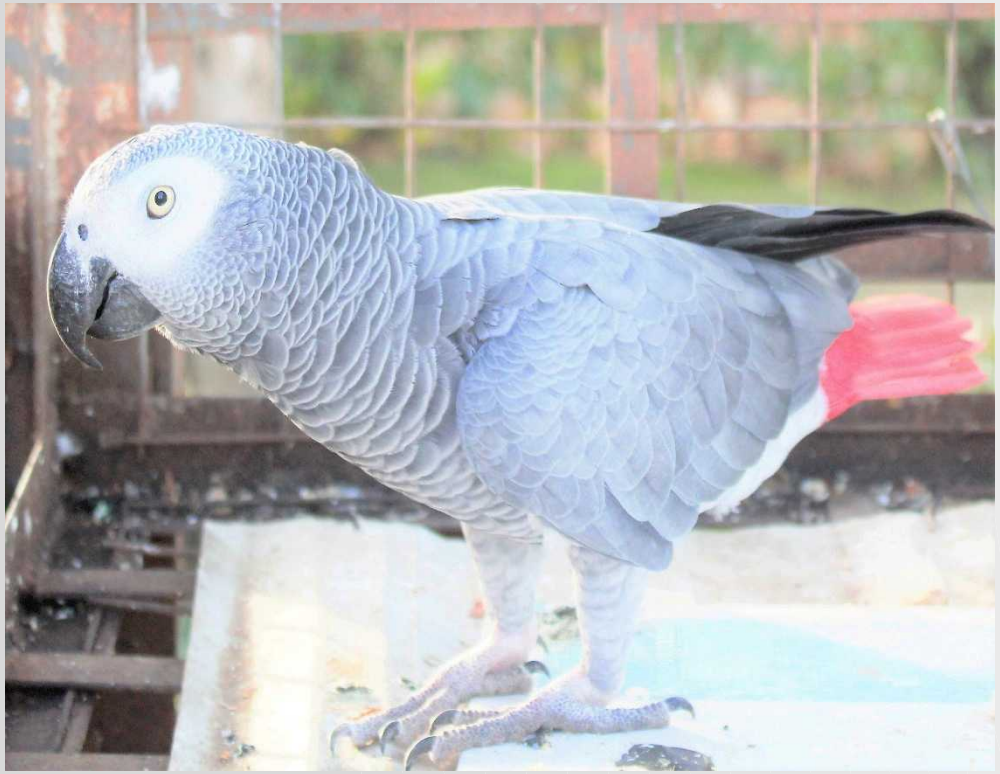
Retrieved on May 22, 2018 from: http://www.nbcnews.com/id/24753683/ns/world_news-wonderful_world/t/lost-parrot-tells-veterinarian-his-address/#.WwWt-0gvzIU

CONVENTION ON THE INTERNATIONAL TRADE OF ENDANGERED FAUNA AND FLORA (CITES)

- In October 2016, the Convention on the International Trade of Endangered Fauna and Flora (CITES) extended the highest level of protection to grey parrots by listing the species under appendix 1, which bans global and domestic trade in the species
- Other parrots covered in the study include Senegal parrot and parakeets, listed under appendix 11
- **CITES** is an international agreement between governments to ensure that international trade in specimens of wild animals and plants does not threaten their survival.

SOCIAL LIFE

- African Grey parrots have been known to live for up to **80 years** in captivity. So it is imperative that those who adopt them can commit to a lifetime of living with a bird.
- It is much older than the University’s biological garden
- It provides amusement to the people of the community.



Parrot in Biological garden of Godfrey Okoye University Enugu, Nigeria

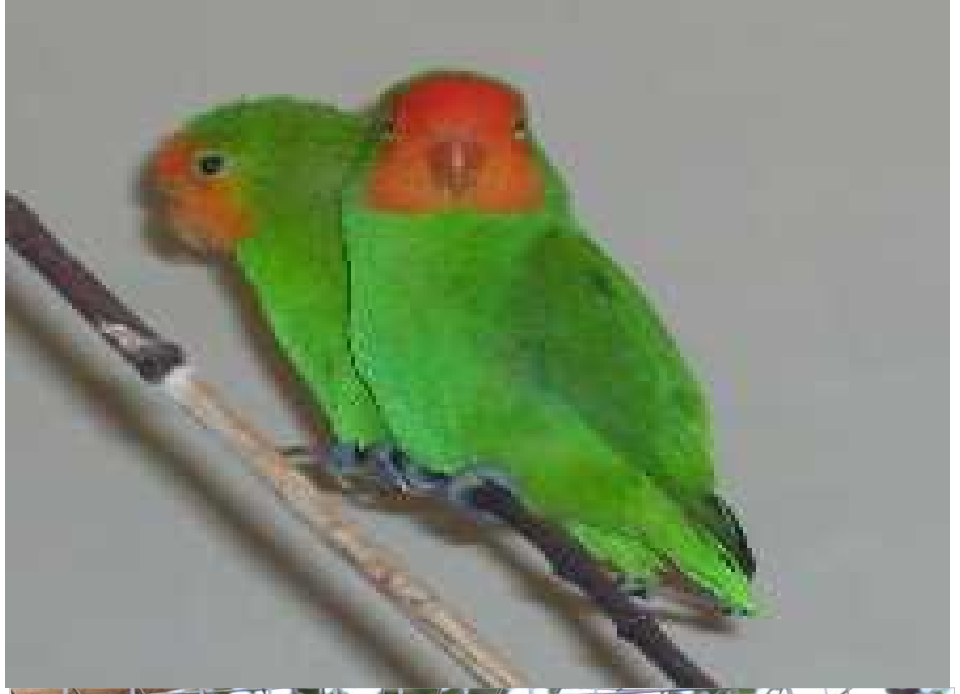
LOVE BIRDS

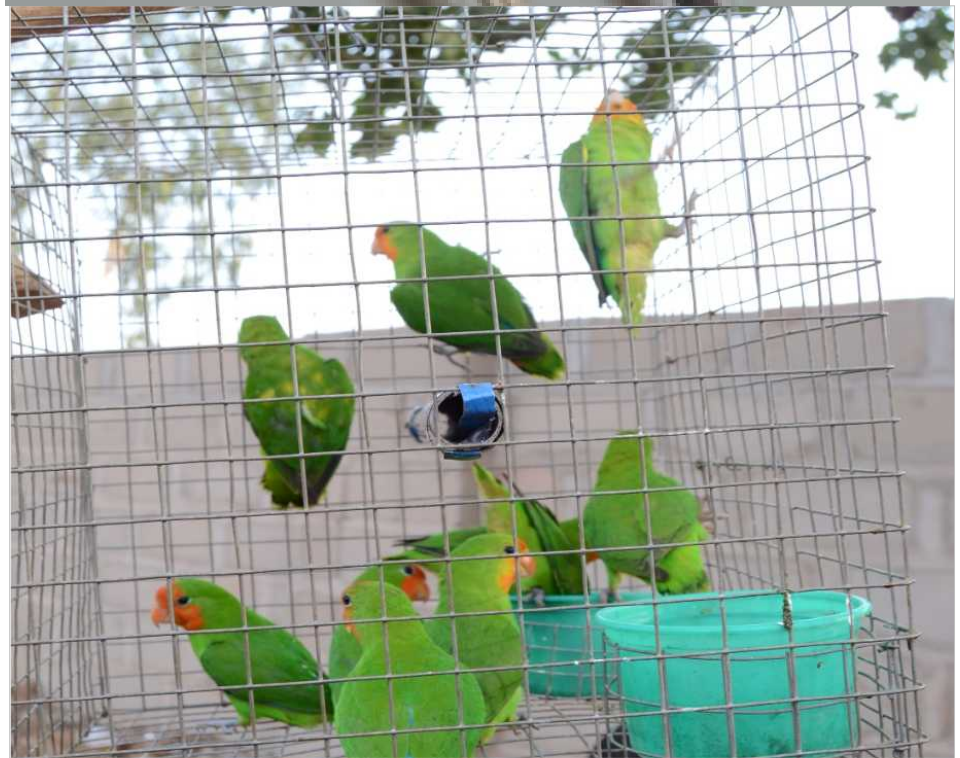
A **lovebird** is the common name of *Agapornis* - a small genus of parrot.

Eight of the nine species are native to the African continent, while the ninth species- the grey-headed lovebird is native to Madagascar.

Love birds are very affectionate, and monogamous in nature, bonding strongly in pairs and can spend long period of time together

Lovebirds live in small flocks and eat fruit, vegetables, grasses, and seeds





PARAKEETS

- There are many different types of parakeets and many are very colorful.
- There are more than 120 species and sub-species.
- Parakeets are small to medium sized, colorful parrots with long, tapered tail feathers.
- The African parakeets inhabit tropical and sub tropic regions.
- They are also traded in international market

SOCIAL LIFE

- Parakeets are very social birds and live in flocks, with each other in the wild and with people, with whom they form close bond.

- They love attention and are some of the most popular pet birds

- They adapt quickly to their cage and can be quite playful. They are very dexterous and are constantly on the go

OBJECTIVES

- To carry out surveillance of all Nigerian bird (Parrot group) species listed in CITES
- To DNA barcode the species hosted in the National Parks (Protected Areas)
- To use the sequence result to protect endangered species from illegal trading

MATERIALS AND METHODS

- Specimen were collected from parrot species hosted in National Parks Services orphanage in Abuja, Nigeria.
- Blood, skin/feather and liver of birds were taken, using syringe and scalpels and stored in the Fluid X tubes containing EDTA.
- Genomic DNA from the animal tissues was extracted using the Qiagen Dneasy Blood and Tissue Kit (cat. 69506).
- All amplification reactions were performed in a GeneAmp® PCR System 9700, Applied Biosystems.
- PCR amplicons were loaded on 1.5% agarose gel and run at 100volts for 2 hours.
- The amplicons with single band were selected from the amplified products and purified using manufacturer’s protocol (QIAquick PCR Purification Kit, cat. No.28106).
- Sequencing was performed by using a Big Dye terminator cycle sequencing kit (Applied BioSystems)

RESULTS

Table 2: Species identification

Species Identification									
Voucher information						Genbank		BOLD	
Sample	Genus	Species	English name	Decimal Latitude	Decimal Longitude	Species	Accession number	Species	BIN (Cluster ID)
1	Agapornis	taranta	Lovebird	7.693	8.65075	*Psittacula krameri*	DQ433148	*Psittacula krameri*	AAE0447
2	Psittacus	erithacus	Grey parrot	9.026222	7.432111	*Psittacus erithacus*	KF381364	*Psittacus erithacus*	AAF6813
3	Poicephalus	senegalus	Senegal Parrot	7.693	8.650806	*Poicephalu senegalus*	KX012808	*Poicephalus senegalus*	ADJ7485

MOLECULAR PHYLOGENETIC ANALYSIS

- The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993).
- The phylogenetic tree involved 16 nucleotide sequences comprising 13 common African parrots sequences sourced from National Center for Biotechnology Information (NCBI) and the nucleotide sequences of the 3 bird species sampled.
- All sequence nucleotides clustered together with Genbank sequences with varying degrees of bootstrap (Fig. 1).
- We observed that one of the samples *Psittacula kramer* was Taxonomically misidentified as *Agapornis taranta* due to close phenotypic characteristics between the species.
- The phylogeny also revealed that common ancestors of the Agapornis lie within the diversity of P*sittacula eques*.

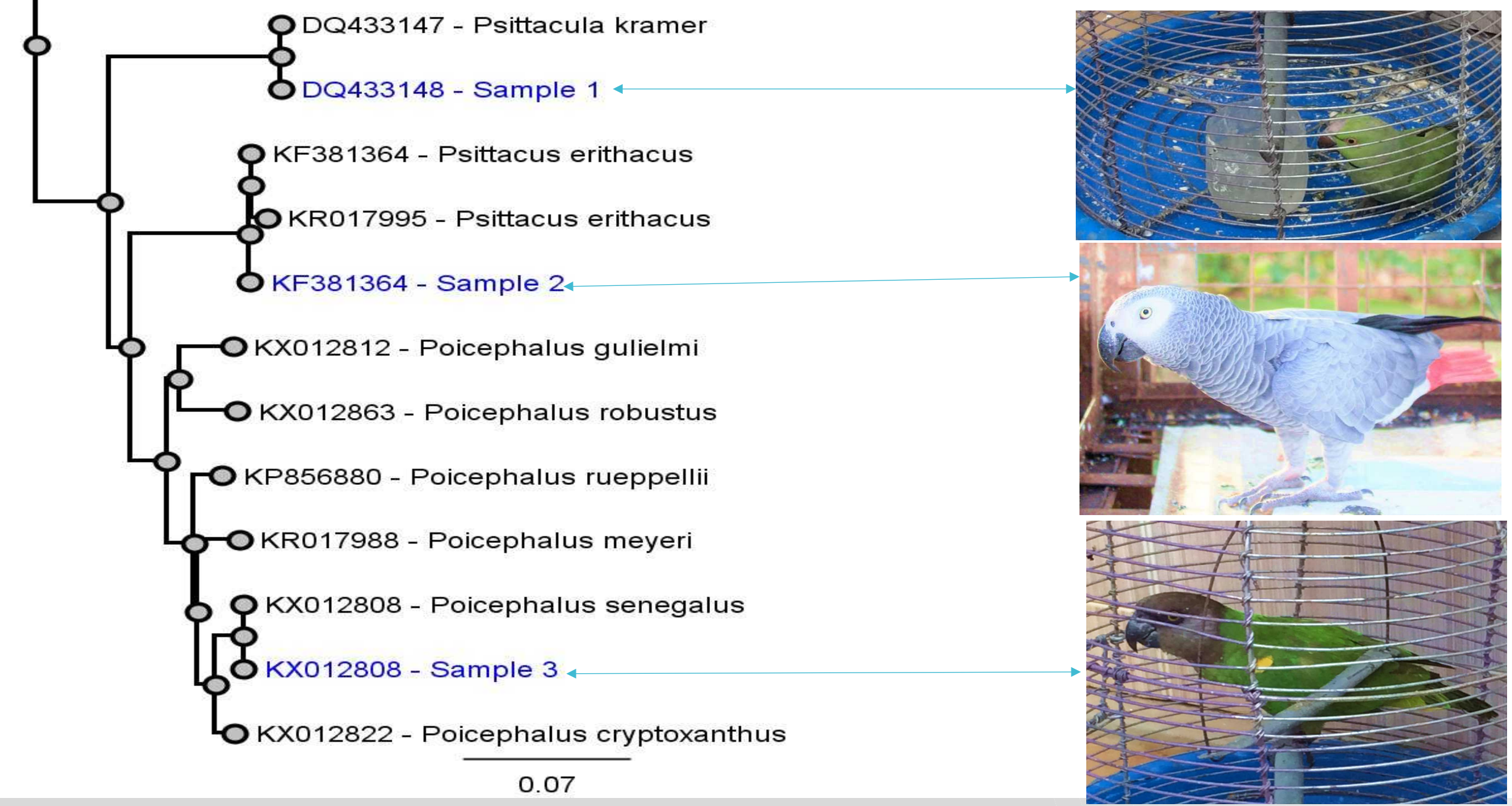


Figure 1:Neighbor-joining tree of 13 common African parrots and the 3 samples studied

DISCUSSION/CONCLUSION

- All the protected parrot species in the Nigeria’s National Park were sequenced and barcoded
- One of the samples, taxonomically identified as *Agapornis taranta* was misidentification of *Psittacula Kramer,* based on the BLAST result
- This study demonstrated the authenticity of DNA barcoding as quality control measure for species identification
- The sequences deposited in the genbank will serve as evidence in a case of poaching or illegal trade against CITES defaulters.

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