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Phylogenetic relationships in the family Felidae in reference to the hybridisation of the Scottish Wildcat, *Felis sylvestris grampia* with the Domestic cat, *Felis catus*

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Abstract

Understanding the evolutionary history and relationships among group or individual animal species is important in making conservation decisions for populations. This study essentially sought to assess the relationship between species in the Felis family using mtDNA from hair and with reference to the hybridization of the Scottish wildcat, Felis sylvestris grampia with the domestic cat, Felis catus. Two reference species, Felis chaus and Felis margarita and one outgroup, Panthera onca were used. The results of the analysis shows a relationship exists between the cat species and that the study of phylogeny could be useful in understanding the distribution and recognition of species.

Keywords: Phylogenetic, relationships, family, Felidae, hybridization, Scottish Wildcat.

Introduction

Also known as interbreeding, hybridization, in this case, has been used to refer to the process where wildcats reproduce with domestic cats[1-3]. This effort to restore the species has left the contemporary wild form difficult to distinguish from many of their domesticated forms, presenting a dilemma for conservationists with respect to defining and enforcing conservation measures[4,5].

Moreover, with decades of hybridization, there are a few wildcats left with estimates for the entire population of feral cats in the UK being between 800,000-1.5 million[6]. *Felis silvestris grampia*, one of Scotland's few remaining native predatory animal species[7,8] is under a continued threat leaving them on the edge of extinction[5,9,10].

The species has been heavily impacted by introgressive hybridization with domestic cats affecting its natural morphological features[11]. Other than the challenge of introgressive hybridization, various researchers highlight several other threats faced by the population of the wildcat.

A research in 1992 detected an infection of several free-living wildcats by the leukemia virus[12]. More so, the wildcat is constantly threatened by persecution through trapping and hunting; and from direct attacks and killing by dogs, snares/poison and road kill[1,13]. This is further supported by studies done in 1992[4], 1994[5], 2004[7], 2011[8], 2015[9,10], 2016[11], and 2017[12].

Methodology

Through a series of experiments, this undertaking sought to investigate into the phylogenetic relationships in the family Felidae with reference to the hybridization of *Felis sylvestris grampia* with *Felis catus.*

This was done through the following steps; i. DNA extraction from felid hair samples, ii. Casting an agarose gel in preparation for electrophoresis of amplified DNA, iii. Amplification of mitochondrial cytB gene fragment by Polymerase Chain Reaction, iv. Gel electrophoresis of amplified DNA, v. DNA Sequencing and pooling of class data. It also involved analysis of genetic data from DNA sequence samples of the mitochondrial Cytochrome b gene of Felis cats. The sequences were BLASTED, aligned and used in the preparation of phylogenetic tree and for species comparison/identification through NCBI database.

Results and discussion

Estimating evolutionary divergence between the sequences: To estimate the evolutionary genetic distances between the species, analysis was conducted using the Maximum Composite Likelihood model (Tamura K., Nei M., and Kumar S., 2004). This involved 20 nucleotide sequences while codon positions included were 1st+2nd+3rd+ Noncoding. All positions containing gaps were eliminated. There were a total of 363 positions in the final dataset. Evolutionary analyses were conducted in MEGA6[14]. The results are as shown in Table-1.

Table-1: Estimates of evolutionary divergence between Sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1																			
2	0.000																		
3	0.000	0.000																	
4	0.000	0.000	0.000																
5	0.017	0.017	0.017	0.017															
6	0.017	0.017	0.017	0.017	0.000														
7	0.000	0.000	0.000	0.000	0.017	0.017													
8	0.006	0.006	0.006	0.006	0.011	0.011	0.006												
9	0.000	0.000	0.000	0.000	0.017	0.017	0.000	0.006											
10	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.006	0.011										
11	0.017	0.017	0.017	0.017	0.000	0.000	0.017	0.011	0.017	0.011									
12	0.006	0.006	0.006	0.006	0.011	0.011	0.006	0.000	0.006	0.006	0.011								
13	0.006	0.006	0.006	0.006	0.011	0.011	0.006	0.000	0.006	0.006	0.011	0.000							
14	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.006	0.011	0.000	0.011	0.006	0.006						
15	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.006	0.011	0.000	0.011	0.006	0.006	0.000					
16	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.006	0.011	0.000	0.011	0.006	0.006	0.000	0.000				
17	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.059	0.065	0.059	0.065	0.059	0.059	0.059	0.059	0.059			
18	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.158	0.150	0.150	0.150	0.158	0.158	0.158	0.168		
19	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.014	0.020	0.014	0.020	0.014	0.014	0.014	0.014	0.014	0.062	0.158	
20	0.059	0.059	0.059	0.059	0.058	0.058	0.059	0.058	0.059	0.058	0.058	0.058	0.058	0.058	0.058	0.058	0.078	0.182	0.055

Note: 1=Captive wild cat 1, 2=Captive wild cat 2, 3=Captive wild cat 3, 4=Black footed cat, 5=Wild cat 1, 6=Wild cat 2, 7=Wild cat 3, 8=Domestic cat 1, 9=Domestic cat 2, 10=Domestic cat 3, 11=Museum cat 1, 12=Museum cat 2, 13=Museum cat 3, 14=Feral cat 1, 15=Feral cat 2, 16=Feral cat 3, 17=Fishing cat, 18=Jaguar (panthera_onca) Out group, 19=Sand cat (felis margarita) Reference, 20=Reed cat (felis chaus) Reference.

Phylogenetic tree for the felids: Studying characteristics of the inherited species could help reconstruct species' evolutionary relationships[2]. This sequence could be used to identify substitutions or mutations characterizing genetic divergences within or between species[15]. The Phylogenetic tree below shows the summary lineage results of the *Felis* family in relation to two reference species (*Felis chaus* and *Felis margarita*) and one outgroup species (*Panthera onca*).

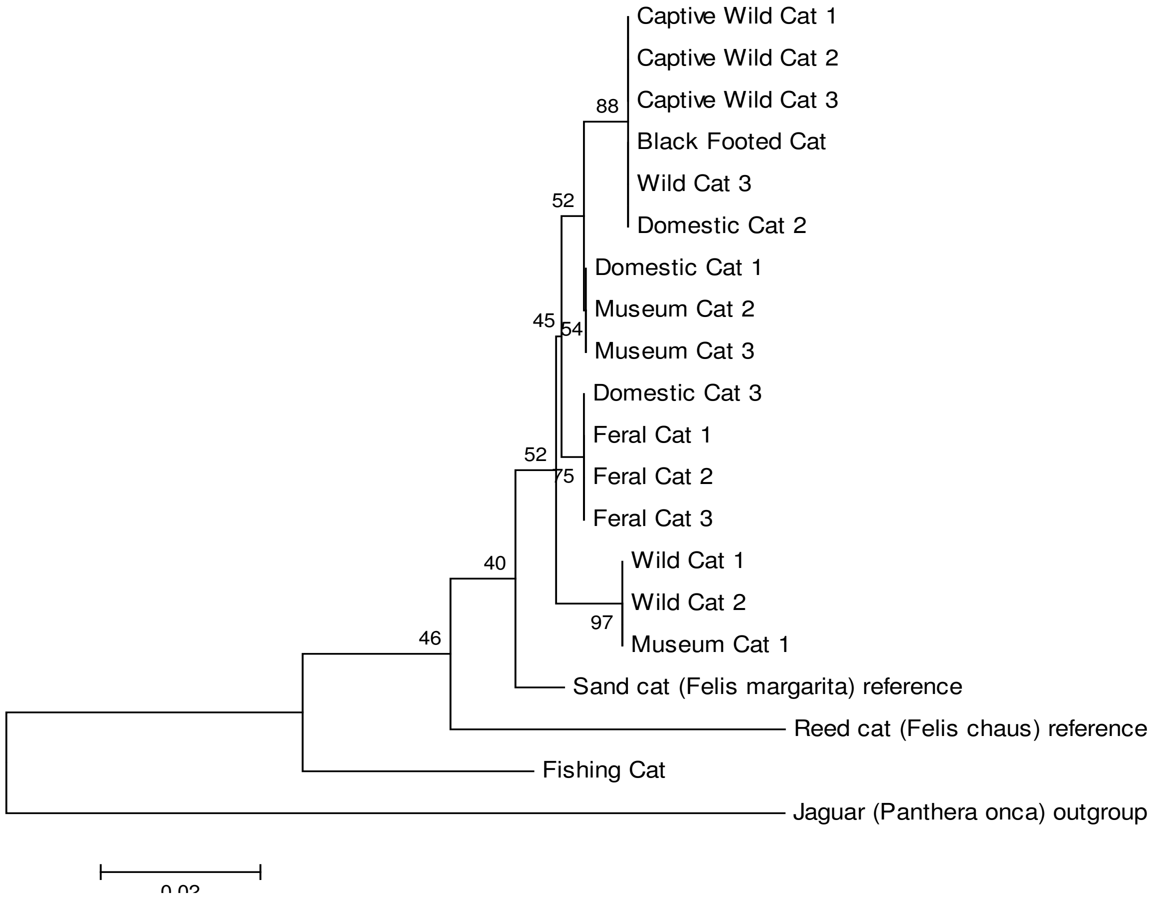

Figure-1: Haplophytic tree (original).

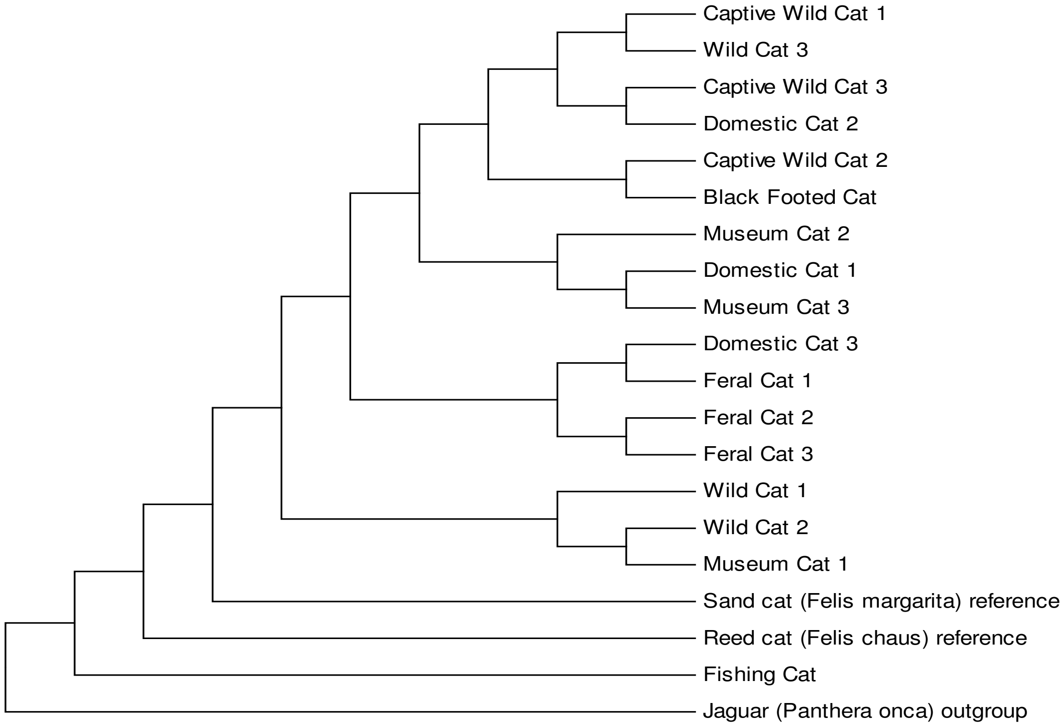

Figure-2: Haplophytic tree (bootstrap consensus).

Species Identification using NCBI: In reference to species identification, the already edited sequence data for the species was compared against the NCBI database. A list of possible matches was derived and analyzed on the basis of species matching scores to produce are given in Table-2.

DNA extraction and amplification results: As highlighted in the methods' section, mtDNA was isolated from the felid hair samples and subjected to site-specific PCR amplification. The result is as shown in Figure-3.

Discussion: In constructing a phylogenetic tree, the maximum Likelihood fit of different nucleotide substitution models was assessed[16]. The model with the lowest BIC scores was considered to describe the best substitution pattern and in this case, HKY (Hasegawa-Kishino-Yano) model was used. Mitochondrion *Panthera onca* (Jaguar), with a 1140 base strand was used as an outgroup[17] while the Jungle cat, *Felis chaus* (16653 bases)[18] and the sand cat, *Felis margarita* (16864 bases) were used as references.

From the phylogenetic trees above, it's evident that all the species share a common ancestor but speciation differentiates them along the lineage. Therefore, in estimating the most likely haplotype where the Scottish wildcat could belong, morphological features of the available species are used[19]. The haplotype bearing wildcat 1, wildcat 2 and museum cat 1 is thus the most probable location of the Scottish cat. This is because all the other haplotypes have species that have quite a distant morphology from the Scottish cat (the black-footed cat, feral cat, and domestic cat) (Figure-2). This leaves the one haplotype which is the best possible match.

Table-2: A list of possible matches was derived and analyzed on the basis of species matching scores.

Description	Max score	Total score	Query score	E value	Indent	Accession
Felis silvestris specimen-voucher SDZ-32188 cytochrome b (cytb) gene, complete cds; mitochondrial gene for mitochondrial product	680	680	100%	0.0	99%	AY170102.1
Felis catus mitochondrial DNA for cytochrome b, complete cds	675	675	100%	0.0	99%	AB004238.1
F.domesticus mitochondrial cytochrome b gene	665	665	100%	0.0	99%	X82296.1
Felis margarita isolate FMA mitochondrion, complete genome	652	652	100%	0.0	98%	KP202276.1
Felis chaus isolate FCH mitochondrion, complete genome	575	575	100%	3e-160	94%	KP202274.1
Felis nigripes isolate FNI mitochondrion, complete genome	564	564	100%	7e-157	94%	KP202277.1

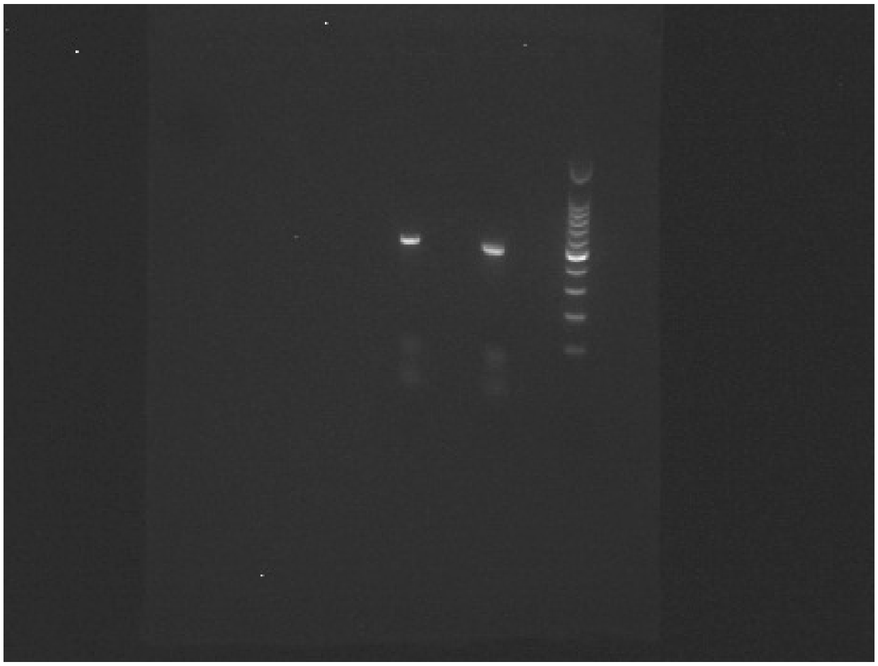

Figure-3: Felid hairs' DNA extraction and amplification results.

In reference to species matching/identification through the NCBI (National Center for Biotechnology Information) database, the sequences with the most significant alignment (having high average scores and complete CDs) were deemed the most likely match[20-22]. This was identified by assessing the results of the NCBI BLAST data and comparing them with the sequence. The species endemic to the UK (especially Scotland) were best considered to be likely matches for the samples used in DNA sequencing.

For instance, although NCBI identified the *ocelot* (Leopardus pardalis), it is native to the southwestern United States and Mexico and therefore not considered. In the same note, *Felis Silvestris bieti* is endemic to China and thus not any close to being a match with the European cat. The same case applies to *Catopuma temminckii*, an Asian wildcat, Felis silvestris lybica (African cat) and *Panthera pardus* cyt b gene because it's a large cat. On the other hand, *Felis silvestris* voucher specimen was considered as a likely match because it is as an identified representative sample/specimen/reference point of a species[23,24].

To determine the extent of intra/interspecies variation between the sequences used in this study, mtDNA was successfully extracted. Through electrophoresis, the DNA fragments were separated resulting in a distinct pattern. In this case, there is a corresponding match (Figure-1) and thus the results may be interpreted as to show some form of similarity between the species represented[25]. Summarily, it can be concluded that there is limited intra/interspecies variation between the sequences used.

The future of Scottish wildcats' conservation in relation to hybridization: There are attempts to rehabilitate the Scottish Wildcat, thanks to the dedicated efforts of conservation agencies, scientists and government institutions[26]. Currently, there are two initiatives seeking to conserve the Scottish cat through pooling together experts and relevant specialist organizations[8].

Wildcat Haven seeks to create wildcats' habitable environments through focusing on areas where pure wildcats appear to survive. They study their genetics with the focus on removing feral cats through neutering and explore opportunities of relocating pure breeds of wildcats to protected regions[8]. The SNH Scottish Wildcat Conservation Action Plan, on the other hand, offers an alternative by conserving the hybrid species of Scottish wildcat.

Species-based conservation efforts (captive breeding) are also viable solutions to conservation of the wildcat[9]. An example is the RZSS's Highland Wildlife Park, a major wildcat-breeding park in Cairngorms, Scotland. The Zoological Society of Scotland also works to buffer the species population. Summarily, future conservation efforts for the Scottish wildcat ought to focus on controlling interbreeding and spread of hybrids and especially limiting habitat loss[2]. This will preserve the existing gene flow in the identified isolated dispersal areas.

Conclusion

The importance of genetics in species conservation cannot be underestimated. Mitochondrial DNA (mtDNA) is particularly useful to provide an understanding of the haplotype distribution and identification of species. However, this comes with its shortcomings because, in comparison to nuclear DNA (nDNA), it is rather difficult to truly identify species' ancestry/lineage using mtDNA.

Additionally, mitochondrial genome has a higher mutation rate, making it more prone to fixation. It also has a lower power of discrimination due to the lack of recombination; a weakness in the identification of species which are closely related to the feral cats. MtDNA also has a comparatively lesser coalescent time and therefore only able to resolve relationships among species that recently diverged. In conservation genetics, therefore, nuDNA is better placed to provide a more coherent understanding of species linkage/relationships than mtDNA.

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