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RESEARCH NOTE

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Genetic support to uplist an African cheetah subspecies, *Acinonyx jubatus soemmeringii*, imperiled by illegal trade

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Abstract

There has been a drastic decline in the number of cheetahs in the past century. Illegal pet trade poses a critical threat to the survival of the species and demands more attention. Genetic analysis of 55 cheetahs confiscated in Somaliland shows 100% as *Acinonyx jubatus soemmeringii*, suggesting that illegal trade subjects *A. j. soemmeringii* to significant pressure. Continued decline of the estimated 260–590 mature individuals qualifies this subspecies as "endangered" versus currently "vulnerable," under criterion C2a(i) of the International Union for Conservation of Nature Red List. A population decline of ≥80% over the next 10 years or 3 generations would even fulfill criterion A3 of "critically endangered." We urge reclassification of *A. j. soemmeringii*, as endangered, and appeal to the cheetah community to investigate further uplisting to critically endangered to reflect the likely extinction risk exacerbated by the illegal trade.

KEYWORDS

Acinonyx jubatus soemmeringii, cheetah cub trafficking, critically endangered, illegal wildlife trade, IUCN red list criteria

The global distribution of cheetahs has decreased drastically during the past century, with current numbers as low as ~7100 mature individuals (Durant et al., 2017), yet cheetahs are only classified as vulnerable by the International Union for Conservation of Nature (IUCN) Red List (criteria A4b and C1, IUCN, 2022). Well-documented threats to cheetah survival include human–wildlife conflict, habitat loss and fragmentation, as well as prey base reduction (Marker et al., 2008). Illegal wildlife trade in live cheetah cubs for the pet market, while far less publicized than the trade of ivory, rhinoceros horn, or pangolin scale, poses a critical threat to the survival of cheetahs and demands more attention. We have therefore performed an initial analysis of cubs affected by the illegal wildlife trade.

Between 2010 and 2019, an estimated 4184 cheetahs were involved in the illegal wildlife trade (Tricorache et al., 2021). Filtering this dataset for confirmed cases that were confiscated/found in Somaliland (a self-declared autonomous region in northwest Somalia that formed in 1990), we identified 489 individual cheetahs affected by trafficking for that time period, in Somaliland alone. Cheetah cubs confiscated from the illegal wildlife trade by the Somaliland authorities require intensive

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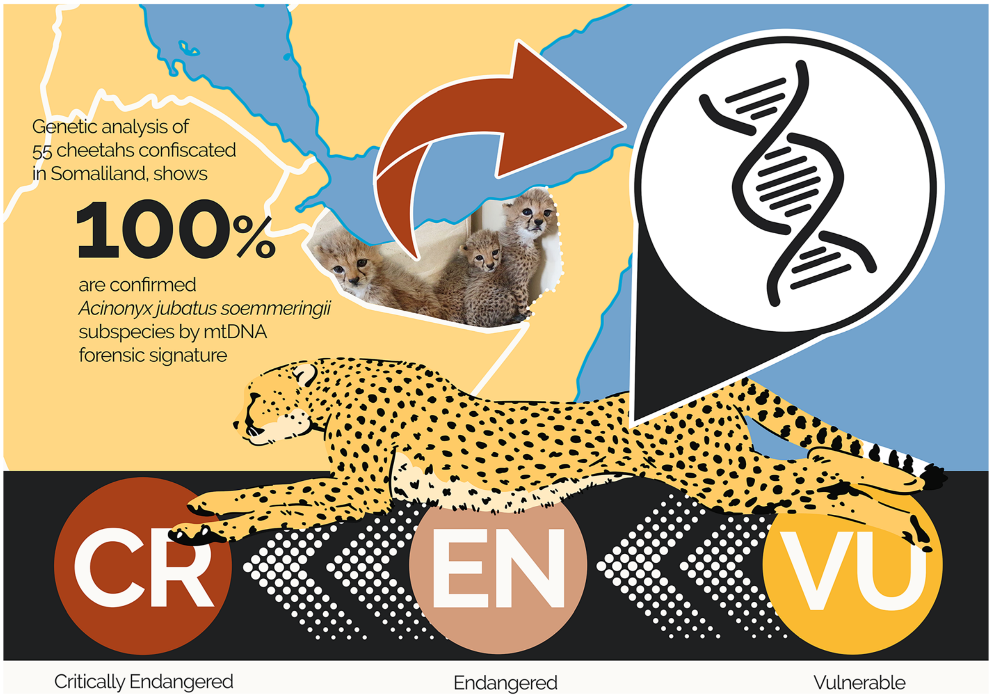

FIGURE 1 Cheetah cubs confiscated from the illegal wildlife trade by the Somaliland authorities need intensive veterinary care. Analysis of their DNA provided evidence that the illegal wildlife trade targets *Acinonyx jubatus soemmeringii*, which provides evidence for the arguments presented for requesting the uplisting of the International Union for Conservation of Nature (IUCN) Red List status of this subspecies.

veterinary care to provide them with a chance to survive (Figure 1). Indeed, mortality in the first month post-arrival is approximately 20% (Cheetah Conservation Fund [CCF] records). As part of the veterinary care provided at the CCF rescue centers in Somaliland, samples are routinely collected from the cubs to assess metabolic health and infectious disease load. For this study, we performed a genetic subspecies assessment on samples we were able to obtain for 55 of the cheetahs confiscated within the time period of the Tricorache et al. (2021) review on illegal trade cases, ranging from 2016 to 2019.

Tissue samples were shipped to the CCF Conservation Genetics Laboratory in Namibia, under the CITES permit #0061077 and processed under the Namibian research permit AN202101032. No animals were kept or sampled for the purpose of the study. We extracted DNA from 53 blood and tissue samples, and 2 hair samples with the DNeasy Blood & Tissue Kit (Qiagen), following a published user-developed protocol (Qiagen DY04, 2006), respectively. DNA was diluted based on relative quantity and quality assessment on a 1% agarose gel. We amplified a subset of the mitochondrial control region (CR) with AmpliTaq Gold™ 360 Master Mix (Applied Biosystems), using touch-down (60–50°C) polymerase chain reaction (PCR) with two published primer pairs (Burger et al., 2004): CR1f and CR1r for amplicon CR1; and CR2f and CR2r for amplicon CR2. Amplicons were purified with ExoSAP-IT™ (Thermo Scientific) prior to sequencing with BigDye™ Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). We sequenced amplicon CR1 with CR1f and amplicon CR2 with CR2r, ensuring coverage of the eight variable sites included in Charruau's amplicons aMT-CR1 and aMT-CR3 (Charruau et al., 2011, tab. 2). An additional three variable sites, including one diagnostic site, were identified from the published sequences (Table 1). The sequencing products were purified with ethanol precipitation and resuspended with formamide prior to capillary electrophoresis on a 3130 ABI genetic analyzer. Base calling and quality control were performed by ABI software Sequence Analysis Software v6.0 (SeqA 6), and sequences were proofread and aligned in Geneious Prime 2020.2.3 (https://www.geneious.com). We included published reference sequences (Charruau et al., 2011) in the alignment to determine the subspecies of those cubs.

Of the 55 samples, 53 samples provided good sequences for both amplicons and included all 11 variable sites, including 2 sites diagnostic for *Acinonyx jubatus soemmeringii*; while 2 samples each only included 1 diagnostic site for *A. j. soemmeringii* (Tables 1 and S1). All 55 haplotypes were a perfect match to published sequences of the subspecies *A. j. soemmeringii*, including the diagnostic sites, and had mismatches to the published reference sequences of all other cheetah subspecies (Table 1). We thereby were able to assign 100% of the 55 cheetahs to mitochondrial haplotypes of *A. j. soemmeringii* (Figure 1), the regional subspecies from the Horn of Africa (Kitchener et al., 2017). Thus, our findings strongly indicate that most trafficked cheetahs detected in the Horn of Africa illegal pet trade are of the subspecies *A. j. soemmeringii* and are sourced regionally.

Given the already small free-ranging population of *A. j. soemmeringii*—an estimated count of 260–590 mature individuals (Durant et al., 2017; IUCN, 2021,

TABLE 1 Haplotypes obtained from 55 cheetahs (*Acinonyx jubatus*) confiscated from the illegal wildlife trade in this study, compared with reference haplotypes.

Amplicon	**CR1**					**CR2**					
Nucleotide position	**16448**[a]	**16454**[a]	**16456**[b]	**16473**[a]	**16474**[a]	**16817**[a]	**16818**[a]	**16828**[b]	**16831**[a]	**16854**[a]	**16865**[b]
Haplotypes from Charruau et al. (2011):											
Acinonyx jubatus venaticus	T[c]	T	C	T	A	T	A	A	A	A	C
Acinonyx jubatus jubatus	C	T	C/T	T	G	T	A	G/A	A	A	C
Acinonyx jubatus soemmeringii	C	T	C	C[c]	G	T	G/A	A	A	A	T[c]
Northern Africa population	C	T	C	T	A	C	A	A	A	A	C
Acinonyx jubatus raineyi	C	C/T	C	T	G	C/T	A	A	G/A	G/A	C
Haplotypes from cheetahs confiscated in Somaliland (2016–2019):											
Hap A ($N = 29$)	C	T	C	C[c]	G	T	A	A	A	A	T[c]
Hap B ($N = 24$)	C	T	C	C[c]	G	T	G	A	A	A	T[c]
IT-AJU1943	C	T	C	C[c]	G						
IT-AJU1944						T	A	A	A	A	T[c]

Note: Nucleotide positions based on GenBank Accession no. GI:38349475.1, as in Charruau et al. (2011).
[a] Informative sites as per Charruau et al. (2011, tab. 2.).
[b] Additional variable sites based on Charruau et al. (2011) sequences.
[c] Diagnostic sites.

Appendix S2)—the illegal trafficking of individuals directly from this subspecies, as established by our genetic analysis, represents a sustained and significant offtake, which will inevitably lead to continued population decline. General threats faced by cheetahs, such as human–wildlife conflict and habitat loss (IUCN/SSC, 2007), further compound the problem. These combined circumstances fulfill criterion C2a(i) of the IUCN Red List (population numbers <2500 mature individuals, continuing decline, no subpopulation >250 individuals; IUCN, 2012) and qualify this subspecies as "endangered" versus currently "vulnerable". Furthermore, it is a realistic possibility that such offtake may fulfill criterion A2 or A3 of "critically endangered" (population decline ≥80% over the last 10 years or three generations, population decline ≥80% over the next 10 years or three generations, respectively; IUCN, 2012).

We urge IUCN to support reclassification of *A. j. soemmeringii*, as "endangered," and appeal to the cheetah community to investigate further uplisting to "critically endangered" to reflect the likely extinction risk exacerbated by the illegal trade. Uplisting will galvanize the support of stakeholders, provide access to funding restricted to species listed as endangered, and increase national and international protection of *A. j soemmeringii* against illegal trade.

AUTHOR CONTRIBUTIONS

Anne Schmidt-Küntzel: Conceptualization, funding acquisition, project administration and supervision, project design and methodology, and first draft. **Laurie Marker:** Conceptualization, funding acquisition, project administration and supervision. **Hafeni Hamalwa:** Laboratory work. **Bruce Brewer:** First draft. All authors have reviewed and edited the manuscript and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All sequences were deposited on GenBank (https://www.ncbi.nlm.nih.gov/genbank/); accession numbers are listed in Table S1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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