



## Short Communication

# The importance of genetic tools when studying the distribution of rare and elusive species illustrated by the Kam dwarf hamster



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## ABSTRACT

Detailed information on the distribution and abundance of animals is often difficult to establish for rare and elusive species. Here we report on genetic analyses confirming the presence of the Kam dwarf hamster 500 km north of its known distribution in China where it was earlier thought to be endemic. Our finding was made during a study on disease ecology in southern Mongolia and illustrates the benefit of genetic approaches when studying rare and elusive species or species that are either difficult to identify or do not elicit public or scientific attention. We suggest that larger ranges than currently known may be a common pattern for a number of rare and elusive species because of ineffective survey methods and lack of sampling effort.

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## 1. Introduction

Proper understanding of the distribution and abundance of animals is fundamental for management and conservation (Sinclair et al., 2006). However, these parameters are often difficult to establish for rare and elusive species and for species that are either difficult to identify or do not obtain public or scientific attention (González and Barbanti Duarte, 2007; McDonald, 2013). Nevertheless, recent developments in camera trapping and genetic analyses provide a means to monitor species that have so far been difficult to study (González and Barbanti Duarte, 2007; Waits, 2013). In fact, genetic analyses are especially useful for small species that may not trigger cameras and for species that are difficult to identify visually (Waits, 2013).

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The Kam dwarf hamster (*Cricetulus kamensis*) has been thought to be endemic to western China where it occurs in grasslands, shrubby marshes, and open steppes in mountainous areas (Smith and Xie, 2008). Despite its presumed restricted distribution, no immediate conservation concerns appear to exist for the species and it is categorized as *least concern* by the IUCN (Smith, 2016). However, the taxonomy of *Cricetulus* is controversial and both the number of species and the phylogenetic relationships are debated (Nuemann et al., 2006; Smith and Xie, 2008). IUCN lists 8 species of *Cricetulus* of which one species is the Kam dwarf hamster (IUCN, 2017). In this paper, we report on encountering the Kam dwarf hamster 500 km north of its known distribution and we discuss the benefits of genetic approaches when studying rare and elusive species that are difficult to identify.

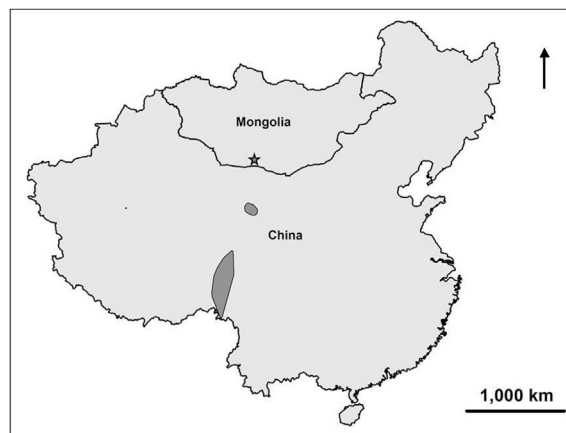
## 2. Material and methods

The observations reported here were made in the Tost Mountains in southern Mongolia (43° 37' N, 100° 12', Fig. 1) and was part of a study on disease ecology of snow leopards (*Panthera uncia*) and their prey. The Tost Mountains consists of several mountain massifs that are surrounded by open steppes. The study area is located ca 60 km north of the border to China, ca 50 km south of the Nemegt Mountains of the Gobi Gurvan Saikhan National Park, and is connected to the Great Gobi Strictly Protected Area A via a fragmented network of hillocks. The Tost Mountains were declared a Nature Reserve in the spring 2016 and is part of the Gobi Desert. The sparse vegetation consists of mountain shrubs and mountain grasslands. The temperature ranges between  $-35^{\circ}\text{C}$  in winter and  $+35^{\circ}\text{C}$  in summer and the altitude ranges between 1800 and 2500 m above sea level. The area hosts numerous small rodents – many of which have similar physical appearance and are difficult to identify by eye. Livestock herding is the primary occupation of local people and the livestock comprise of goats (*Capra aegagrus hircus*), sheep (*Ovis aries*), camels (*Camelus bactrianus*), and horses (*Equus ferus caballus*).

In June 2012, June 2013, and April 2015, we live-captured small rodents by using two sizes of Sherman traps ( $5.1 \times 6.35 \times 16.5$  and  $7.6 \times 9.5 \times 30.5$  cm) that were baited with peanut butter and rolled oats. Traps were set in locations that showed signs of use by small rodents and we checked traps twice daily. We collected a small sample of blood from captured rodents and cut a small piece of skin from their ear to (1) assure that rodents were not sampled twice (i.e. we did not sample animals that had a notch in the ear) and (2) use for genetic identification of species (see details below). We sexed the animals based on external genitalia and we aged them based on development of external genitalia and size.

We performed identification analyses based on genetic markers following Gillet et al. (2015). Briefly, DNA was extracted from a piece of ear tissue using the DNeasy extraction Kit (Qiagen Inc., Hilden, Germany). An illumina amplicon sequencing was then performed following a modified Miseq protocol (Metagenomic Sequencing Library Preparation). Total genomic DNA of the studied samples were subjected to PCR amplification targeting a ~133-bp fragment of the cytochrome oxidase I gene (COI) using a modified forward primer LepF1 (Hebert et al., 2004) and a modified reverse primer EPT-long-univR (Hajibabaei et al., 2011). Purified products were quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit on a fluorimeter (FilterMax F3, Molecular Devices). Quantified products were then pooled in equimolarity and sent to the GIGA Genomics platform (ULg) for sequencing on an ILLUMINA MiSeq V2 benchtop sequencer.

Raw sequences were processed using a script consisting of a mix of the Fastx-toolkit ([http://hannonlab.cshl.edu/fastx\\_toolkit](http://hannonlab.cshl.edu/fastx_toolkit)) and the Usearch function (Edgar, 2010). Processed sequences were then compared with published sequences in the BOLD databases (Ratnasingham and Hebert, 2007) where we considered sequences that had identity scores of  $\geq 98\%$  to be positive matches.



**Fig. 1.** The known distribution of the Kam dwarf hamster outlined in dark grey (Smith, 2016) and our observation of the species in southern Mongolia highlighted by the star.

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