

# **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.

**Keywords:** conservation, methodology, species distribution, rare and elusive species

## 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).

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 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.

## Material and Methods

The observations reported here were made in the Tost Mountains in southern Mongolia (43° 37' N, 100° 12', Figure 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°C in winter and +35°C in summer and the altitude ranges between 1,800 and 2,500 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 × 6.35 × 16.5 and 7.6 × 9.5 × 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.

## Results

Our genetic analyses showed that 5 of 142 rodents captured and samples during 5,040 trap-nights were the Kam dwarf hamster with up to 99% homology with the sequences in the BOLD database. This extends the range of the species by about 500 km to the north of its known distribution in China (Figure 1). All five of the hamsters appeared to be in good condition and we captured both female and male hamsters (Table 1). Other *Cricetulus* species captured during this study were the long-tailed dwarf hamster (*Cricetulus longicaudatus*) and the grey dwarf hamster (*Cricetulus migratorius*) with 60 and 30 individuals captured, respectively. We have added the COI sequences of the 5 Kam dwarf hamsters encountered during this study to GenBank (number XXXXX).

## Discussion

Knowledge on the distribution and abundance of animals is crucial for management and conservation (Sinclair et al. 2006). Here our observation of the Kam dwarf hamster in southern Mongolia not only extends the range of the species about 500 km to the north and shows that it is not endemic to China but also illustrates the benefits of genetic approaches when studying rare and elusive species or species that are difficult to identify or does not obtain public or scientific attention (González and Barbanti Duarte 2007, Waits 2013). In fact, genetic approaches may be the only technique available to monitor small animals that may not trigger cameras or are difficult to identify by eye (Waits 2013) and we suggest that the knowledge on such animals can be enhanced significantly by using genetic tools. However, the taxonomy of *Cricetulus* is debated (Nuemann et al 2006, Smith and Xie 2008) and we stress the importance of proper sampling techniques and analyses as well as clear information to which genetic sequences the data were compared to avoid sample contamination and analytical pitfalls that may result in wrong conclusions (Waits and Paetkau 2005).

Our observation of the Kam dwarf hamster 500 km north of its known distribution suggest that the hamster occurs also in other areas outside its known distribution but has not been detected because of ineffective survey methods or lack of sampling effort (sensu McDonald 2013). Similarly, we suggest that this may be a common pattern for a number of rare and elusive species that still remain to be detected outside their presumed ranges. Here recent development in camera trapping and genetic techniques provide us with new tools to monitor animals that have so far been difficult to study (González and Barbanti Duarte 2007, Waits 2013) and we predict that we will see an increase in the knowledge on rare and

elusive species in the near future that will be important for the conservation of these species.

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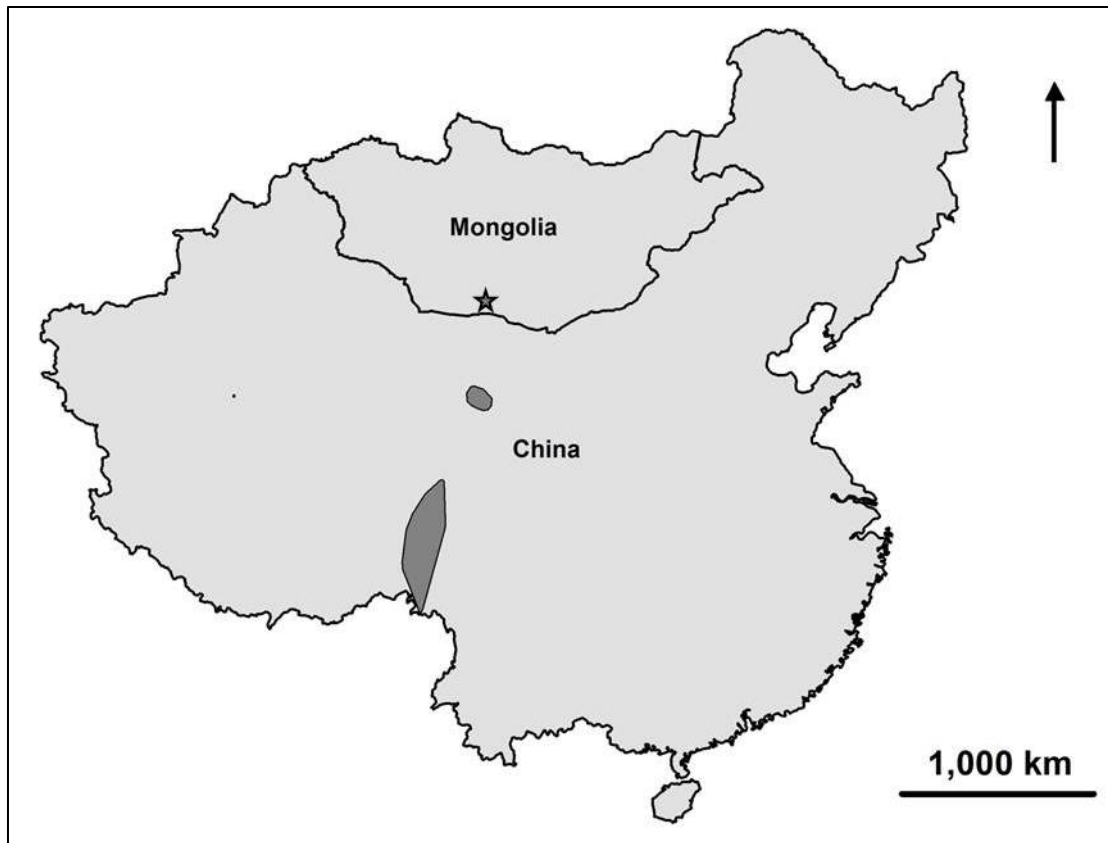
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**Table 1.** Capture date, sex, and age of the Kam dwarf hamsters captured in the Tost Mountains in Southern Mongolia in spring and summer in 2012 and 2013.

<b>ID number</b>	<b>Capture date</b>	<b>Sex</b>	<b>Age</b>
2012-23	15 June 2012	F	adult
2012-34	20 June 2012	M	adult
2013-06	28 May 2013	F	adult
2013-08	29 May 2013	M	adult
2013-85	18 June 2013	F	adult



**Figure 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.