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Detecting Domestic Dog (*Canis lupus familiaris*) in Diet of Persian Leopard (*Panthera pardus saxicolor*) Using DNA Tools

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Short Communication

Abstract

Persian leopard (*Panthera pardus saxicolor* Pocock, 1927) is distributed in different ecosystems, and Golestan Natural Park is one of the main habitats of the species in northeast Iran. A precise knowledge of diet is a critical part of conservation plans for endangered species. *P.pardus* has varied diet and preys on various items such as wild ungulate and livestock. In this report, we discovered an unusual prey in diet of *P. pardus* using genetic analysis. Domestic dog (*Canis lupus familiaris*) has been identified in diet of *P. pardus* through the use of sequencing the control region of mtDNA in Golestan National Park. This is the first record of domestic dog in the diet of *P. pardus* in Iran, although many different preys have been detected in other studies. Existence of dog in leopard's diet is remarkable while many favourite preys of leopard exist in the same habitat.

Keywords: Diet analysis, Carnivora, Predation, mtDNA, Golestan National Park

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1. Introduction

The leopard (*Panthera pardus*); which is the best adaptable felid in Panthera genus, exists in a wide range of climate types (Beer *et al.,* 2005; Bailey, 1993). Although Persian leopard (*Panthera pardus saxicolor* Pocock, 1927) is widely distributed and occupies large territories in Iran, it is classified as an endangered subspecies by IUCN (2010). Furthermore, the animal's natural preys are mainly wild ungulates that have become very rare (Kiabi *et al.,* 2002; Sanaei and Zakaria, 2011). However, there are remarkable populations of leopard's preys in some protected area such as Golestan Natural Park.

The diet analysis can improve our knowledge about ecosystem's functions; and especially it becomes more essential in the context of studying endangered species (Valentini *et al.*, 2008). A precise knowledge of the diet of species can disclose specific behaviours and interactions in the predations. DNA tools are very useful for species identification when only hair, feces or urine left behind by animals are available (Valentini *et al*., 2008). Morphological traits approach for identification of species has numerous difficulties; for instance, misidentification of a taxon because of the plasticity of studied traits, or ambiguous taxa (Knowlton, 1993). DNA tools can be very practical to identify elusive and endangered carnivores in particular when only biological remains are available (Valiereet *et al*., 2003; Sugimoto et al., 2006). In this paper, we present the result of DNA analysis on remains of the leopard in Golestan National Park of Iran.

2. Materials and methods

2.1. Golestan National Park

Golestan National Park is the first national park in Iran, located among Golestan, Khorasan and Semnan Provinces in northeast Iran (Majnoonian, *et al.,* 1999). The park lies between of 37° 16' 43"N to 37° 31' 35"N and 55° 43' 25"E to 56° 17' 48"E, and its area is more than 91000 hectares (Fig. 1). Golestan Natural Park is one of the most prominent habitats of Persian leopard in Iran, and the largest skull as well as the largest specimens in terms of body weight have come from this area (Tajbakhsh and Jamali, 1995; Kiabi *et al*., 2002).

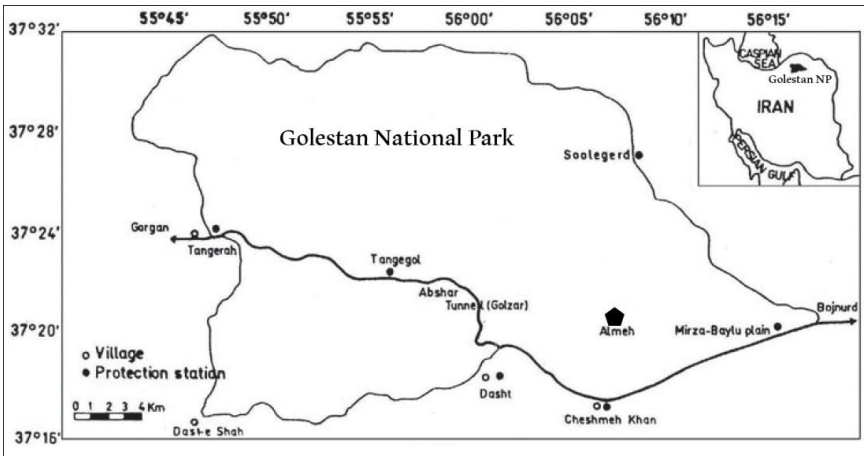

Figure 1. Location of Golestan National Park

The park covers forested areas in the east, semi forested to bushlands in the center and semi desertic areas in the east. Natural and scenic beauty together with high biodiversity are features of the park (Fig. 2).

Figure 2. Almeh Valley, Golestan National Park

2.2. DNA extraction and PCR amplification

Samples of the prey remains were collected and preserved using ethanol 96% for 24 hours, then alcohol was removed and the samples were kept in silica gel until DNA extraction (Fig. 3). Whole genomic DNA was extracted from remnants of prey using Bioneer DNA extraction kit (Takapozist) following the manufacturer's

protocol. The Control region of mtDNA was PCR-amplified using primers including L15995 (Taberlet *et al.*, 1994) and H16498 (Fumagalli *et al.*, 1996).

Figure 3. The prey remains of *P. pardus* in Golestan National Park.

The PCR reactions were performed in a final volume of 25 µl containing 1 µl of DNA, 1 µl of each primer, and 22µl of water using the Bioneer PCR kit. Amplifications were performed under the following conditions: initial denaturation (5 min at 94°C); then for 35 cycles, denaturation, 95°C, 30 s; annealing, 54°C, 30 s; extension, 72°C, 60 s; a final extension, 5 min, 72°C. PCR product was purified using the Bioneer kit (Takapozist) following the manufacturer's protocol. Purified PCR product was analyzed on an ABI 3130 automated sequencer. The sequence was edited for correction with the SecScape v2.6 software (Applied biosystems), and then BLAST software was used to determine unknown prey's mtDNA sequence in GenBank. The sequence was deposited in GenBank (Accession Number KF553665).

3. Results and Discussion

The DNA of samples were successfully extracted with high quality and quantity, and the accuracy of sequences were adequate. The BLAST of unknown prey's sequence in GenBank revealed that it was domestic dog (*Canis lupus familiaris)*. mtDNA control region could identify dog (Sindicic *et al*., 2011), and many studies have demonstrated that there is no common haplotype of mtDNA control region which have been shared by wolves and dogs (Okumura et al., 1996; Vila and Wayne, 1999; Randi *et al.,* 2000). Thus, there is no uncertainty between wolf and dog, and we concluded that our sample was definitely domestic dog.

This is the first observation of domestic dog in diet of leopards. Various preys have been recorded in diet of leopards such as birds, rodents (Ott, 2004 cited in Hayward *et al*., 2006), catfish, hares (Shenton and Uys, 1965 cited in Hayward *et al.,* 2006), giraffe calves and adult male eland (Hirst 1969; Kingdon 1977; Scheepers and Gilchrist 1991 cited in Hayward et al. 2006). In Iran, according to Sanaei. (2007); *P. pardus* attacked a local shepherd in 2001, a dog in 2002, also a

goat and sheep in 2002-2003. Sharbafi (2011) analyzed diet of leopards in Golestan National Park and revealed that wild boar (*Sus scrofa*) was the most frequent prey, although Wild sheep (*Ovis vignei*), wild goat (*Capra aegagrus*) and livestock were detected in the diet of leopard as well.

4. Conclusion

Although leopards have a varied diet (Mills and Hsrvey, 2001; Hayward et al. 2006); dog had not been recorded before in diet of leopards. Our result is striking because in spite of the fact that many favourite preys of leopard such as wild ungulates exist in Alme Valley (37° 21' 07.47"N and 56° 10' 54.77"E; Fig. 2) of Golestan National Park (Fig. 1), *P. pardus* hunt on domestic dog as well. Furthermore, this area which is near the villages locates in the buffer zone of Golestan National Park and has big flocks of sheep and goat; therefore, there is no shortage of prey in this valley. Other notable matter is that *P. pardus* in this instance has preferred domestic dog as a small size carnivore rather than wild and domestic ungulates that are larger and easier to catch. This finding adds to our understanding of diet of *P. pardus*, and we believe that our method could probably be usefully employed in more extensive analyses of *P. pardus* diet.

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