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Conservation Biology of the Caracal (*Caracal caracal*) in Iran: action plan and conservation genetics

Mestrado em Biologia da Conservação

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Abstract

The caracal is the third biggest of the eight felids present in Iran after the leopard and the cheetah. Its habitat is mostly arid areas and it has a key role in the control of rodent populations. The conservation status of caracal populations is not clear across most of the range, but the Asiatic population is threatened and listed in CITES appendix I. The main threats for the caracal are habitat loss and human conflict due to frequent livestock attacks. Lack of knowledge about the caracal and the unknown impacts of the conflict on its population may drive the species to an endangered situation. The main goals of this study are to review the biology, conservation status and conflict with humans of the caracal in Iran, suggest practical measures to reduce such conflict, and conduct a preliminary genetic study. The suggested conservation measures include improving the livestock husbandry system to avoid attacks, predation and thus conflict, education of the local people about carnivores and their importance, and promoting their participation in conservation actions. Genetic diversity and structure in Iran was analyzed based on 24 samples from six provinces. Mitochondrial DNA (mtDNA) and four microsatellite markers were used to assess genetic variation and the results indicated low mtDNA diversity, possibly due to a historical bottleneck of a reflection of the colonization (founder effect and serial bottlenecking) of the Middle East from Africa, or alternatively to the low mutation rate of the genes studied. Forensic genetic analyses of hair and saliva from wounds of livestock carcasses to identify predator species is also recommended to provide insight on the dimension of the conflict. The conservation genetics study initiated here is a starting point for subsequent work with additional samples from across Iran and more microsatellite markers.

Keywords: Caracal, Conservation genetics, Conflict, Action plan, Iran

Resumos

O caracal é um gato de tamanho médio e o terceiro maior entre oito felinos no Irão, depois do leopardo e da chita. O seu habitat é principalmente em zonas áridas e a espécie tem um papel fundamental no controlo das populações de roedores, e uma reputação de predar em animais domésticos. O caracal tem hábitos solitários, nocturnos, e a sua reprodução é anual. A distribuição deste animal é extensa em quase toda a África, com excepção dos desertos do Saara e do Namib, e na Ásia ocorre no Médio Oriente até à Índia no leste. O caracal como espécie não está classificado como ameaçado pela IUCN, mas as populações asiáticas estão listadas no apêndice I e as populações africanas no apêndice II da CITES. Na verdade, o estatuto das populações de caracal em grande parte da sua área de distribuição é desconhecido, sendo esse também o caso no Irão. As principais ameaças para o caracal são a perda de habitat, a mortalidade rodoviária e os conflitos com os agricultores e pastores por observados e supostos ataques a animais de criação. O desconhecimento sobre a ecologia e estatuto populacional do caracal e sobre o impacto do conflito com os humanos sobre os efectivos de caracal podem ocultar um declínio acentuado das populações da espécie que ainda não foi detectado.

Os principais objetivos deste estudo são rever a biologia, estatuto de conservação e conflito com humanos do caracal no Irão, sugerir medidas prácticas para a redução desse conflito, e realizar um estudo genético preliminar da população local de caracais.

O caracal no Irão está aparentemente presente na maioria do país com excepção de algumas florestas a norte e nos desertos extremos de Lout e Kavir no centro. A informação sobre o estado da população de caracal no Irão é escassa mas os relatórios anuais do Departamento do Ambiente revelam uma elevada mortalidade associada ao conflito com humanos, e consequentemente a espécie foi categorizada como de alta prioridade para a investigação e conservação. Uma revisão da informação disponível e dados por mim recolhidos permitem propor algumas medidas prioritárias para a conservação do caracal no Irão e que também deverão beneficiar outros carnívoros selvagens. Alterar as prácticas de alojamento dos animais de criação, promovendo a segurança dos celeiros e dos rebanhos, é essencial para reduzir os ataques e perdas para predadores. Outra acção essencial é educar a sociedade em geral para diminuir a atitude negativa relativamente aos carnívoros e envolver a população local em acções de conservação, informando sobre o valor da vida selvagem e os benefícios a ela associados. Um plano nacional de conservação com a criação de uma plataforma web de fácil utilização para reunir os registos de observação de caracais e outras espécies é crucial para motivar o público para a conservação.

Este primeiro estudo genético sobre o caracal no Irão procurou fornecer uma visão preliminar da diversidade genética, estrutura populacional e filogeografia. Foram testadas 24 amostras provenientes de seis províncias usando dois genes do DNA mitocondrial (NADH5 e citocromo b) e quatro microssatélites. Os resultados sugerem uma baixa diversidade no DNA mitocondrial, possivelmente devido a uma contração demográfica histórica ou reflexo da colonização (efeito

fundador e perda de diversidade na expansão) do Médio Oriente a partir de África, ou alternativamente consequência da baixa taxa de mutação dos genes estudados.

Estudos futuros em genética de conservação do Irão devem revisitar as questões abordadas nesta tese pela primeira vez (diversidade genética e filogeografia) com mais amostras e com melhor cobertura geográfica de todo o país. Adicionalmente, outros vectores de trabalho em genética de conservação seriam: i) análises forenses de pêlos e saliva em feridas em animais de criação causadas por predadores para identificar a espécie ou espécies responsáveis pelos ataques para uma mais correcta quantificação do impacto do caracal e da dimensão do conflito, e ii) avaliar o efeito das auto-estradas e estradas na demografia e conectividade genética das populações de caracal, visto que estudos anteriores de modelação de adequabilidade do habitat inferiram um impacto negativo das estradas na selecção de habitat pela espécie. O trabalho genético aqui iniciado é um ponto de partida para tais estudos em genética das estradas que podem utilizar os microssatélites já testados, aos quais se tem que adicionar outros para aumentar o poder analítico.

Como conclusão, o caracal aparenta uma baixa diversidade genética no Irão, tal como a chita com quem partilha habitats similares em zonas áridas. A importância de conservar ambas as espécies deve ser transmitida como crucial para a preservação das áreas selvagens onde elas persistem. Elevar o perfil do caracal, também tirando partido da sua beleza estética, aumenta o número de espécies carismáticas e emblemáticas para uma protecção global e generalizada dos ecossistemas nas áreas secas do Irão, facilitando a implementação de um plano de acção nacional de conservação.

Palavras-chave: Caracal, genética da conservação, Conflito, plano de ação, Irão

Chapter 1

General introduction: Caracal biology, ecology, taxonomy and conservation

1.1. Target species

The caracal *(Caracal caracal)* is a slender, long-legged, medium-sized felid carnivore that belongs to the caracal lineage, which includes the caracal, African golden cat (*Caracal aurata)*, and serval (*Leptailurus serval)*. The caracal is known for its extraordinary ability to capture birds by jumping 2 meters or more into the air (Fig 1) (Sunquist and Sunquist 2002). This cat has an affinity for dry habitats, being an important member of the arid fauna with a key role in the control of small and medium-sized mammals, particularly in areas where large carnivores disappeared. However, the caracal often attacks livestock and poultry near human settlements. This behavior causes a conflict with people that threatens its persistence in many areas of its range.

Figure 1. Caracal in the wild (Yazd, Iran) with winter fur ©Hasan Moghimi

1.2. Biology

1.2.1 Size

The caracal in Asia is smaller and in arid areas has a paler fur than those in sub-Saharan Africa, and females are markedly smaller than males (Stuart and Trever 1982; Nowell and Jackson 1996). Body mass range in South Africa is 7.2-19 kg (mean 12.9 Kg) for males and 7-15.9 kg (mean 10 Kg) for females (Stuart and Trever 1982), while in Israel the average weight of male caracal is 9.8 kg and the average female weight is 6.2 kg (Nowell and Jackson 1996), although an individual with 25kg has been reported from western Iran (Moqanaki, et al. In press).

1.2.2 Morphology

The caracal is a slim cat with longer hind legs than forelegs so that a standing caracal seems taller at the rump than at the shoulders. The structure of its hindquarters enable the caracal to make spectacular leaps. The caracal coat has a light brown, gray or reddish color, which is more whitish in the chest, belly and the undersides of legs and spotted with pale red markings that vary among individuals. They have tufts of stiff hairs on the paws between the pads as an adaptation to live in arid habitats for moving through soft sandy ground. The caracal has a broad face and tall triangular ears that are topped by a long black tuft of hair. The ears tufts hang down like tassels in older animals. On the face, a dark line from the center of the forehead turns down to near the nose and another line starts from the inner edge of the eyes to the nostrils (Sunquist and Sunquist 2002).

1.2.3. Reproduction

The reproduction season in caracal is probably year-round. Estrus lasts 1-3 days, its cycle is 14 days and the estrus condition in absence of fertile copulations persists for 3-6 days. In captivity, gestation lasts 78-81 days and litter size is 1-3 (mean 2.2) kittens (Bernard 1987; Nowell and Jackson 1996; Sunquist and Sunquist 2002). Age of independence is 9-10 months and females can have their first pregnancy when 14-16 months old, while males are sexually mature when 12.5-15 months old (Bernard 1987; Nowell and Jackson 1996; Sunquist and Sunquist 2002). The oldest captive female that could give birth was 18 years old. Longevity in captivity is up to 19 years (Nowell and Jackson 1996).

1.3. Ecology

1.3.1. Activity pattern

Caracals are usually nocturnal, being active from dusk to dawn and early morning and resting during the day in dense vegetation or rock crevices in areas where arid habitats are predominant, but are often observed in daytime, particularly in protected areas. The activity pattern can be influenced by the environmental temperature and activity pattern of their prey (Nowell and Jackson 1996; Sunquist and Sunquist 2002; Singh, et al. 2014).

1.3.2. Movement and home range

In South Africa, male home range size varies between 15.2 to 312.6 km^2 and for females between 4.31 to 7.39 km^2. In Asia home ranges may be larger as the home ranges in Israel for males are 221± 1.32 km^2 and for females 57 ± 55 km^2 (Nowell and Jackson 1996; Marker 2005). Still in Israel, it has been reported that males travel 10.4± 5.2 km daily while females move 6.6±4.1 km (Weisbein and Mendelssohn 1990). Sapozhenkov (1960) reported a nocturnal travel by an individual of up to 20 km in Turkmenistan.

1.3.3. Habitat

The caracal is able to conceal itself even in scarce vegetation on bare ground by laying down. Although habitat preferences are typically associated with dry lowlands, including savanna, coastal scrub and semi-arid woodlands, or rocky areas, caracals have been also recorded in evergreen and montane forest and at altitudes up to 2500 m (exceptionally 3300 m) in Ethiopia. Singh, et al. (2014) suggested that forest and rugged habitats in semi-arid landscapes of India provide increased foraging opportunities.

1.3.4 Diet

The caracal mainly preys on mammals with less than 5 kg, such as rodents, hares, hyraxes, and on birds, but also reptiles and insects and occasionally larger animals such as goitered gazelle, wild sheep and wild goat are part of its diet. Scavenging behavior is also reported. In some parts of the caracal distribution the species attacks livestock, particularly in areas close to human settlements, and the persecution by humans in return is a major driver of decline of caracal populations, particularly in Asia (Stuart and Trever 1982; Nowell and Jackson 1996; Sunquist and Sunquist 2002).

1.4. Biogeography

1.4.1. Current distribution

The caracal is widely distributed in most of Africa except in the true deserts of Sahara and Namib, and in the Congo and equatorial forest belt of western and central Africa. Beyond Africa, the caracal is found in Asia from Turkey in the west and Arabic peninsula in the south-west to India in central Asia and to Turkmenistan in the northern edge of the species range (Nowell and Jackson 1996; Sunquist and Sunquist 2002). The caracal is native in these countries: Afghanistan; Algeria; Angola; Benin; Botswana; Burkina Faso; Cameroon; Chad; Democratic Republic of the Congo; Côte d'Ivoire; Djibouti; Egypt; Eritrea; Ethiopia; Gambia; Ghana; Guinea; Guinea-Bissau; India; Iran; Iraq; Israel; Jordan; Kazakhstan; Kenya; Kuwait; Lebanon; Lesotho; Libya; Malawi; Mali; Mauritania; Morocco; Mozambique; Namibia; Niger; Nigeria; Oman; Pakistan; Saudi Arabia; Senegal; Somalia; South Africa; South Sudan; Sudan; Swaziland; Syrian Arab Republic; Tajikistan; Tanzania, United Republic of; Togo; Tunisia; Turkey; Turkmenistan; Uganda; United Arab Emirates; Uzbekistan; Western Sahara; Yemen; Zambia; Zimbabwe (Fig 2) (The IUCN Red List of Threatened Species 2015).

Figure 2. Global distribution of Caracal caracal; (The IUCN Red List of Threatened Species 2015)

North African populations are disappearing, but caracals are still abundant in other African regions. Conversely, Asiatic populations are less abundant and more sparsely distributed than those of Africa and many are under threat (Breitenmoser-Wursten, et al. 2008; Sunquist and Sunquist 2002). The historical range of caracal and cheetah were similar and seemingly coincide with the availability of small desert gazelles with 15-30 kg of weight as prey. In some areas wild prey have been replaced by livestock, which is a good alternative food for caracal (Sunquist and Sunquist 2002).

1.4.2. Historical movements

During the first migration wave of felines around 10 MYA, the precursor of the caracal lineage spread trans-continentally from Asia into Africa between 10-8 MYA. (Fig 3). During this period of time, sea level lowered to 60 meters below modern levels creating a land bridge between Africa and the Arabic Peninsula in the southern Red Sea. In Africa, the serval lineage diverged 5.5 MYA from the ancestor of caracal and African golden cat. Around 2-3 MYA, the two latter lineages separated and expanded in Africa. The African golden cat stayed in Africa but the caracal migrated back to Asia. It is possible that this caracal migration was contemporary with the second migration wave of felines that occurred 4-1 MYA in the late Pliocene when sea level again dropped and East Africa and Arabian were again connected (Johnson, et al. 2006; O'Brien and Johnson 2007; Werdelin, et al. 2010).

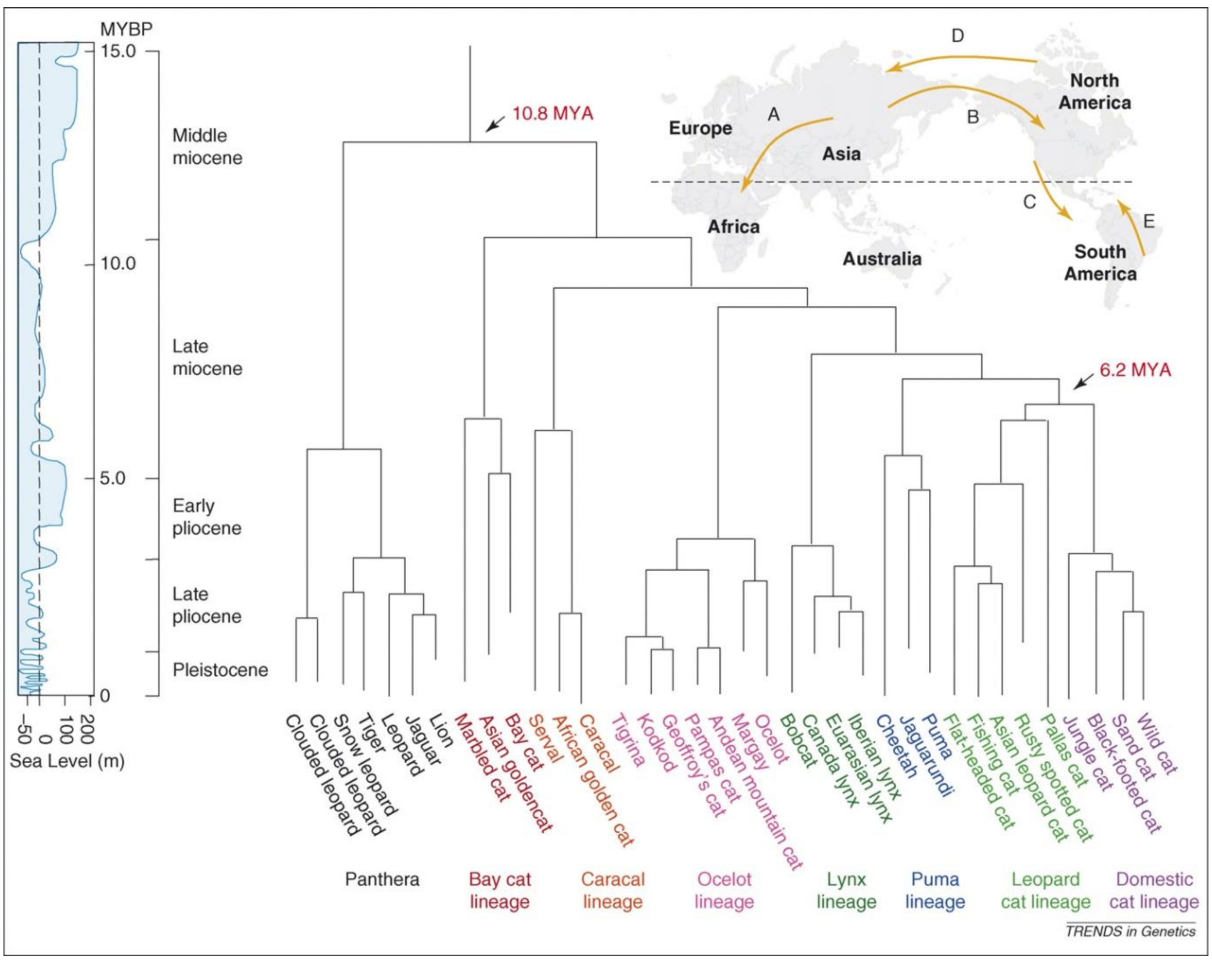

Figure 3. Molecular phylogeny of Felidae and historical global sea levels; arrows A-E show the migration routes of felids (O'Brien 2008)

1.5. Taxonomy

The caracal (currently *C. caracal* Schreber, 1776) has been classified variously with *Lynx* and *Felis* in the past, but molecular evidence supports a distinct genus. The caracal is closely related to the African golden cat (*C. aurata* Temminck, 1827) and the serval (*L. serval* Schreber, 1776) (Johnson, et al. 2006). The ancient root of this lineage is supported by 3-5 MYA fossils and was estimated to have branched off at 8.5 MYA from the other Felidae, with the serval being basal to the other two species.

Although the taxonomy and geographical distribution of putative caracal subspecies are not well known, the IUCN SSC Cat Specialist Group recognizes eight subspecies (Fig 4) (Wilson and Reeder 2005; Breitenmoser-Wursten, et al. 2008; www.itis.gov).

C. caracal algira (Wagner, 1841); North Africa

C. caracal caracal (Schreber, 1776); east and South Africa

C. caracal damarensis (Robert, 1926); Namibia

C. caracal limpopoensis (Robert, 1926); Botswana

C. caracal lucani (Rochebrune, 1885); Gabon

C. caracal michaelis Heptner, 1945; Turkmenistan

C. caracal nubica (Fischer, 1829); Ethiopia, Sudan

C. caracal poecilotis Thomas and Hinton, 1921; western Africa

C. caracal schmitzi (Matschie, 1912); Israel, western Asia, Iran, Arabian Peninsula, Pakistan, India

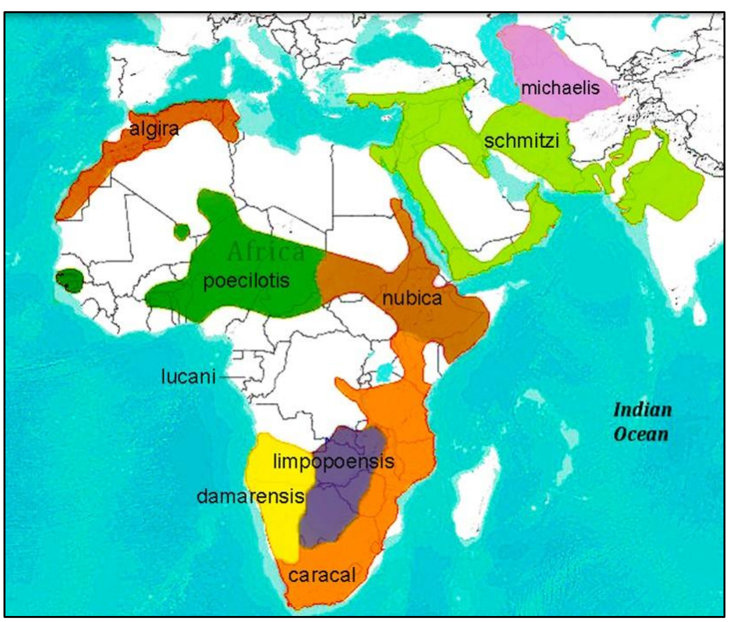

Figure 4. Distribution of subspecies of caracal in the world.

1.5.1. Names

Caracal [kârâkâl], desert lynx, African lynx, Persian lynx (English); karakulak, step vasagi (Turkish); Siahgush [black-ear], Yuzou [cheetah-shape] (Persian: Iran); Ajal, Anaq al ardh, Washag (Arabic); Warsal, bousboela, Mousch, Nouadhrar, Aousak (Berber: Algeria); Psk garh gol (Dari: Afghanistan); Harnotro [killer of blackbuck] (Kutchi dialect of Gujarati: India); Wüstenluchs (German) Степная рысь (Russian), lince do deserto (Portuguese), Lince africano (Spanish); Gazelle cat (in Africa); Rooikat, lynx (Afrikaans: South Africa); Delg ambassa (Amharic: Ethiopia); Djime taikorlo (Baguirmien); Soumoli (Bornouan); Guette anasa (Chad); Filiki (Djerma); Pyaberi (Gourmanche: Burkina Faso); Messo (Hausa: Sahel); !hab (Hei//kum Bushman: Namibia); Simbamangu (Kiswahili); =ui (Ju/'hoan Bushman: Botswana, Namibia); Mwai (Luo: Kenya, Uganda); Indabutshe, intwane (Ndebele: Zimbabwe); Ayuku (Ovambo: Namibia); Safandu (Peul/Foulbe); Thwane (Setswana: Botswana); Hwang, Twana (Shona: Zimbabwe); Gedudene, Maharra (Somalia); Daga (Toucouleur: northwest Africa); Ngada (Xhosa: South Africa) (Nowell and Jackson 1996; Ziaei 1996).

1.6. Conservation

The IUCN lists the caracal as a species of least concern, but the fact is that the status of most populations across the range is unknown and many may be threatened. Accordingly, other sources list the species as threatened since habitat loss is occurring systematically over most of the African continent (Sunquist and Sunquist 2002). Caracal populations in Asia are in general even rarer than those in Africa, due to habitat loss and human conflict, and thus of greater concern (CITES Appendix I).

Because the caracal is capable of preying on small domestic livestock, it is often in conflict with human communities. A study in the Cape Province of South Africa reported an annual loss of up to 5.3 domestic stock animals per 100 km^2 that could be attributed to the caracal (Brand 1989). Therefore, farmers in South Africa traditionally kill caracals; for example, between 1931 and 1952 around 2,219 caracals per year were killed in the Karoo during population control operations. Similarly, farmers in Namibia killed about 2,800 caracals in 1981 (Stuart and Trever 1982; Nowell and Jackson 1996).

1.6.1. Caracal in Iran

Of the nine recognized caracal subspecies, two of them are described to occur in Iran: *C. c. schmitzi* (Matschie, 1912) (data deficient) and *C. c. michaelis* (Heptner, 1945) (endangered) (www.cites.org). The caracal population in Iran is threatened by human activity, habitat loss and conflict with shepherds, leading to significant mortality with an unknown impact on its abundance. Because conserving the caracal across the country is challenging, it is crucial to investigate the population genetic and phylogeographic structure of the species in Iran to eventually define and allocate areas and corridors that should be priorities for conservation.

The caracal has also the potential to join the cheetah and leopard as flagship species in Iran, with an umbrella role for the protection of wildlife and biodiversity in general.

1.7. Literature sources

During the last three decades literature about the caracal has increased. Sunquist and Sunquist (2002) discussed the global status of the species and reviewed previous studies on its biology, ecology and distribution. Other local studies have been conducted, mostly in southern Africa (Shortridge 1934; Roberts 1951; Smithers 1971; Rosevear 1974; Kingdon 1977; Visser 1978; Stuart 1981, Joubert, et al 1982; Bernard 1987; Rowe-Rowe 1992; Avenant and Nel 1998), in Saudi Arabia (van Heezik and Seddon 1998; Stuart and Stuart 2007), Turkey (Ilemin and Gurkan 2010), Israel (Weisbein and Mendelssohn 1990), India (Mukherjee, et al. 2004), Iraq (Thalen 1975) and Iran (Farhadinia, et al. 2007; Ghoddousi, et al. 2009).

Chapter 2

The caracal in Iran: review of the biology and conservation status

2.1. Introduction

The first studies on Iranian caracals suggested the presence of two subspecies in the country: *C. c. michaelis*, and *C. c. schmitzi* (Ellerman and Morrison-Scott 1951; Karami, et al. 2008). The earliest scientific reports on the species are for the provinces of Khuzistan, Kerman, and Tehran (Lay 1967; Etemad 1985; Harrison and Bates 1991). Later, Farhadinia, et al. (2007) gave an account on the population in the Abbas-abad Naein Reserve, Esfahan province, and Ghoddousi, et al. (2009) surveyed the status of the caracal in the Bahram-gur protected area, Fars province. More recently, conflict of caracal and local people have been studied in southern Khurasan and in Esfahan (Hassan-Beigi, et al. 2013; Hassan-Beigi, et al. In Prep).

Besides some genetic studies that have clarified the phylogenetic placement of the caracal within the Felidae (e.g. (Janczewski, et al. 1995; Johnson and O'brien 1997), there is a lack of molecular systematics and phylogeographic data on the caracal and the reality of the two subspecies described for Iran has never been genetically assessed.

2.2. Caracal in history

The caracal has been a constant presence in the culture and art of Iran. Several historical references show the role of caracal as a hunting animal in the company of royal and noble hunting groups. There are unfortunately no known text references left from before the Arabic presence in Iran ended (7th century AC), but several rhytons have been found across Iran in which the caracal is represented. Figure 5 shows a silver rhyton from the 1st century AC representing a caracal catching a rooster (Golshan 2011).

Persians trained caracals and cheetahs to hunt birds and mammals and passed this knowledge and tradition to India. Historical documents explain how to catch an adult caracal and train it for hunting. Caracals were mostly used to hunt rabbits, hares and birds like francolin, duck, goose and crane. Like the cheetah, they were seen as faithful and reliable pets to their owners.

In the past, when the Asian lion *(Panthera leo persica)* was still present in Iran, the caracal was called “Shäter- shir”, which means lion’s vanguard. This association may have originated from the fact that caracals were known to follow lions to feed on their prey remains and even to hassle them so that they leave the carcasses of the preys, but more probably stems from the belief that the appearance of the caracal near to livestock herds could sign the subsequent arrival of lions.

2.3. Biology and ecology

Caracals in Asia are comparatively smaller than those in Africa (Sunquist and Sunquist 2002), with a database for 19 individuals of mixed genders in Iran showing a range of 7.3- 13.6 kg (mean = 10.6), which is at the lower end of the global range of 8-20 kg mentioned by Sunquist and Sunquist (2002), but with caracal males in Iran possibly reaching weights of more than 20

Kg (Moqanaki, et al. In press). Body length range in Iran is between 61 and 108 cm, tail length between 18 and 34 cm, and shoulder height from 38 to 50 cm. Length of hair tufts on ears can reach 5-6 cm (Moqanaki, et al. In press). Etemad (1985) compared a skull from the British Museum with a database of Iranian caracals (table 1), this study being the only published one on the craniometrics of Iranian caracal.

Table 1. Skull morphology information for caracal (Etemad 1985)

Variables	Size range (mm)	Sample in British Museum (mm)
Condylobasal length	100-120	119.5
Zygomatic breadth	70-91	79.5
Length of bulla	40-52	23.3

A general survey on felids with 400 camera-nights yielded a total of seven caracal photos from three events. A total of 20 verified observations derived from interviewing 25 local people showed that caracals were mostly alone (80% of observations) confirming the solitary lifestyle (Farhadinia, et al. 2007). Reproduction is not well studied but according to Etemad (1985) mating occurs in winter and breeding in late winter. Farhadinia, et al. (2007) suggests that the birth peak in semi-arid areas might be in April. Reproduction is annual and litter size is mostly two kittens (six cases) but instances of three and five cubs were also observed (Farhadinia, et al. 2007; Karami, et al. 2008).

A study based on limited number of scats and direct observation in Bahram-gur Protected Area indicates that Cape hare, rodents and birds play a key role in the caracal diet. Noteworthy, no livestock predation was reported by local people, but the main threats to caracal were domestic herding dogs and road traffic (Ghoddousi, et al. 2009).

The caracal is a generalist feeder and its food diet is highly variable, including mammals, birds, reptiles and insects. Various rodents and hare, plus partridges, appear to be the main prey for caracal. There are nevertheless cases of uncommon prey in different areas of Iran: gray francolin (*Francolinus pondicerianus*), hedgehogs (family Erinaceinae), Rueppell’s fox (*Vulpes rueppellii*), stone marten (*Martes foina*), and gazelles such as chinkara (*Gazella bennetti*) and goitered gazelle (*G. subgutturosa*). (Farhadinia, et al. 2007; Karami, et al. 2008; Ghoddousi, et al.2009; Farhadinia, et al. 2012). Analysis of stomach content of a dead specimen in Naein, Esfahan, showed that the last meal contained insects, lizard and a small mammal (personal observation, unpublished).

2.4. Distribution

Of the two recognized caracal subspecies in Iran, the most widespread in the country is *C. c. schmitzi*, while *C. c. michaelis* may be restricted to the northeast near the border with

Turkmenistan (Ellerman and Morrison-Scott 1951; Etemad 1985; Nowell and Jackson 1996; Farhadinia, et al. 2007; Karami, et al. 2008).

Lay (1967) attempted to gather all known observation records of the caracal in Iran between 1876 and 1963, and the author only found evidence for the presence of the species in Khuzestan and Kerman, as reported by Blanford (1876) Birula (1912) and Siddiqi in (1961). Lay also found a skin in Tehran but it was from an animal shot in Khuzestan in 1959. Although the records did not confirm a wide distribution, Lay agreed with the range described by Ellerman and Morrison- Scott (1951) and that the caracal occurred more widely in Iran than suggested by the records (Lay 1967). Currently it is well established that the caracal is widely distributed in Iran and its distribution ranges from the temperate Kopet Dag plains in the northeast to the semi-arid mountainous woodlands of Central Zagros in the west, and from the Alborz forest in the north through the central extreme dry lands down to the semi-desert coast of the Persian Gulf in south. It is possibly absent only in the Caspian region, Iranian Caucasus, and extreme central deserts of Iran. In some regions, the caracal is the largest or top predator (Farhadinia, et al. 2007; Moqanaki, et al. In press).

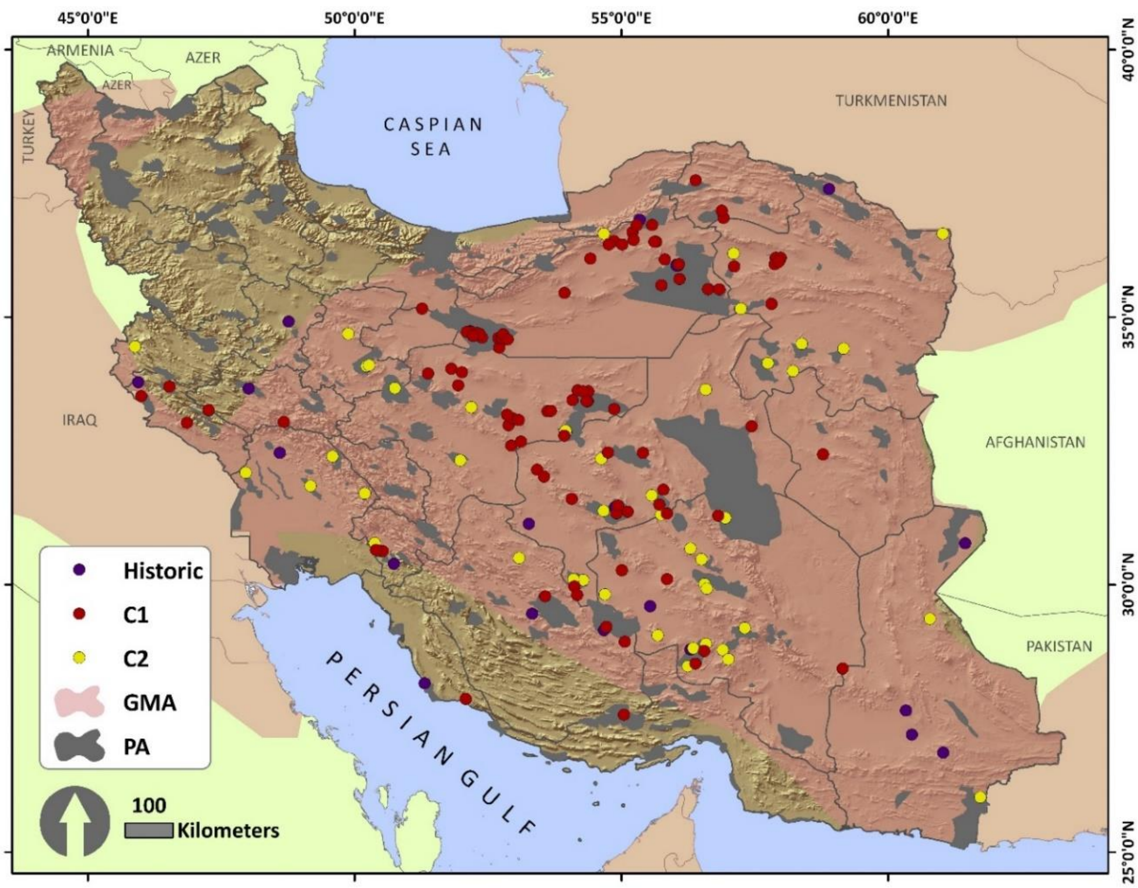

Figure 6. Distribution of caracal in Iran. C1: Hard fact data, C2: Confirmed data, GMA: Potential global area, PA: Protected areas (Moqnaki, et al. In press)

Moqanaki (in press) updated the historic and recent records of caracal in Iran (Fig 6) and collected data from about a total of 122 individuals in a camera trap survey conducted between 2003 and 2013 (Conservation of Asiatic Cheetah Project (CACP) and Iranian Cheetah Society (ICS)). This review confirmed the species in 11 protected areas, wildlife refuges or no-hunting areas, from five provinces: Esfahan, Semnan, North Khorasan, Fars, and Yazd. Sporadic observation records reported by media have also supported the present distribution map.

2.5. Habitat

The habitat of the Asiatic caracal has been summarized by Heptner and Sludskii (1992) as typically well vegetated or rocky lands that provide a good cover for hunting and shelter. During a study about the status of the caracal in the Esfahan Province, Farhadinia, et al. (2007) concluded that the dry mountains and hilly terrains are a suitable habitat based on observation records, while the species was never seen in flat areas except to cross them when moving between hills.

Ghoddousi, et al. (2009) surveyed the status of the caracal in the Bahram-gur Protected Area (BPA) in the Fars Province, southern Iran. Results of 22 days of field surveys and 97 trap-nights in two sites plus 50 hours spot-lighting over 750 km on road showed that among the five resident felids in this area (cheetah, leopard, wildcat, jungle cat and caracal), the caracal was the most common species. It was mainly found in the core zone of the protected area, with 325.76 km^2, due to a high density of prey such as Cape hare and various rodents, which were the main items in the caracal diet, based on a limited number of scat analyses. Caracal density was lower out of the core zone due to lower prey abundance and safety from humans (Ghoddousi, et al. 2009).

A status survey of cheetah and other carnivores using camera-traps has been performed in the Abbas-abad Wildlife Refuge, Esfahan province, central Iran (Farhadinia, et al. 2012). In this study, the presence of caracal was recorded and a habitat suitability model for the species has been constructed. By using 23 points in the study area and a neighbor area (Siah-kuh Protected Area), the analysis estimated that of a total of 5037.12 km^2 in the two areas, only 33.2% (1675.15 km^2) is suitable for caracal. The parameter altitude had a negative relation with the presence of caracal, probably due to a decrease in the density of wild sheep and chinkara at lower altitudes. Overall, the study showed that flat areas and short distance to roads were negative features for habitat suitability and previous reports of casualties due to traffic accidents confirm this result (Farhadinia, et al. 2012). The study also pointed to a direct relation between distance from villages and habitat suitability, meaning that far from human settlements is a better habitat for caracal. But the few number of records near villages may be also related to the fact that camera traps were mostly installed inside the protected area and less near its edge, as cheetah was the main target of the study. Indeed, a more recent study in the same study area found a significant presence of caracal around villages and human settlements due to the presence of livestock (Hassan-Beigi, et al. In Prep).

2.6. Conservation priorities

The caracal is a protected species according to the Iranian Department of Environment (DoE) laws and hunting them is prohibited, with an associated fine of 1500 € (50 million Rials). The conservation status of the caracal in Iran is poorly known and the last DoE report on the conservation of felids places the caracal, together with the wildcat and Pallas cat, as a priority among the 8 wild cats present in Iran (Table 2) (Mohammadi 2012).

Annual unpublished reports clearly show that there is strong human pressure on caracal persistence in the form of shooting, poaching, traffic-related mortality and taking cubs from wild. Although these reports do not offer much additional information, they highlight the urgency for conservation research on the caracal to inform measures and plans for protecting the species.

Main priorities listed in these reports are research, monitoring and raising awareness about the caracal across Iran. Crucial data is needed from habitat use studies using radio-tracking, diet studies, and taxonomic and genetic status assessments. Mohammadi (2012) underlines areas and provinces that should be priority targets for such investigations: Siahkuh and Abbas-abad (Esfahan Province), Bahram-gur, Gode Ghoul and Gare Tappeh (Fars Province), Turan and Kavir (Semnan Province), and Ilam Province.

2.7. Human-caracal Conflict

Human-wildlife conflict is a global and serious menace to the survival of many threatened species, with those more exposed to conflict more likely to go extinct. Conflict can result from livestock predation but also of the collision between human settlement, road and railway expansion and the requirements of space and habitat of wildlife. Conflicts such as the former lead to persecution, hunting, poaching and poisoning, while those of the latter type may encroach and fragment habitat to such an extent as to render unsustainable the population persistence of some species (Distefano 2005). Livestock growth also leads to competition with wild herbivores and sometimes to their persecution and exclusion by humans. In turn, lack of wild prey and the fact that livestock may be easier to capture and kill by predators often leads to conflict between humans and carnivores (Inskip and Zimmermann 2009).

Inskip and Zimmerman (2009) define human-wildlife conflict as ‘the situation that arises when behavior of a non-pet, wild animal species poses a direct and recurring threat to the livelihood or safety of a person or a community, and in response, persecution of the species ensues’. Human wildlife conflicts most commonly involve damage to crops or killing of livestock or game, and occasionally involve attacks on people. They are of particular concern when the animal persecuted in retaliation for these events is a threatened species.

The attitude of people towards wildlife is determined by social and economic factors, education, and religious, ethical and cultural views. So, tolerance toward wildlife damages can be increased by acting on factors such as educational level, extent of benefit that people gain from wildlife and amount of costs that they have to pay due to wildlife (Oli, et al., 1994). Participation of local communities and support for wildlife conservation depend on the value that people attribute to wildlife and biodiversity. Such value is also a function of the costs and benefits associated with the presence of wild species (Gusset, et al. 2009). Understanding which factors drive attitudes and tolerance in each context is key for choosing and targeting the most appropriate solutions, which may include mitigation to reduce losses, education to improve awareness, or generation of benefits and income to provide incentives for conservation (Zimmermann, et al. 2005). Among wildlife species, carnivores are one of the most prominent groups of animals involved in human-wildlife conflict. The history of conflict between human and carnivores is as old as the history of domestication and in many areas is currently a major threat for carnivore conservation. In this regard, modifying livestock husbandry to practices compatible with the presence of wild carnivores in those areas is a critical step (Breitenmoser, et al. 2005; Baker, et al. 2008; Boitani & Powell, 2012).

2.7.1. Case studies on the caracal in Iran

To evaluate attitudes of local people towards the caracal, I conducted two questionnaire surveys throughout the villages of two protected areas. For these surveys a questionnaire was designed consisting of five parts to assess the following aspects: personal and livestock information, knowledge about wildlife, conflict and its cost, attitude towards wildlife, conflict management approaches. To build trust, questions on personal information were made at the end of the interview. The results were:

2.7.1.1. Ark and Korang Protected Area, Birjand, South Khorasan (Hassan-Beigi, et al. 2013)

For some years no official records of caracal were reported from this region, but in 2013 unofficial news started about several caracals killed by local people, both inside and outside of this Protected Area. The results the questionnaire showed that there is a significant negative view of people concerning carnivores, especially about wolf and jackal, and 54% of the interviewed persons have had losses in their herds). Caracals were held responsible for 18.7% of the attacks in 2011-2013 and they occurred mostly inside barns (Figure 7). Although we could not find hard evidence for the presence of caracal in the area, local reports supported by different persons suggested that in 2011-2013 at least 3 caracals were trapped and killed. This may suggest a low caracal density in the area but this needs to be properly investigated.

A strategy suggested to local people to conserve their livestock could be to promote livestock safety. By visiting the barns and checking their safety it was obvious that improving them is practical and could be effective in reducing the attacks in barns. Nevertheless, the high number of conflicts in this area is probably caused by decrease in wild prey such as wild sheep due to illegal hunting by local people. The results of the questionnaire showed that attacks on livestock occurred frequently and resulted of lack of protection and attention to the barns or herds outdoors, and supported the notion that wild carnivores lived close to the human settlements and depended on the domestic animals for survival. The habit of leaving the carcasses of dead domestic animals, even those killed by predator attacks, near the villages also clearly contributed for the return of predators to the villages and to keep them in the vicinities. The use of guarding and shepherd dogs is not implemented by local people because of the associated expenses and given the low income obtained from agriculture, and also because of the risks of dogs being killed by wolf packs.

2.7.1.2. Abbas-abad Wildlife Refuge, Naein, Esfahan (Hassan-Beigi, et al. In Prep)

The study to evaluate the attitude and interaction between local communities and the caracal and other carnivores in the Abbas-abad Wildlife Refuge, western border of the Esfahan province. This area, with 4000 km^2, a high biodiversity and rugged topography, is considered one of the most suitable areas for the caracal and a major priority for research on the species (Mohammadi 2012). It contains a stable and varied prey basis for the nine species of carnivores occurring in the area, gray wolf, golden jackal, red fox, sand fox, Blandford's fox, striped hyena, caracal, wildcat, sand cat, and for cheetah and leopard individuals eventually crossing through. The Abbas-abad Wildlife Refuge has an arid climate with a long warm summer and its landscape is 44% mountainous and rocky crops, the rest being flat and often covered by saxaul (*Haloxylon* spp.) (Akbari & Jalalpor, 2011).

The study was conducted by interviewing environment experts, game guards and local people (livestock owners and non-owners) over 18 years old from the communities inside and around the Wildlife Refuge. Results showed a significant relation between livestock losses and negative views about carnivores in the whole area and across age and education categories, but young people had more positive opinions on carnivores. Almost half of the interviewed people had suffered losses of domestic animals to wild carnivores. Attacks mainly occurred during the night (80%) and inside barns (65.4%), highlighting again the inefficiency of the livestock protection methods. In most of the cases (85%), people did not see the attacking species and its identity was guessed by the 'hunting style', with definitions for each species being ambiguous. Data regarding caracal attacks followed the same general trend, with the species claimed to be involved in 12% of the attacks, but only one event (0.8%) could confirm this by direct observation of a caracal inside the barn (Figure 8). Only few people use insurance due to the bureaucracy of the process and 26% of people believe that they cannot prevent the carnivore attacks. The main conflict management suggestions from people were promoting the safety of barns and herds (35%) and killing the attacking carnivores (30%). Because in most of the events the attacking carnivore could not be identified, the latter respondents suggested killing any carnivore coming closer to the villages by shooting, poisoning, etc. Few people believed that asking help from the DoE game guards could be helpful, indicating that, although there is a seemingly positive relationship between game guards and local people, the DoE has been unable to find and implement a successful plan and working solution for the conflict; this particular issue is common to many protected areas in Iran. On the other hand, the option of increasing the safety of the livestock has had here positive experiences, with some people stating that after improving their barn safety they did not have livestock losses to carnivores anymore.

2.8. Conservation action plans for the future

The situation of the caracal in Iran is still unclear and there is presently no estimation about its population status and trend. It is apparent that the caracal is threatened in many areas but because it is not a charismatic animal in Iran, news about caracal deaths caused by humans do not appear in the media like in the cases of the cheetah, leopard and brown bear. Also, in general, people bury or destroy the carcasses of caracals after killing them because it is a protected species in Iran. Instead of communicating about caracal presence in the area to game guards and local DoE offices, local people often attempt themselves to remove the animal(s) from the area.

Listed below are some suggestions for short- and long-term measures for the conservation of the caracal until additional and more systematic studies are conducted on the species:

1. The caracal is mostly trapped and killed due to attacks on barns and herds, so a practical, low-cost, and straightforward action would be improving livestock barns and sheds by fencing the roofs and elevating the walls (Fig 9). As shown by the questionnaire in Abbas-abad, local people can be aware already that this method works and be receptive to it. The reason for most people to use traditional barns is due to the fact that many of them possess only a few domestic animals, and thus there is no incentive to improve the old barns. The gains in terms of predator conservation and safety of the local people and livestock justifies that improvements on barns and sheds are at least partly funded by the national or local governments.

2. Education of the society and general public about the unknown, but likely declining, status of caracal in Iran and its importance in the wild to control rodent populations. In particular, education must focus on issues known to be threats and causes of population decline, such as hunting and poaching of wildlife, particularly of potential prey as their rarity pushes wild carnivores closer to villages in search of food, poisoning of carcasses to kill predators, capturing cubs to sell them as pets, and indiscriminate shooting of carnivores approaching villages.

3. Training local people about wildlife and inform them on the value of biodiversity, specifically the role of carnivores in nature, and carrying out educational programs and meetings with shepherds and farmers about practical procedures to increase the safety of their livestock, such as abandoning the habit of leaving carcasses of dead animals in the field close to villages (Fig 10).

4. The caracal is capable of living in dry areas and there are not many large carnivores that have this ability. Together with the cheetah, which habitat is similar to the caracal, the importance of conserving both species should be transmitted as crucial for the preservation of the wild areas where they persist. Raising the profile of the caracal, also taking advantage of its aesthetic beauty, will add another flagship and umbrella species to the preservation of dry areas in Iran. In the meanwhile, building more water sources for wildlife inside protected areas in dry regions and addressing the problem of road mortality are important components of a national conservation plan (Fig 11).

5. The Iranian Cheetah Society (ICS) manages the Cats Information Database that gathers together data collected by experts and volunteers across Iran for several years now. It is now time to develop a user-friendly web platform for an easy reporting of observations so that local people can join national conservation plans. Educated local elements have the potential to become citizen scientists, gather data and report observation records from different regions. The EcoRelevé platform is an example of this strategy that allows users to input their observation data about any species and send it to the main database for further scientific studies.

Chapter 3

Conservation genetics of Iranian caracal: genetic diversity and phylogeography

3.1. Introduction

Conservation genetics is the application of genetic theory and techniques to the goal of reducing the risk of extinction of populations and species from genetic causes. Typically, genetic studies in conservation biology focus on rare or endangered species, assess their genetic status and suggest ways to maintain or increase genetic diversity (Frankham. 2004; Avise and Hamrick 1996). Determining the conservation genetic status of endangered populations and species is now just one of the applications of a discipline that has burgeoned and became subdivided in specific sub-disciplines contributing for more effective solutions to the multiple challenges associated with the conservation of biodiversity and natural areas (DeSalle and Amato 2004). One of the main premises and objectives of conservation genetics is, respectively, that there is a relationship between genetic diversity and population viability, and finding out which genetic diversity is critical for population viability (Beebee and Rowe 2005) and maintenance of evolutionary potential.

Loss of genetic diversity often occurs in parallel with inbreeding to concurrently cause a reduction in fitness. The recognized importance of genetic diversity is evident by its classification as one of the three global priorities for conservation by the IUCN. Genetic diversity, also called genetic variability or genetic variation, describes the variety of alleles and genotypes present in a population or species. Genetic diversity in endangered species is lower than in non-endangered ones because there is a relationship between genetic diversity and population size, mediated by genetic drift. Smaller population sizes also often imply reduced dispersal among populations, and thus lower gene flow to counteract the effects of drift (Frankham, et al. 2002).

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3.1.1. Phylogeography and subspecies

There is no universally accepted definition to answer the question ‘what is a species?’ and many definitions exist, ranging from those based on morphology, ecology and other biological aspects, to the ones based on genetic, evolutionary inference, and phylogeny. Many of the definitions of what is a species use, explicitly or implicitly, phylogeographic reasonings. Phylogeography is the discipline that studies the principles and processes that govern the geographic distributions of genealogical lineages within species but also among closely related species (Avise 2000). Phylogeography is a sub-discipline of biogeography that emphasizes the tight historical connections between population demography and genealogy, and builds bridges between the fields of population genetics and phylogenetics. Phylogeography is a powerful theoretical and analytical framework to study allopatric and parapatric divergence, subspecies, speciation, historical hybridization, hybrid zones, introgression, and many other research questions (Hewitt, 2001 and 2004; Frankham. 2004; Hickerson, et al. 2010).

Avise and Hamrick (1996) argued that two factors render the subspecies category very important in conservation biology. First, the potential of subspecies to become distinct species in the future, and second, the fact that subspecies should represent local or regional adaptive divergence among allopatric or parapatric populations. Of course it is not possible to predict which subspecies will speciate, and the adaptive divergence of subspecies should be assessed to evaluate their evolutionary and thus conservation relevance (Avise and Hamrick 1996). Traditionally, designation of species and subspecies was based on phenotypes, leading to a severe proliferation of sub-specific names, for instance in the leopard (*Panthera pardus*), where 27 subspecies have been described but only five are currently accepted, and in the puma (*Puma concolor*), in which 32 subspecies were described and now only six are thought to exist. To guide the tasks of subspecies revision, O'Brien and Mayr (1991) attempted to provide an operation definition of subspecies through the following definition: "populations that share a unique geographic range or habitat, a group of phylogenetically concordant phenotypic characters and a unique natural history relative to other subdivisions of the species".

3.1.2. Population genetics

To preserve the genetic diversity of a species it is crucial to find out its current genetic status and how genetic variability is distributed along the different populations (Avise, et al 1987; Avise 2000). Important parameters that inform about population genetic status are the level of inbreeding, the effective population size (Ne), and the ratio effective population size to census size (Nc). In contrast to interspecific phylogenetic studies, which evaluate the relationship among species, intraspecific phylogenies are about populations from different parts of the distribution of a given species or species complex. While mitochondrial DNA (mtDNA) has been traditionally the central molecular marker in phylogeographic studies, microsatellites have been hugely popular in population genetic studies during the last two decades. Microsatellites provide estimates of contemporary genetic variation, but also inform on recent population history, including demographic events such as population bottlenecks, inbreeding, hybridization, reproductive behavior, social structure, and dispersal (Beaumont and Bruford 1999).

3.1.3. Molecular markers

In the past few decades, the molecular markers that became more popular are those that accumulating studies showed to be unequivocally the most efficient, powerful and flexible to answer ecological and evolutionary questions and estimate multiple population genetic parameters (Selkoe and Toonen 2006). Molecular markers are hereditary and their characters states reflect mutation, drift and selection through evolutionary time. Different molecular markers, such as allozymes, microsatellites, mtDNA, and nuclear DNA exons and introns, have different sets of merits and disadvantages (Avise 1994). Most of the currently more popular molecular markers have become easily and routinely screened in the lab due to the advent of the polymerase chain reaction (PCR) technique, which allowed amplifying minute genetic samples without the need to resort to cloning or other laborious and time-consuming approaches. Following the appearance of the PCR technology, it was critical that evolutionarily conserved sets of primers for mtDNA and nuclear genes started to be developed by pioneering labs and became available to the research community. The development of microsatellite loci as hyper variable markers and the expansion of automated sequencers for straightforward DNA sequencing in the lab were two other crucial steps in the explosion of phylogeographic and population/conservation genetic studies of wildlife (Hillis, et al. 1996; Sunnucks 2000).

3.1.3.1 MtDNA

Avise et al. (1987) discussed that an ideal molecular marker for genealogical analyses could have: i). a simple genetic structure lacking complicated features such as repetitive DNA, transposons, pseudogenes and introns; ii) a straightforward mode of genetic transmission without recombination or other genetic rearrangements; iii) an abundance of qualitative character states whose genealogical relationships could be inferred by reasonable parsimony criteria; and iv) a relatively rapid evolutionary rate, so that new character states commonly arise within the lifespan of species, making it useful for purposes of microevolutionary analysis. The authors the noted that, to a remarkable degree, the mtDNA of higher animals meets all of these criteria. mtDNA is a circular chromosome/genome abundant in the mitochondria of eukaryotes, which has maternal inheritance, lack of recombination, and high variability due to a relatively high mutation rate, 5-10 times that of single-copy nuclear (Kim, et al. 2006; Jansen, 2000; Qiu-Hong, et al. 2004; Galtier, et al. 2009).

Typically thus, mtDNA carries phylogenetic signal and can help to resolve evolutionary histories from the recent past (Quaternary), while still being often useful to shed light on older (e.g. Neogene) processes and patterns. Besides the phylogenetic and genealogical insights it provides, mtDNA can also be used to assess genetic variation and population genetic differentiation and divergence (e.g. Fst and related statistics and allele frequency-based genetic distances), to infer demographic histories (population bottlenecks and expansions), to estimate historical effective population size (Ne), and to quantify the level and direction of gene flow among populations.

3.1.3.2. Microsatellites

Microsatellites, also known as short tandem repeats (STRs) or simple sequence repeats (SSRs) are popular molecular markers for population genetic studies because of their high polymorphism, due to an extremely fast mutation rate, which is reflected on a usually high number of alleles and heterozygosity. On the other hand, their fast evolutionary rate generates sizable allele homoplasy and genealogical patterns are difficult to infer. Typically, a microsatellite locus consists of five to 40 repeats of mono, di-, tri-, tetra-, penta- or hexanucleotide motifs, with dinucleotides and tetranucleotides being the most commonly used in studies in mammalian species (Li, et al. 2002).

They are suitable for the study of recent events (i.e. Late Pleistocene and Holocene) in population history and biogeography (Hewit 2001, 2004), and especially suited to assess contemporary population structure and migration (Selkoe and Toonen 2006).

Of advantages of working with microsatellites is easy sample preparation and high information contents. Microsatellites are species-specific and risk of cross-contamination by non-target species is less than other universal primers techniques. But this species-specific isolation trait of microsatellite brings difficulty to work with it. Other drawbacks of this marker are unclear mutational mechanism, hidden allelic diversity, and different problems with amplification. (Selkoe and Toonen 2006).

3.1.4. Wildlife genetic studies in Iran

Only recently genetic studies on wild mammals in Iran have started to be published. Farhadinia et al. (2015) used the mtDNA gene NADH5 to study the phylogeography of the leopard and the results supported a single subspecies of Persian leopard and showed a significant differentiation of this subspecies from other leopard subspecies, particularly from the Arabian leopard. A study using mtDNA and microsatellites on the cheetah showed that the Iranian cheetah, currently with only about 70 individuals, is the last representative of the Asiatic subspecies (*venaticus*), which diverged from the African cheetahs 67,000-32,000 years ago, and that this population has suffered a recent demographic bottleneck (Charruau, et al. 2011). Another study on the genetic diversity and population structure of the goitered gazelle using microsatellites revealed a high differentiation between populations west and east of the Zagros mountain chain (Hayatgheib 2011).

3.1.5. Aims

In Iran, the caracal was once a common inhabitant of the arid regions of the country. The Iranian caracals are believed to mainly belong to the subspecies *C. c. schmitzi*, but in the northern areas bordering Turkmenistan it has been suggested that a different subspecies, *C. c. michaelis*, might be present (Ellerman & Morrison-Scott 1951, Nowell & Jackson 1996, Karami 2008). Due to the lack of genetic and taxonomic data about the caracal in Iran, this initial investigation on the genetic status of the Iranian caracal is aimed at providing preliminary insights into the following questions:

i) What seems to be the level of current genetic diversity?

ii) Are there any signs of population structure and genetic differentiation across Iran?

iii) How the results relate with the described subspecies?

3.2. Materials and methods

3.2.1. Study area

Iran, the 18th largest country in the world with a width of 1,648,195 km^2, lies between latitudes 24° and 40° N, and longitudes 44° and 64° E and is delimited by the Caspian Sea in the north and the Persian Gulf and Oman Sea in the south. The topography of Iran includes rugged, mountainous rims surrounding elevated interior basins and plateaus. Given the caracal's distribution in Iran, the middle part of the Zagros Mountains in the east should be an important barrier, although there are some records from that region. The Zagros Mountains is a major mountain chain, a series of parallel ridges interspersed with plains that bisect the country from northwest to southeast, with many peaks exceeding 3,000 meters above sea level. The range of the caracal is limited in the north by the Alborz Mountain, a narrow but high chain that separates the northern Caspian coast region from the rest of the country. The center of Iran consists of several closed basins that are collectively referred to as the Central Plateau. The average elevation of this plateau is about 900 meters, but several of the mountains that tower over the plateau exceed 3,000 meters. The eastern part of the plateau is covered by two salt deserts, the Dasht-e Kavir (Great Salt Desert) and the Dasht-e Lut. Except for some scattered oases, these deserts are uninhabited.

The caracal also lives in neighboring countries, such as Iraq and Turkey in the west and northwest, Afghanistan and Pakistan in the east, and Turkmenistan in the north-east, and there is possibly gene flow between Iran and these countries.

3.2.2. Sampling

I collected a total of 24 samples (11 tissue samples from fresh specimens and 13 skin samples from museum specimens) from museums and the DoE genetic sample bank, and also from road-kills found across 6 provinces in Iran (Fig 12) (table 3). All tissue samples were preserved in 96% ethanol and kept frozen at -20ºC, until DNA extraction.

We extracted genomic DNA from the tissue samples using the E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek) and from the skins using the Tissue and Hair Extraction IQ™ Kit (Promega), following the respective manufacturer's protocols. To monitor potential contaminations, we included one negative extraction control per extraction session. The quantity of extracted DNA from each sample was measured by spectrophotometry.

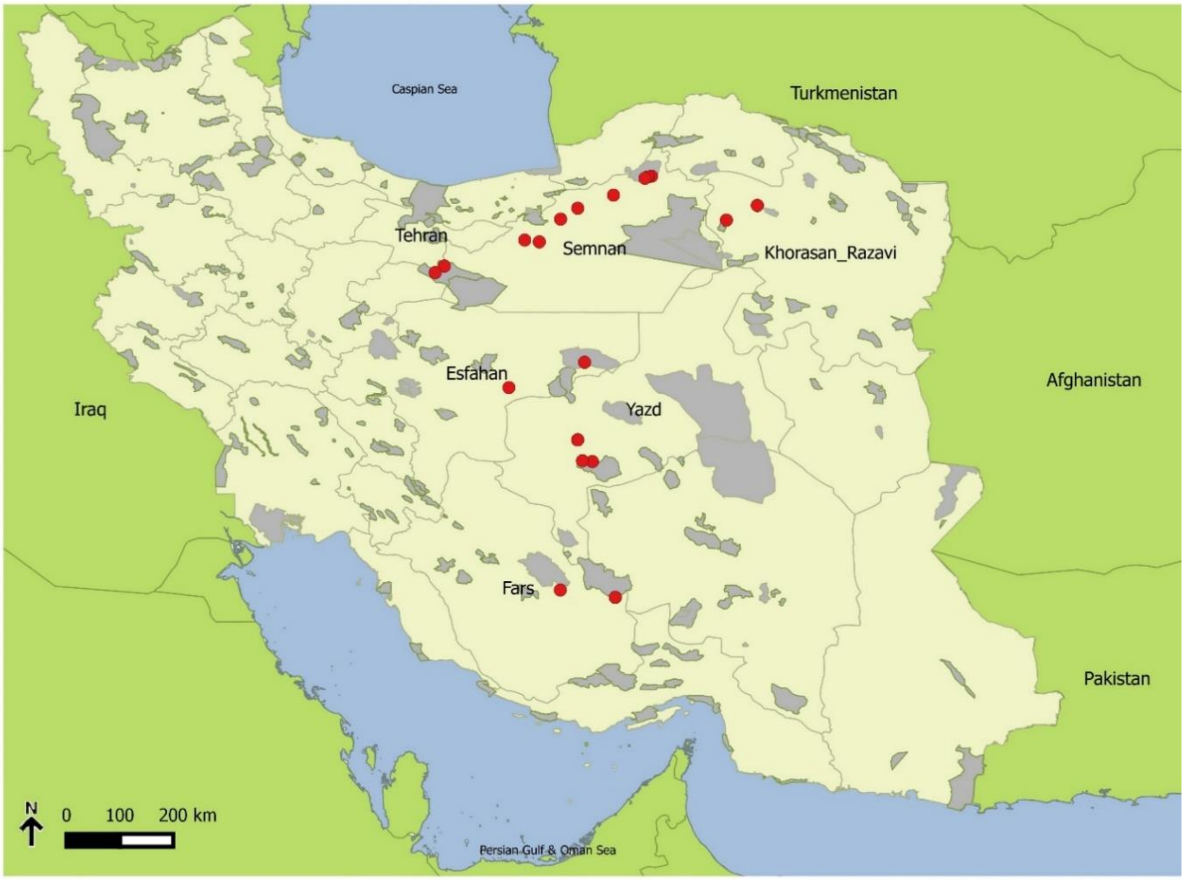

Figure 12. Location of samples within Iran

3.2.3. Mitochondrial DNA sequencing and microsatellite genotyping

For the tissue samples, we amplified and sequenced a fragment of about 600 bp of the mitochondrial NADH5 gene using primers L12656 and FelismtDNAR (Carlos Fernandes, personal communication), and a fragment of about 700 bp of the mitochondrial gene Cytochrome b using the primers L14724 and H15791 (Carlos Fernandes, personal communication). Polymerase Chain Reactions (PCRs) were carried out in a total volume of 20 µl containing 2U of Supreme NZYTaq DNA polymerase (NZYTech), 4µl of 10x PCR reaction buffer with MgCl2, 1.6µl of 10mM dNTPs mix, 0.2µl of each primer at 50uM, and 4µl of DNA extract. The thermal cycling was as follows: initial denaturation at 95 °C for 5 min, followed by 55 cycles of 94 °C for 10 s, 55 °C for 10 s, and 72 °C for 20 sec, and a final extension at 72 °C for 1 min.

Due to the expected fragmented DNA in the skin samples, two internal primer pairs targeting smaller NADH5 fragments (of about 200 bp) were used: L12656/PpardusR1 and PpardusF3/PpardusR3 (Carlos Fernandes, personal communication). PCRs for skin samples were carried out in 15µl volumes using the QIAGEN Multiplex PCR Kit and the following thermal cycling: initial denaturation at 95° C for 15 min, followed by 60 cycles of 94 °C for 30 s, 50 °C for

30 s, and 72 °C for 30s, and a final extension at 72 °C for 7 min. PCR products were analyzed by electrophoresis in 1.5% agarose gels. Positive results were purified using ExoSAP and sequenced at Macrogen Inc.

Microsatellite genotypes for four loci were obtained from eight tissue samples. The loci, F53, FCA124, FCA723, and FCA742, were developed in *Felis catus* (Menotti-Raymond, et al. 1999 and 2005). PCRs were carried out in a total volume of 10µl containing 0.18µl of Supreme NZYTaq DNA polymerase (NZYTech), 1µl of 10X PCR reaction buffer, 0.7µl of 50mM $MgCl_2$, 0.2µl BSA at 10mg/ml, 0.8µl of 10mM dNTPs mix, 0.5µl of each primer at 50uM, and 1.5µl of DNA extract. The thermal cycling was as follows: initial denaturation at 95 °C for 5 min, followed by 10 cycles of 94 °C for 30 s, 63 °C for 30 s, 72 °C for 30 s, and 30 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 30 sec, and a final extension at 72°C for 20 min.

3.2.4. Statistical data analysis

3.2.4.1. MtDNA

The sequences were aligned with Sequencher 4.1.4 (Gene Codes Corporation). The alignment was analysed with FaBox 1.41 (Villesen 2007) to collapse identical individual sequences into representative haplotypes.

3.2.4.2. Microsatellites

GeneMapper 4.1 was used to analyse the microsatellite fragments and score the genotypes. GeneAlEx 6.5 (Peakall and Smouse 2006) was used to calculate the summary statistics of genetic variation. Genetic structure in the analyzed samples was probed using Structure 2.3.4 (Pritchard, et al. 2000) and a factorial correspondence analysis (FCA) in Genetix 4.05 (Belkhir, et al. 2000).

3.3. Results

3.3.1. MtDNA

Nine of the 11 samples yielded usable sequences and all of them had the same haplotype. Partial sequences from the skin samples confirmed the results from the fresh samples by suggesting a single haplotype.

3.3.2. Microsatellites

Eight of the 11 tissue samples worked for the four microsatellite loci and the summary statistics for the loci are given in table 4. N is sample size, Na is number of alleles, Ne is effective number of alleles, I is the information index, Ho is the observed heterozigosity, uHe is the unbiased expected heterozigosity, and F is the fixation index.

Table 4. Summary statistics for microsatellite loci in Iranian caracals

	F53	FCA124	FCA742	FCA723
N	8	8	8	8
Na	7	3	6	4
Ne	6.095	2.133	4.129	2.723
I	1.873	0.900	1.561	1.143
Ho	0.250	0.750	0.875	0.250
uHe	0.892	0.567	0.808	0.675
F	0.701	-0.412	-0.155	0.605

The posterior probability of the Structure analysis suggested a single population (K=1), while the FCA placed the individuals in separated areas of the ordination space but there was no clear geographic pattern in the arrangement (Fig 13).

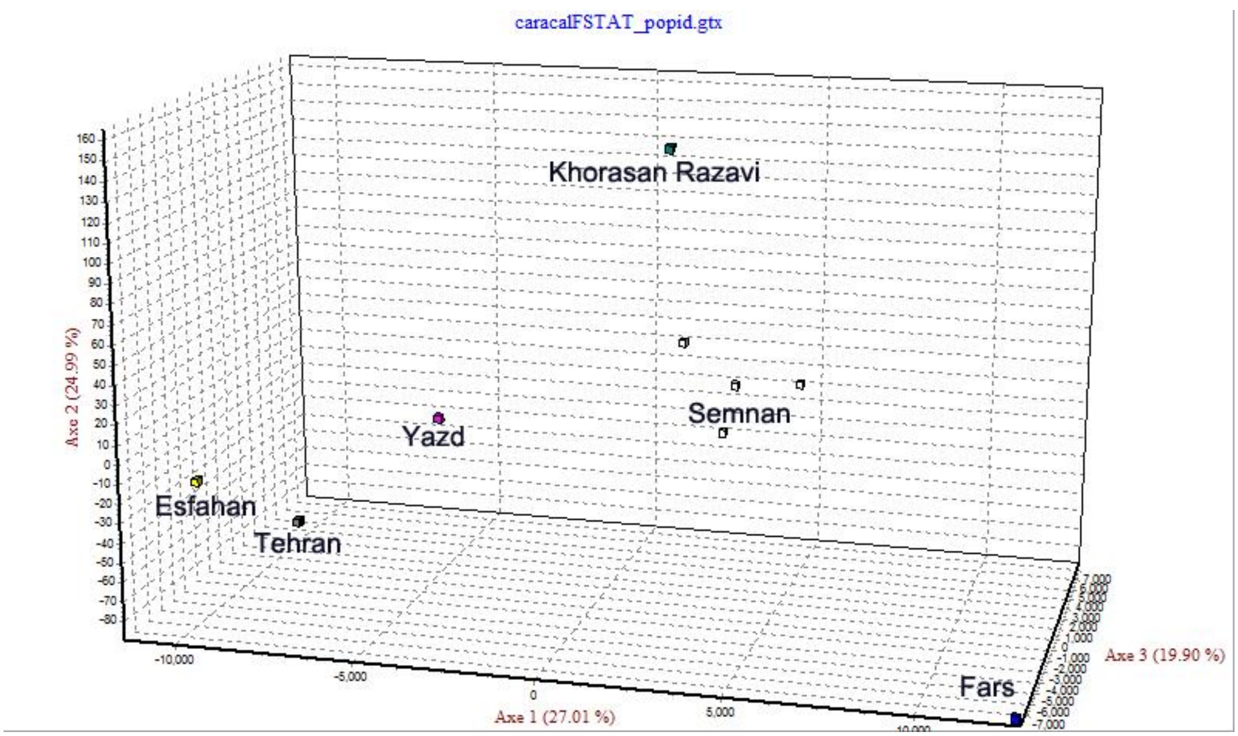

Figure 13. Three-dimensional factorial correspondence analysis (FCA) of Iranian caracals based on four microsatellite loci.

3.4. Discussion

The results of the genetic analysis suggest a very low mtDNA variation in the Iranian caracal, which may suggest a historical demographic contraction, both ancestral and associated to the expansion from Africa into Asia or due to a later population crash in Iran. The gene used is a protein-coding gene with a relatively low mutation rate and this may also contribute to the observed low variation; analysis of the faster evolving control region of mtDNA would be desirable but this region is rich in repetitive regions in felids and not amenable to straightforward PCR amplification and sequence analysis.

Since there are no microsatellite markers described for the caracal, we tested domestic cat loci that have been used in other felid species. Of the eight loci tested we could only obtain successful results for four of the markers. The next step of this research is to test additional domestic cat primers among the many available in the literature.

All the analyzed samples were from east of the Zagros mountain chain and subsequent effort will be to gather and analyze samples from the Zagros and to the west of the Zagros. The Zagros is probably not an impermeable barrier but certainly restricts gene flow between western and central Iran. A wider sampling and also a larger number of samples is needed to ascertain the lack of mtDNA variation observed and to properly assess the genetic diversity and population structure using microsatellites in the Iranian caracal.

3.5. Prospects for future conservation genetics research

Besides continuing the preliminary research initiated here, other conservation genetic studies are relevant for the caracal in Iran. Given that much of the mortality of caracals caused by humans is due to persecution and retaliatory killings for supposed attacks in barns, genetic analysis of predator saliva on carcasses of livestock could identify the true culprits of livestock attacks and clarify the frequency of caracal involvement in those events. Since the livestock owners are often unsure about the carnivore species behind a given attack, this approach can be really useful to help resolving the conflict situation. Similar assessments have been done in Sweden to find out whether wolves or dogs were responsible for attacks on sheep, and in many instances result showed that a same dog was responsible for many attacks (Sundqist et al. 2008). In the case of the Iranian carnivore community, it should be possible to design a species-specific mtDNA marker set (e. g. Fernandes et al. 2008) to identify which species are behind the attacks by analyzing saliva or hair samples found at the wounds of the livestock carcasses.

Roads are a serious threat to wildlife, not only because of vehicle collisions causing mortality but also because they can sever dispersal and migration between populations (e.g. Riley et al. 2006). Indeed, the results of a habitat suitability model showed that roads are a negative factor for caracal in Iran (Farhadinia et al. 2012). The older and wider highways in Iran may have a strong impact on the population demography and genetic of caracals and other carnivores, as well as of their prey.

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Appendix

Figure 5. A silver rhyton with protome of a caracal catching a fowl, Provenance from Iran, Late 2nd - 1st century B.C., Miho museum, Tokyo, Japan

Figure 7. Two samples of typical herds in the area, short walls and non-practical roofs to save domestic livestock during the days and nights. © Y. Hassan-beigi

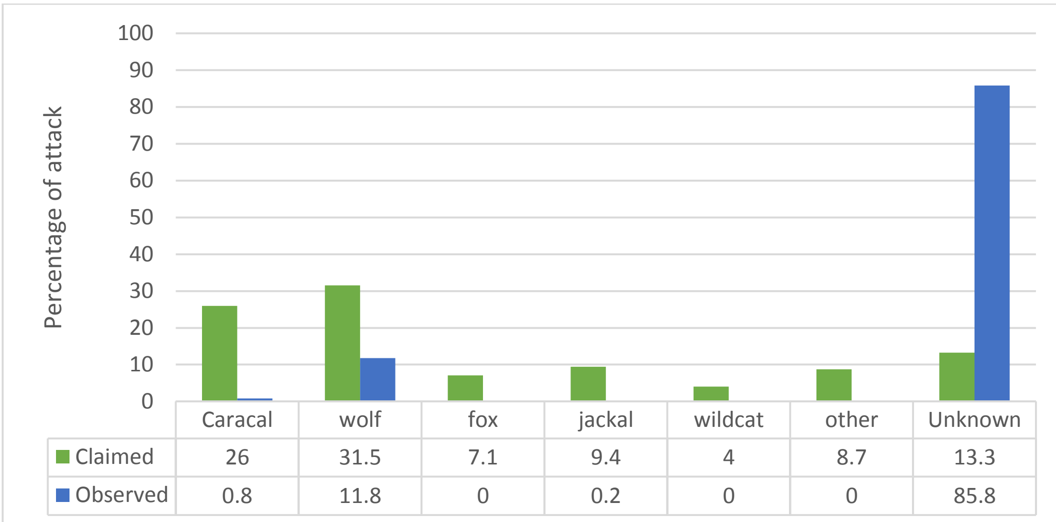

Figure 8. Persentage of claimed and observed carnivores attacking the livestock

Figure 9. Example of a promoted barn by fence after late attach by an unknown carnivore. © Y. Hassan-beigi

Figure 10. Rest of the carcass of a camel, left near the village, Southern Khorasan. © Y. Hassan-beigi

Figure 11. A caracal killed in road accident. Abbas-abad P.A. Esfahan. © M.R. Halvani

Table 2. Status of information and knowledge about the Iranian cat species. (Aj) Acononyx jubatus venaticus Asiatic cheetah, (Cc) Caracal caracal caracal, (Ll) Lynx lynx Eurasian lynx, (Om) Octocolobus manul Pallas's cat, (Pp) Panthera pardus saxicolor Persian leopard, (Fc) Felis chaus jungle cat, (Fm) Felis margarita sand cat, (FS) Felis silvestris wildcat. -2 means that information knowledge is very poor, 0 that it is acceptable and 2 that it is very good. Topics in blue were not included in the overall total as they were not assessed for all species (Mohammadi 2012)

No.	Topic	Species								
		Aj	Cc	Ly	Om	Pp	Fc	Fm	Fs	∑
1.	Distribution	0	0	1	0	1	0	-1	-1	0
2.	Abundance	0	-2	-2	-2	-2	-2	-2	-2	-14
3.	Population trends	0	-2	-2	-2	-2	-1	-1	-2	-12
4.	Diet	1	-1	0	-1	0	0	0	-1	-2
5.	Status of main prey	1	0	-1	-2	1	2	-1	-1	-1
6.	Habitat preference	0	-1	0	-1	1	0	0	-1	-2
7.	Land tenure system	0	-2	-1	-2	-1	-2	-2	-2	-12
8.	Demography	0	-2	-1	-2	-1	-2	-1	-2	-11
9.	Competition/-titors	0		0				-1	-1	
10.	Taxonomy (e.g. ssp)	1	-2	0	-1	2	-1	-2	-2	-5
11.	Genetic (e.g. inbreeding)	-1	-2	-1	-2	-2	-2	-2	-2	-14
12.	Health/diseases	-2	-2	-1	-1	-2	-2	-2	-2	-14
13.	Threats	1	0	2	0	1	0	0	0	4
14.	Conflict	1	0	1	0	1	0	2	-1	4
15.	Human attitude	0		0						
16.	Local people knowledge		-1	0						
∑		2	-16	-5	-16	-3	-10	-12	-19	-79

Table 3. Details of the samples used in the study

No.	ID	Type	Origin	Collector
1	Cc1_Fa	Tissue	Fars	Hassan-Beigi
2	Cc2_Se	Tissue	Semnan	Kaveh
3	Cc3_Se	Tissue	Semnan	Adibi
4	Cc4_Is	Tissue	Isfahan	Farhadinia
5	Cc5_Th	Tissue	North Khorasan	Khanloo
6	Cc6_Is	Tissue	Isfahan	Farhadinia
7	Cc7_Yz	Tissue	Yazd	Dehghan
8	Cc8_Se	Tissue	Semnan	Zaheri
9	Cc9_Th	Tissue	Tehran	Jebeli
10	Cc10_Fa	Skin	Fars	Hassan-Beigi
11	Cc11_Fa	Skin	Fars	Hassan-Beigi
12	Cc12_Kh	Skin	North Khorasan	Khanloo
13	Cc13_Se	Skin	Semnan	Hassan-Beigi
14	Cc14_Se	Skin	Semnan	Hassan-Beigi
15	Cc15_Se	Skin	Semnan	Hassan-Beigi
16	Cc16_Yz	Skin	Yazd	Fathollah zade
17	Cc17_Se	Skin	Semnan	Zahri
18	Cc18_Se	Skin	Semnan	Farhadinia
19	Cc19_Th	Skin	Tehran	Adibi
20	Cc20_Yz	Skin	Yazd	Hassan-Beigi
21	Cc21_Yz	Skin	Yazd	Hassan-Beigi
22	Cc22_Yz	Skin	Yazd	Hassan-Beigi