



# Distribution patterns and genetic structure of golden jackal in Europe and Asia

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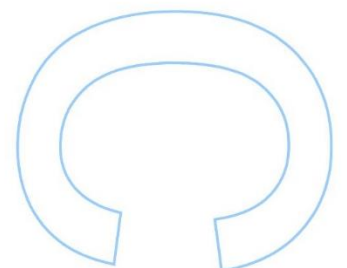
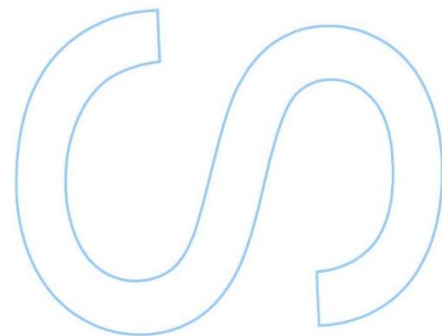
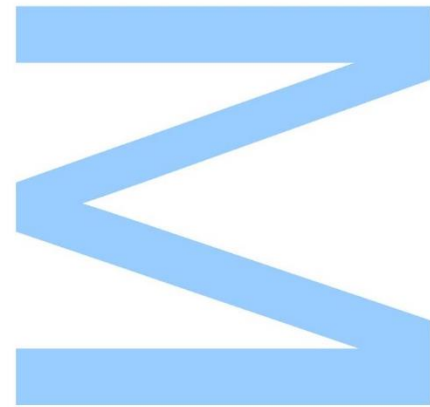
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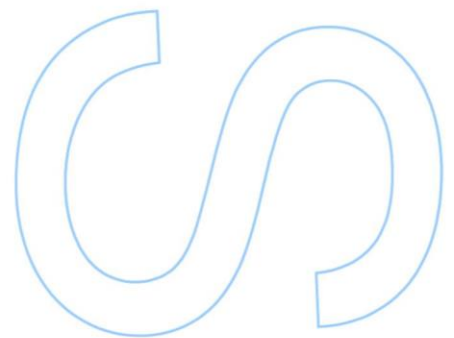
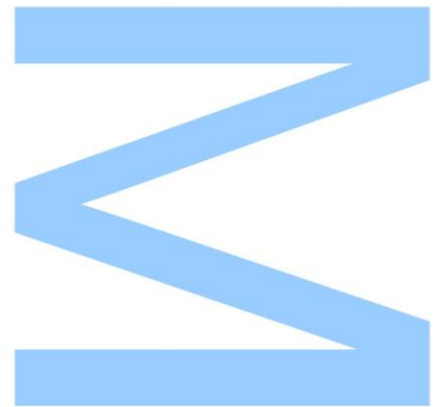
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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.  
O Presidente do Júri,

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# Abstract

Organisms with great ability to mobility and dispersion are able to adapt to various habitats and ecosystems, surviving to climate and environmental changes caused mainly by man. The carnivores are a good example of organisms which cover long distances, occupy wide areas and inhabit different habitats, thus prompt to find barriers to gene flow and dispersion, which can cause isolation of populations. Applying molecular genetic tools enables to acquire a wide knowledge on carnivore populations, relevant to develop effective management and conservation strategies. However, these studies are often related to iconic carnivores, with little available information on other species with wide occurrence areas, such as the Golden Jackal.

Golden Jackal *Canis aureus* is one of the most widespread canid species with a range that covers Central and Eastern Europe to Middle East and Southeast Asia, as can be seen in the information provided by IUCN. In Asia there is a lack of information on the populations of golden jackal, but it is estimated that this species is abundant, especially in Turkey, Iran and India. In Europe there is more information, however the recent expansion and distribution are not known. Recently, several studies claimed that the jackal has expanded from South East Europe to more central areas and to northern Europe, particularly in the Baltic region. There are few genetic studies on golden jackal, mostly at local scale, and no intercontinental or long distance studies is available.

The objective of this study was to make a compilation of presence records from Eurasian golden jackal across their worldwide range and measure the population structure and genetic diversity of this species in Europe and the Middle East. A total of 1842 presence records were compiled to update the global range extension, evaluate distribution trends, and evaluate areas of potential distribution. Furthermore, 184 samples of golden jackals from Southeastern Europe, the Baltic region, Caucasus and the Middle East, were genotyped for 43 microsatellites to assess population structure and genetic diversity.

Results confirm the prehistoric occurrence of jackals on coastal areas of Croatia and Greece since the Holocene, and a strong recent expansion occurring in Europe over the past decades, where jackals have colonized new areas in Central and Northeast Europe. In Asia, the presence of records were generally older and scarce, but well distributed across the continent from Middle East to Southeast Asia. Genetic analysis revealed a division between European and Asian populations, with Asian populations presenting a higher genetic diversity. The genetic results, together with the

presence records and the distribution model confirm that the Asian jackals colonized Europe, apparently in different occasions, with potential corridors located both at the south and north coast of the Black Sea. The recent expansion of jackals to Baltic region result from dispersal of individuals belonging to both southeast European population and Caucasian populations. Our results also reveal several relict populations, with low genetic diversity, in coastal Croatia, mainland Greece and Samos Island, which raises the need for regional assessments to access their conservation status need to be preserved. This study contributed for a deeper knowledge on the population history of Eurasian Golden jackals and provided an update of the global distribution, in relation to the available range information from IUCN.

Future research should be focused in further sampling for genetic analyzes in relict populations of coastal Southeastern Europe and in the expanding populations that assure the connection between Asia and Europe, in order to better understand the Europe's colonization processes by this species. The golden jackal in Europe is often considered an invasive species, being heavily hunted by humans. However, jackals already inhabit Europe since the Holocene and as jackal expansion in Europe occurs without human intervention, future management actions should not consider this species as invasive and should reassess their conservation status.

**Keywords:** Golden Jackal, *Canis aureus*, dispersal, distribution trends, Europe, Asia, genetic structure, genetic diversity, potential distribution, range extant

## Sumário

Os organismos com grande capacidade de mobilidade e dispersão são capazes de se adaptar a diferentes habitats e ecossistemas, sobrevivendo, assim, às mudanças climáticas e ambientais causadas, principalmente, pelo homem. Os carnívoros são um bom exemplo de organismos que percorrem longas distâncias, ocupam vastas áreas e habitam diferentes habitats, podendo encontrar barreiras ao fluxo gênico e dispersão, causando o isolamento das populações. Aplicando técnicas de genética molecular é possível adquirir um amplo conhecimento sobre as populações de carnívoros, relevante para o desenvolvimento de estratégias eficazes de gestão e conservação. No entanto, estes estudos estão frequentemente relacionados com carnívoros icónicos, com pouca informação disponível sobre outras espécies com grandes áreas de ocorrência, como a Chacal Dourado.

O Chacal Dourado *Canis aureus* é uma das espécies de canídeos mais amplamente distribuídas, abrangendo a Europa central e oriental, bem como o Médio Oriente e o Sudeste Asiático, como se pode verificar na grosseira informação disponibilizada pelo IUCN. Recentemente descobriu-se que os chacais que habitam no Norte e Este de África correspondem a outra espécie, mais relacionada com o lobo. Na Ásia existe falta de informação sobre as populações de chacal dourado, mas estima-se que esta espécie seja abundante, principalmente na Turquia, Irão e Índia. Na Europa já existe mais informação não se conhecendo, no entanto, as recentes expansões e a atual extensão da distribuição. Recentemente, vários estudos afirmam que o chacal se tenha expandido do Sudeste Europeu para áreas mais centrais e a norte da Europa, nomeadamente para a região dos países Bálticos. Até ao momento são poucos os estudos genéticos sobre o chacal dourado e os que existem focam-se em estudos locais e nenhum estudo entre continentes ou de longa distância está disponível.

O objetivo deste estudo foi efectuar uma compilação de registos de presença de chacal dourado em toda a sua distribuição e avaliar a estrutura da população e diversidade genética desta espécie na Europa e no Médio Oriente. Um total de 1842 registos de presença foram compilados para atualizar a distribuição global, avaliar as tendências de distribuição e avaliar a potencial distribuição da espécie. Além disso, 184 amostras de chacais dourados do Sudeste da Europa, da região do Báltico, Caucaso e do Médio Oriente, foram genotipadas para 43 microssatélites, com o intuito de avaliar a estrutura genética e diversidade genética da população.

Os resultados mostram a ocorrência de registos pré-históricos de chacais em áreas litorais da Croácia e da Grécia, desde o Holoceno, bem como uma forte expansão recente na Europa ao longo das últimas décadas, onde os chacais colonizaram novas áreas na Europa Central e Nordeste. Na Ásia, os registos de presença são geralmente mais antigos e escassos, mas bem distribuídos por todo o continente a partir do Médio Oriente até ao Sudeste Asiático. A análise genética revelou uma divisão entre as populações europeias e asiáticas, com as populações asiáticas apresentando uma maior diversidade genética. Os resultados genéticos, em conjunto com os registos de presença e do modelo de distribuição confirmam que os chacais asiáticos colonizaram a Europa, aparentemente, em diferentes ocasiões, com potenciais corredores localizados tanto a sul e a norte da costa do Mar Negro. Além disso, a expansão recente na região do Báltico resulta da dispersão de indivíduos pertencentes a ambas as populações do Cáucaso e do Sudeste da Europa. Os nossos resultados também revelaram várias populações relíquia, com baixa diversidade genética, na costa da Croácia e ilha de Samos, o que aumenta a necessidade de avaliações regionais para acessar o seu estado de conservação. Este estudo contribuiu para um melhor conhecimento sobre a história das populações de chacais dourados e forneceu uma atualização da distribuição global, em relação às informações disponíveis pelo IUCN.

Futuros projectos deverão incidir em mais amostras para análises genéticas das populações relíquia da costa do Sudoeste da Europa e nas populações em expansão que asseguram a ligação entre a Ásia e a Europa, a fim de compreender melhor os processos de colonização da Europa por esta espécie. O chacal dourado na Europa é muitas vezes considerado uma espécie invasora, sendo muitas vezes caçado. No entanto, os chacais já habitam na Europa desde o Holoceno e como a sua expansão na Europa ocorreu sem a intervenção humana, não se deve considerar esta espécie como invasora e serão precisas futuras ações de gestão, bem como reavaliar o seu estado de conservação.

**Palavras-chave:** Chacal Dourado, *Canis aureus*, dispersão, padrões de distribuição, Europa, Ásia, estrutura das populações, diversidade genética, alcance de distribuição

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# 1. Introduction

## 1.1. Challenges to conserve carnivores

Facing environmental and climate change, both by geological processes or human action, organisms can adapt locally, migrate or become extinct (Hughes *et al.* 2003; Davis *et al.* 2005; Ryan and Cunningham 2012; Vavrus *et al.* 2012; Ledevin and Millien 2013). In this context, mobility and dispersal influence the long-term survival and adaptation of species facilitating population persistence, thus being suggested as important evolutionary forces (Sharbel *et al.* 2000; Ramachandran *et al.* 2005; Hardy *et al.* 2006; Hellberg 2009; McManus *et al.* 2015). In fact, dispersal influences the distribution and abundance of populations, as well as their persistence and structure (Coulon *et al.* 2004). Moreover, it determines gene flow within and between populations, assuming that reproduction occurs following dispersal, and affects processes such as local adaptation, speciation and the evolution of life history traits (Slatkin 1987; Bohonak 1999; Dieckmann *et al.* 1999; Lomolino *et al.* 2001; Nichols *et al.* 2001; Gaston 2003). These traits are well exemplified by large carnivores, which have the ability to roam extensive distances and occupy a variety of environmental conditions (Sunquist and Sunquist 2002; Sillero-Zubiri *et al.* 2004). Larger ranges are linked with high gene flow (Rosenzweig 1995; Gavrillets *et al.* 2000; Gaston 2003; Birand *et al.* 2012), nevertheless, there is also an increasing number of evidences showing various barriers to gene flow and effective dispersal, including both physical obstacles and ecological conditions, that eventually promote speciation (Cegelski *et al.* 2003; Stenseth *et al.* 2004; McRae *et al.* 2005; Pilot *et al.* 2006; Musiani *et al.* 2007; Schmidt *et al.* 2011). Furthermore, carnivores are thought to be particularly vulnerable to local extinction in fragmented landscapes due to their relatively large spatial requirements, low numbers, and direct persecution by humans (Noss *et al.* 1996; Woodroffe and Ginsberg 1998; Stronen *et al.* 2013).

Large carnivore populations have been expanding and recolonizing former habitats in the last few decades, namely in Europe and North America (Stahl *et al.* 2001; Crooks 2002; Cardillo *et al.* 2005; Dalerum *et al.* 2009; Traill *et al.* 2010; Linnell and Boitani 2012; Chapron *et al.* 2014). This event occurs after massive historic declines in population sizes and geographic ranges due to their biological traits such as small population size, long generation times, large home ranges, and high levels of

human persecution (Crooks 2002; Miller and Waits 2003; Kendall *et al.* 2009; Liberg *et al.* 2012; Proctor *et al.* 2005). These dynamic population trends in large carnivores are expected to shape their genetic characteristics (Brooker and Brooker 2002; Mills *et al.* 2003; Bonte *et al.* 2004; O'Connell *et al.* 2007). Moreover, this recent expansion have incited increasing human-wildlife conflicts, rendering the need for new effective management and conservation strategies (White and Ward 2010; Cromsigt *et al.* 2013), for which the understanding of the distribution patterns and their impact on the genetic population structure is essential.

### **1.1.1. Relevance of dispersal in large carnivores**

The expansion of carnivores might follow a propagule model, first the early dispersal of vagrants, usually young males in search of new available territories (Fabbri *et al.* 2013), then the settlement of stable reproductive familial units, which may rapidly expand and saturate all suitable areas (Mech 1970). A variety of factors can influence patterns of dispersal, including home range attributes (Bowman *et al.* 2002), connectivity of landscapes or habitats (Gibbs 1998; Riley *et al.* 2006), and even large scale climatic patterns (Geffen *et al.* 2004). Though, even carnivores are highly mobile by definition, discontinuous habitat and anthropogenic-associated barriers constrict movements and reduce population densities and connectivity (Walker *et al.* 2000; Sinclair *et al.* 2001; Woodroffe *et al.* 2005; Loxterman 2011; McManus *et al.* 2015). Subsequent human activity has resulted in further and more recent fragmentation in species ranges such as European brown bears (*Ursus arctos*) (Zedrosser *et al.* 2001), Eurasian lynx (*Lynx lynx*) (Schmidt *et al.* 2011) and wolves (*Canis lupus*) (Stronen *et al.* 2012).

Assessing distribution trends and species distribution models are a precious and increasingly used tool for access distribution, dispersal patterns and connectivity. Species distribution models ideally can predict the most likely areas for species presence from a sample of distribution data and their correlation with environmental variables (Guisan and Zimmermann 2000). The knowledge of species distribution patterns and the identification of factors influencing these patterns are crucial for managing biodiversity (Krebs 1978; Brown 1984). In recent years, the modelling of species distributions has been increasingly used to identify suitable habitat (Rushton *et*

*al.* 2004), to predict potential distributions (Austin 2002; Robertson *et al.* 2003) and identify broad-scale management units (Williams and Araújo 2002).

### **1.1.2. Effects of regressions and expansions on genetic parameters**

Genetic approaches have been used to identify factors that promote gene flow and connectivity between populations, as well as its overall effect on genetic structure of broader or fine-scale populations (Manel *et al.* 2003; Broquet and Petit 2009; Row *et al.* 2012). When gene flow between populations is prevented, two major genetic threats may occur: alleles become randomly fixed or are lost due to genetic drift, and prejudicial mutations may accumulate (McManus *et al.* 2015). It is also known that some species are particularly sensitive to loss of connectivity (Traill *et al.* 2010): For example, Canada lynx (Schwartz *et al.* 2002), badgers, long-tailed weasels, spotted skunks, mountain lions, bobcats, and coyotes (Crooks 2002),

To assess the impact of genetic variation in populations, particularly in comparison with environmental factors, inferences on the genetic structure of populations are often done to look for possible genetic clusters and their number. Population genetic structure is determined by the interaction between gene flow and genetic drift (Wright 1943; Kimura and Weiss 1964; Slatkin 1985). Knowledge of the within-population genetic structure should always be a prerequisite for decision making in the management of breeding and natural populations (Hamrick and Sherman-Broyles 1992; Williams and Hamrick 1996). Data from highly mobile large carnivores, such as the Canada lynx (*Lynx Canadensis*) (Row *et al.* 2012), the bobcat (*Lynx rufus*) (Reding *et al.* 2012), mountain lions (Castilho *et al.* 2011), wolverines (Kyle and Strobeck 2002), the grey wolf (*Canis lupus*) (Vila *et al.* 1999) and the red fox (*Vulpes vulpes*) (Teacher *et al.* 2011), indicate weak population structuring prior to the last glacial maximum (11.700 years ago), suggesting that the current genetic distributions appeared in consequence of the most recent glaciation (Hofreiter *et al.* 2004). However, the same species may display limited gene flow in the presence of geographical barriers. Geographical barriers, like mountains and seas, may prevent gene flow between populations and induce isolation, which can result in a cryptic population structuring like has been shown for the Canada lynx (Rueness *et al.* 2003; Row *et al.* 2012), the grey wolf (Pilot *et al.* 2006; Munoz-Fuentes *et al.* 2009), the Scandinavian lynx (*Lynx lynx*) (Rueness *et al.* 2003) and coyotes (*Canis latrans*) (Sacks *et al.* 2008).

Additionally, recent studies have revealed cryptic population structures correlating with transitions in habitat types and prey species composition. This is the case of the Canadian lynx, for which three demographically distinct populations were described, inhabiting three climatic regions with differential snow conditions, leading to different dynamics between predator and prey (the snowshoe hare *Lepus americanus*) (Rueness *et al.* 2003) and to genetic structure (Stenseth *et al.* 2004). In another example, Dalen *et al.* (2005) showed that genetic differentiation in the Arctic fox appears to be directly correlated to prey specialization, revealing that the genetic structure of this species throughout its range corresponds to two ecotypes, one specialized on lemmings and another feeding on coastal food. Habitat and dietary differentiation has also been shown to correlate with genetic structure in the grey wolf *Canis lupus* (Pilot *et al.* 2006; Carmichael *et al.* 2007; Musiani *et al.* 2007; Munoz-Fuentes *et al.* 2009).

The genetic diversity of expanding populations is influenced by the rates of long-range dispersal and by founder effects (Fabbri *et al.* 2007, Cohen *et al.* 2013). On the other hand, relict and isolated populations might face losses of genetic diversity if population expansion is delayed by years (Vila *et al.* 2003). This is in accordance with recent findings on the grey wolf genetic structure in Europe, for example the wolves in Alps, that suffered isolation, have significantly lower genetic diversity (heterozygosity, allelic richness, number of private alleles) than wolves in the Apennines (Fabbri *et al.* 2007). Small, isolated, and fragmented populations suffer loss of genetic diversity over time through inbreeding and genetic drift (Ellstrand and Elam 1993; Lacy 1997; Frankham and Ralls 1998; Chevolut *et al.* 2008). When population size suffers a deep reduction is normally referred to as a population bottleneck, which can result in low levels of genetic diversity (Nei *et al.* 1975; Chakraborty and Nei 1977; Nei 1977; Leberg 1992). Expected and observed heterozygosity, effective and private alleles, and genetic distances between populations are an example of diversity analysis that give us important information about populations and their connectivity.

Most commonly, genetic studies have employed neutral markers such as microsatellites, which are useful for addressing questions relating to the origin of founders, inbreeding and relatedness (Jarne and Lagoda, 1996; Marsden *et al.* 2013). Microsatellites are tandemly repeated sequences found in all eukaryotes and also in the chloroplastic genome of plants. In general, microsatellite loci are highly polymorphic in natural populations, with average expected heterozygosity well above 50% which is a consequence of their high mutation rates. Microsatellites have been widely used to investigate the population structure, including the genetic relationships, among subpopulations (Forbes *et al.* 1995; Estoup *et al.* 1996) and the estimation of genetic

distances among closely related populations (Bowcock *et al.* 1994; Buchanan *et al.* 1994; Ciampolini *et al.* 1995; Estoup *et al.* 1995; Forbes *et al.* 1995; Arranz *et al.* 1996; Barker *et al.* 1997; MacHugh *et al.* 1997).

Applying molecular genetic tools has made possible to gain greater knowledge of the current status of populations of carnivores and to harness this information to develop efficient management strategies that may be of assistance in wildlife conservation. However, genetic studies across continents and with broader scale have mainly focused iconic carnivore species, such as lynxes (Schwartz *et al.* 2002; Rueness *et al.* 2003; Reding *et al.* 2012; Row *et al.* 2012; Ratkiewicz *et al.* 2014), bears (Kopatz *et al.* 2014), wolves (Vila *et al.* 1999; Pilot *et al.* 2010; Stronen *et al.* 2013), coyotes (Sacks *et al.* 2008) and foxes (Teacher *et al.* 2011; Statham *et al.* 2014), but other carnivore species with large occurrence areas, such as Eurasian Golden Jackal, are still lacking comprehensive information.



## 1.2. The Golden Jackal as a case study

The Golden jackal *Canis aureus* (Fig. 1) is one of the most widely distributed canid species although there is little available information on its natural history. Golden jackals were considered to be found across many areas of Europe, Asia and Africa (Sillero-Zubiri *et al.* 2004). However, jackals from North and East Africa have been recently recognized as a different species, more related to wolves and with distinct evolutionary history than Eurasian jackal despite a high morphological similarity (Koeplli *et al.* 2015).



Figure 1 – Phenotypic variation on the Eurasian Golden Jackal *Canis aureus*, in Europe (A; © Carlo Galliani), Iran (B; © Fariborz Heidari), Israel (C; © Yoav Perlman) and India (D; © Victor Szatornili).

Eurasian golden Jackal is a medium-sized carnivore with mean body mass for females as 5.8kg, and for males 6.6kg, and a total length up to 74-84 cm (Sillero-Zubiri *et al.* 2004). Basic coat color is golden but varies from pale creamy yellow to a dark tawny hue on a seasonal basis (Sillero-Zubiri *et al.* 2004). Due to their tolerance of dry habitats and their omnivorous diet, the golden jackal can live in a wide variety of habitats. They occupy forested, mangrove, agricultural, rural and semi-urban habitats, they are omnivorous and opportunistic foragers, and their diet varies according to season and habitat, between invertebrates, fruit, rodents, hares, lizards, snakes and birds (Sillero-Zubiri *et al.* 2004). Jackals are territorial animals but their social

organization is extremely flexible depending on the availability and distribution of food resources (MacDonald, 1979; Sillero-Zubiri *et al.* 2004). The basic social unit is the breeding pair, which is sometimes accompanied by its current litter of pups and/or by offspring from former litters. Golden jackal is classified by the IUCN as Least Concern, due to its widespread range in areas with optimum food and shelter (Jhala and Moehlman 2008), but is listed as “vulnerable” in the Red Data Book for Greek Vertebrates (Karandinos and Parasch 1992), where suffered a huge population decline induced by human activities. Moreover, in Europe, jackal has become the most popular predator for hunting due to its valuable skin (Trouwborst *et al.* 2015).

The Eurasian golden jackal (*Canis aureus*) occurs in South Asia eastward to Thailand, Middle East, southeastern and central Europe (Demeter and Spassov 1993; Jhala and Moehlman 2004; Stoyanov 2012). Based on fossil records, it has been suggested that jackals have colonized Europe during the Upper Holocene (Bauer 2001), constituting a relict population, namely in Dalmatia (Coastal Croatia) and Greece, that reveal regression in last decades (Giannatos *et al.* 2005). Currently, golden jackal is most common on Southeastern Europe, with increasing expanding populations towards central and northeastern Europe (Arnold *et al.* 2012; Trouwborst *et al.* 2015). These assessments of expansions and regressions in European populations together with the fact that the available ecological information about this species actually include two different taxons (the African jackal mentioned above), confirms the necessity of studies that approach the ecology, distribution patterns and genetic characteristics to identify management units and potential colonization routes.

### **1.2.1. Distribution patterns of Eurasian golden Jackal**

The golden jackal's distribution is under documented in their global range. It's known only a rough area available in IUCN, showing Southeast Europe, Middle East and Southeast Asia with presence of Golden Jackal (Fig.2). The exact boundaries of the occurrence area are not known, namely the expansion front in Europe, the northern range limit in Asia and the potential connection between Europe and Asia.

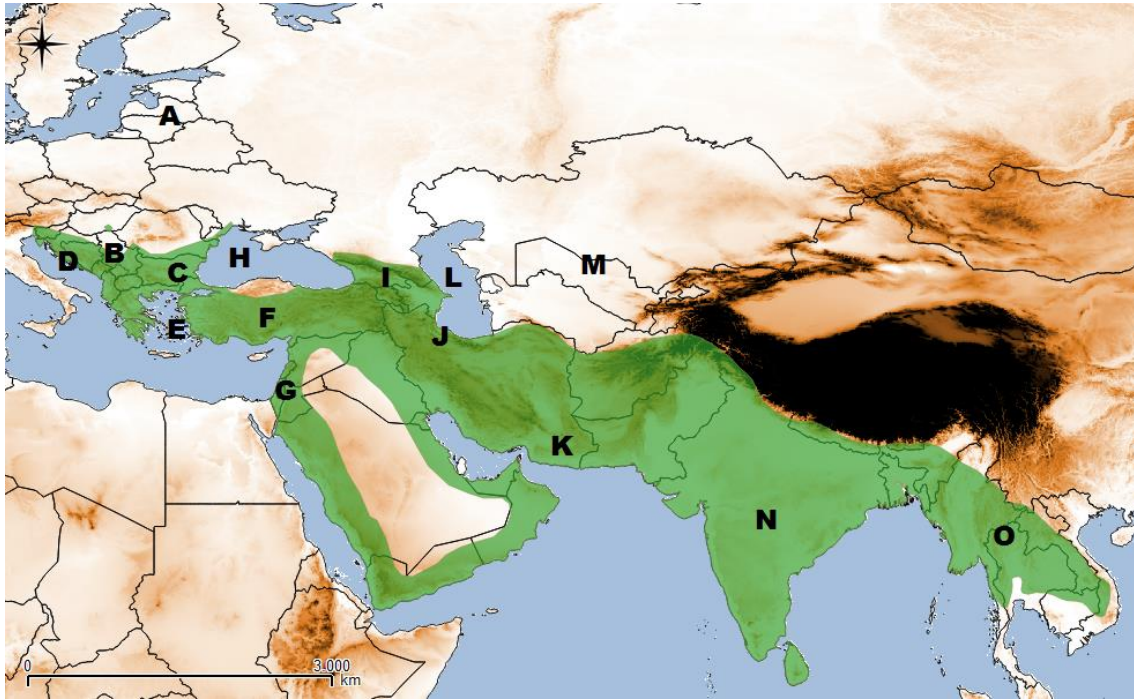


Figure 2 - Geographic range of the Eurasian Golden Jackal according to IUCN Red List of Threatened Species (<http://www.iucnredlist.org/>) and main regions mentioned along the text: A – Baltic region; B – Panonian Basin; C – Danube river; D – Dalmatia (Coastal Croatia); E – Samos Island (Greece); F – Turkey; G – Levant; H – Black Sea; I – Caucasus; J – Elbrus Mountain (North Iran); K – Baluchistan (South of Iran); L – Caspian Sea; M – South former-URSS; N – Indian subcontinent; O – Southeast Asia

In Asia, there are few studies addressing jackal distribution and often involve a few number of records. Nevertheless, Golden Jackal's documented range in Asia is vast, with records from the Middle East to Southeast Asia (Table 1). There are no evidences of expansions or regressions of populations in Asia, except at local scale in Israel (Cohen et al. 2013). In Europe, golden jackal appears better documented than in Asia, but their recent expansions and range extent are poorly known. Golden jackals nearly became extinct during the 1960s, in Europe, due to habitat loss and persecution with poisoned baits (Spassov 1989). Nucleus populations were found only in some dispersed regions, for example, Strandja (Bulgaria and Turkey), Dalmatian (coastal Croatia), Aegean Macedonia and the Peloponnesus (Spassov 1993; Giannatos 2004; Spassov 2007; Arnold *et al.* 2012). Apart from Greece, where the jackals are on the decline and listed as vulnerable in the national Red List, the species has expanded its European distributional range, most notably in Bulgaria, which now supports the largest jackal population in Europe (Krystufek *et al.* 1997) (Table 1).

Table 1 – Documented distribution of golden jackal by regions in Asia and Europe.

Region	Countries	Distribution	References
<b>Southeast Asia</b>	India	Considered as well distributed throughout India, even with scarce available information.	Mukherjee <i>et al.</i> 2004 Jhala and Moehlman 2008 Vanak and Gompper 2009 Kait and Sahi 2010 Majumder <i>et al.</i> 2011 Mondal and Qureshi 2012 Gupta <i>et al.</i> 2014 Alam <i>et al.</i> 2015 Prerna <i>et al.</i> 2015
	Nepal, Bangladesh, Bhutan, Cambodia, Myanmar and Thailand.	Considered widespread but with scarce available information	Heptner and Naumov 1996 Rabinowitz and Khaing 1998 Jaeger 2007 Gray and Phan 2011 Jenks <i>et al.</i> 2011 Katuwal and Dahal, 2013 Thapa <i>et al.</i> 2013 Chutipong <i>et al.</i> 2014
<b>Caucasus</b>	Georgia, Armenia and Azerbaijan	Considered widespread but with scarce available information	Vershechagen 1959 Heptner and Naumov 1996
<b>Western Asia</b>	Turkey	Along the coasts of the Black Sea and Aegean Sea, being absent in the area between Tekirdag and Istanbul, and also in the central steppe plateau	Krystufek <i>et al.</i> 1997
	Israel	Appears in large numbers, because of the implementation of laws that protect wildlife in conjunction with conservation actions, but in 1964, a large-scale poison eradication program resulted in the near-complete extermination of populations	Mendelssohn 1972 Cohen <i>et al.</i> 2013
	Jordan, Syria and Iraq	Considered widespread but with scarce available information	Hatt 1959 Qumsiyeh <i>et al.</i> 1993 Bunaian <i>et al.</i> 2001 Amr <i>et al.</i> 2004 Masseti <i>et al.</i> 2009
<b>Europe</b>		Reported on the east coast of Adriatic Sea Dalmatia, north-eastern Italy, south of Hungary, Serbia and on the left bank of Danube River in southern part of Romania. Recent population expansions have been recorded in Central Europe (Austria, Swizerland, Poland) and Northeast Europe (Baltics).	Krystufek <i>et al.</i> 1997 Angelescu 2004 Krofel and Potocnik 2008 Lapini <i>et al.</i> 2009 Szabo <i>et al.</i> 2009 Zachos <i>et al.</i> 2009

Detailed assessments of the jackal's distribution have been done only in Europe (Fig. 3). Krystufek *et al.* (1997) (Fig. 3A) published the first review on jackal distribution based in a compilation of records without systematic survey and presenting course range maps. The first systematic survey for this species was conducted by Mitchell-Jones *et al.* (1999) (Fig. 3B) in the scope of the "Atlas of European Mammals" presenting confirmed records of golden jackal before and after 1970 at a 50x50 km UTM grid, and showing the first evidences of population expansion. More recently, Arnold *et al.* (2012) (Fig. 3C) presented an updated review on jackal distribution records and areas and showed that golden jackal was expanding towards north and west. Finally, a recent article presents a detailed and updated account of the current distribution of golden jackal in Europe (Trouwborst *et al.* 2015) (Fig. 3D), considering for the first time both areas occupied by territorial groups and records of vagrant animals, revealing new areas of occurrence in the Baltic region and in central Europe, such as Switzerland and Germany.

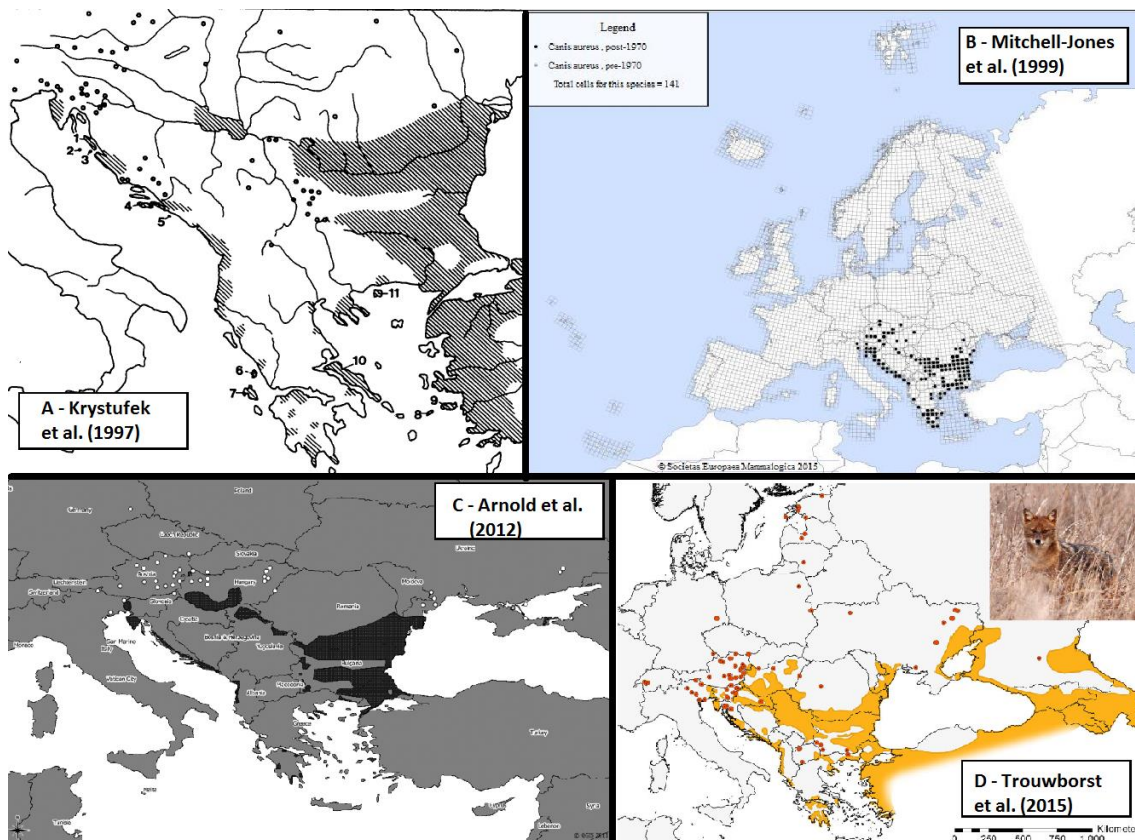


Figure 3- Main studies with distribution assessments of Golden Jackal in Europe: A – Krystufek *et al.* (1997); B – Mitchell-Jones *et al.* (1999); C – Arnold *et al.* (2012); D - Trouwborst *et al.* (2015)

In Europe, various populations are in expansion (towards central and northeast Europe) while others have evidences of regression (e.g. Greece and Balkan region), revealing different population histories and most likely different genetic characteristics which should be reflected in different managements and legal measures. This new

expansion from Baltic region raised new legal issues, namely the consideration of golden jackal as invasive species and its inclusion as a hunting species in several European countries. Trouwborst *et al.* (2015) demonstrated that jackals are not an invasive species and because of that the governments of Baltic region, where population persist for centuries, should reevaluate hunting laws.

### 1.2.2. Genetic characterization of the Eurasian Golden Jackal

The golden jackal belongs to genus *Canis*. Phylogenetic studies show that grey wolf, coyote and Ethiopian Wolf (*Canis simensis*) form a monophyletic group, with the golden jackal as the most likely sister taxon (Fig. 4) (Sillero-Zubiri *et al.* 2004, Lord *et al.* 2013; Koepfli *et al.* 2015). The golden jackal is currently not considered an African inhabitant. Very recently, Koepfli *et al.* (2015) show that African and Eurasian golden jackals are genetically distinct and independent lineages, and that African golden jackals likely represent a separate species, based on their results of mtDNA, nuclear loci, and whole genomes. Using morphologic data, they also demonstrate a spectacular morphologic similarity between East African and Eurasian golden jackals, suggesting parallelism, which may have mistaken taxonomists.

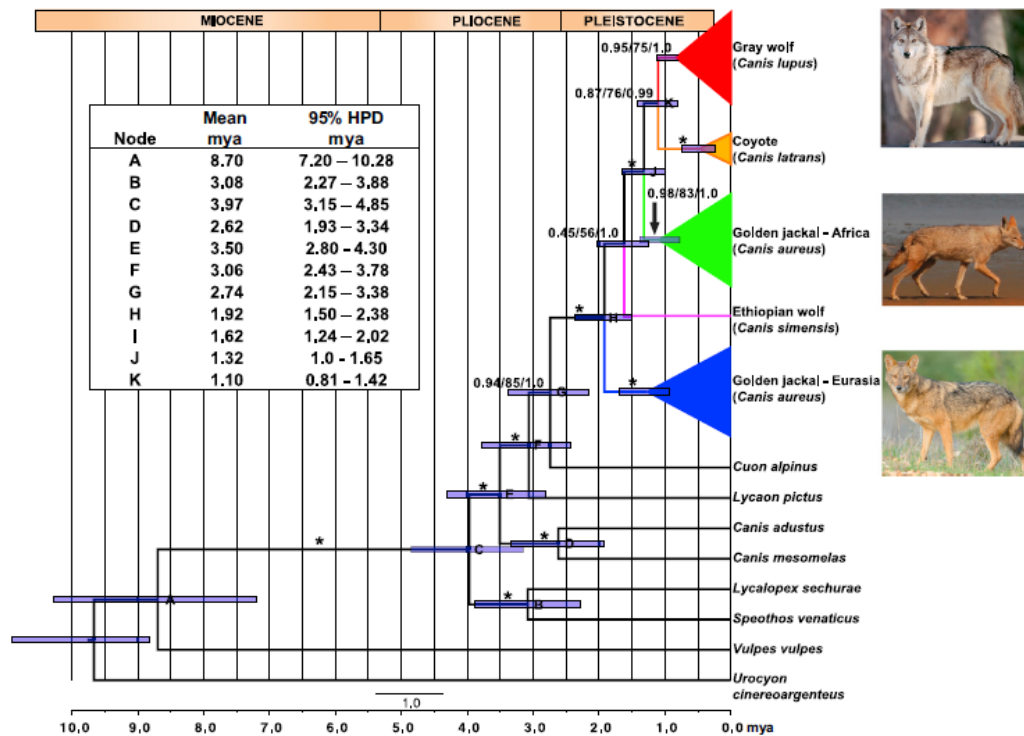


Figure 4 – Phylogenetic tree based on analysis of twenty nuclear gene segments (adapted from Koepfli *et al.* 2015)

The genetic structure and diversity of golden jackal populations still remains largely unknown and merely genetic studies from regional character exist. Confirming this, a unique genetic study with golden jackals in Israel suggested that five subpopulations could be discerned among local individuals, and although values of genetic diversity were generally low between geographically adjacent subpopulations, were higher in comparisons involving the center subpopulation suggesting the genetic isolation of canids in this region. (Cohen *et al.* 2013).

In Europe, Zachos *et al.* (2009) demonstrated very low genetic variability among subpopulations in Serbia, and an Austrian vagrant jackal was genetically indistinguishable from the Serbian animals with respect to both mtDNA and microsatellites. This is interesting because it has been assumed that the golden jackals found in Italy, Slovenia and Austria came from Istria in northwestern Croatia (Krystufek *et al.* 1997). In other genetic study, bayesian and landscape genetics models showed a weak population subdivision between Bulgaria, Serbia and Italy, but golden jackals from Dalmatia clustered separately from the other samples (Fig. 5) (Fabbri *et al.* 2013). Moreover, allele richness and heterozygosity were smaller in Dalmatia than in the other populations. Reduced genetic diversity suggests that jackals in Dalmatia might have experienced a demographic bottleneck in the past (Cornuet and Luikart 1996) and have survived in isolation for centuries. Population structure and assignment tests concordantly indicated that jackals colonizing Italy have admixed genotypes that originated from the Dalmatian and Slavonian populations.

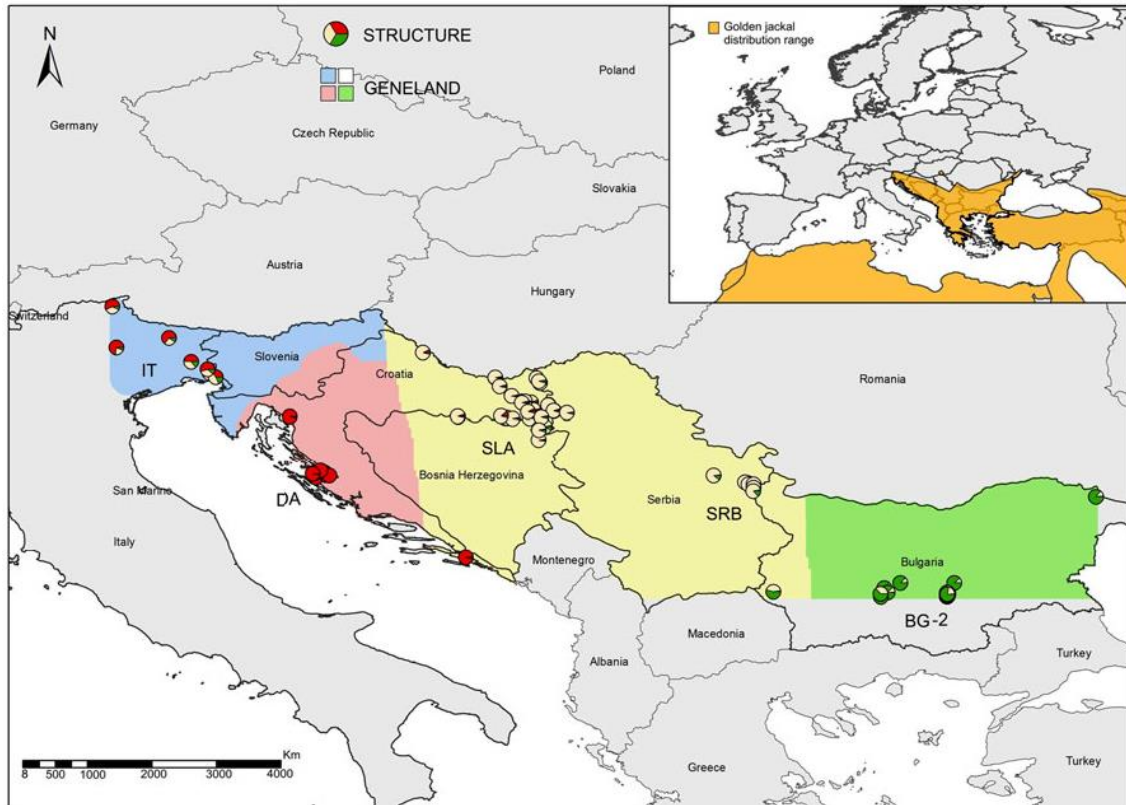


Figure 5 – Patterns of genetic structure of Golden Jackal in Europe based in the analysis of 14 microsatellites and according to results from Structure and Geneland (shaded areas) (adapted from Fabbri *et al*, 2013).

Nevertheless, further genetic analyses of jackals from other countries in southeastern Europe are needed, and there are also missing studies addressing distribution patterns and genetic traits of golden jackals in intercontinental scale, including Middle East and Southeast Asian populations. Several sub-species were described based on morphology, but genetic studies are missing (Ellerman and Morisson-Scott 1951; Coetzee 1977; Sillero-Zubiri *et al*. 2004).



### **1.3. Objectives**

This study aims to investigate the distribution patterns and genetic structure of the Eurasian golden jackal by focusing three main goals:

- 1) Assess the past and current range of golden jackals in Europe and Asia by an exhaustive bibliographic compilation of presence records. With this approach we expect to improve the knowledge on the current distribution of the Eurasian golden jackal, evaluate the distribution trends of the expanding golden jackal population in Europe and identify areas with fossil records of this species.
- 2) Determine the potential distribution of golden jackal in Europe and Middle East by modelling known presence records. With this approach, we intend to identify areas of potential connectivity between European and Middle East populations and to provide insights on the potential distribution in the areas where presence is poorly known, such as Turkey, Belarus and Ukraine.
- 3) Analyze population structure and genetic diversity in golden jackals from Europe and Middle East based on microsatellite data. With this approach, we expect to determine local and large scale patterns of genetic diversity and differentiation, major population clusters and make inferences on source populations and routes of expansion in recently colonized European areas.

Considering these three main goals, we intend to address the following questions:

What are the time periods with a higher number of presence records and bibliographic sources?

What is the degree of updated of IUCN distribution range of golden jackal updated and how much area are outdated?

Are all golden jackal populations in Europe a result of recent expansions or there are some relict populations?

What are the potential areas of connectivity between Europe and the Middle East?

What is the degree of connectivity and isolation between golden jackals populations in Europe and Middle East?

What is the source of the recent population occurring in Central and Northeastern Europe?

What is the degree of genetic diversity across Europe and Middle East and how is it geographically distributed?

Is genetic variability geographically structured?

The results of this study are expected to contribute to increase our understanding on the range patterns as well as the genetic diversity and population structure of the Eurasian golden jackal, in order to increase the available knowledge on this species and to improve our capabilities for its efficient management and conservation.

## 2. Materials and Methods

### 2.1. Distribution patterns

#### 2.1.1. Data collection

A compilation of presence records from golden jackal was done based in an intensive literature review, focused both in scientific journals and grey literature. For that, search engines were used such as Google Scholar, Web of Science, Sci-Hub and BookSc. In this search, the keywords used were “golden jackal”, “*Canis aureus*”, “carnivore”, “mammals”, each combined with the country name within this species known range. The compilation of presence records was obtained based on both text and figures from various scientific articles, technical reports and websites (references in Annex 1). For records present in text, an excel database was constructed in which country, locality/city/region, continent, latitude, longitude, date of occurrence, reference of article or journal and type of record (territorial group, vagrant animal, photographed, shot, runover or fossil record) was annotated. When latitude and longitude values were missing, we considered the geographic coordinates of the locality, city or region that was mentioned. Figures were georeferenced to extract the coordinates of presence records. Tissue samples used for genetic analysis were also considered as presence records whenever the exact geographic coordinate was known. Based on these presence records, a new global range of Eurasian golden jackal was constructed to update global range maps available at the IUCN. Furthermore, it was made a comparison between the total areas of update and present IUCN global range, as well as, for a continental scale in Europe and Asia (the whole Russia and Turkey were considered in Asian continent, and Ukraine and Belarus in Europe), using the IUCN criteria (<http://www.iucnredlist.org/documents/RedListGuidelines.pdf>).

Considering the timescale of each presence record in all jackal range in Europe and Asia, maps were made for prehistoric records (Late Postglacial, Holocene, Neolithic and Bronze age; from 20,000 years ago to 2,600 years ago) and historic-present records (from 1384 to present). Only for Europe, where more data are available, distribution trends of the species were accessed by mapping records to the scale of UTM 50X50 km and considering the following time intervals: prehistoric records, between 1384-1850, 1850-1950, 1950-1980, 1980-2010, and after 2010. All spatial analysis were done using a Geographic Information System (QGIS 2.8.).

Furthermore, number of presence records per time intervals were analysed to Europe and Asia.

Based on literature search, the number of bibliographic sources per decade and per type of publication (Scientific Citation Index (SCI) journal, non-SCI journal, Book, Report or Website) were performed simultaneously to Europe and Asia. A journal was considered a non-SCI journal when the impact factor was 0.

### **2.1.2. Modelling potential distribution**

To model the potential distribution of golden jackals in Europe and Middle East, we used 653 presence records and 11172 absences (generated by subtracting the total of squares with the number of presence records) at a scale of UTM 50km×50km, obtained from the compilation of bibliographic records mentioned above. We used 22 predictor variables related to topography, climate and human activity, which are expected to affect jackal occurrence (Table 2). All 19 climatic variables and altitude were obtained from WorldClim – Global Climate Data (Hijmans et al. 2005) and averaged on the UTM cells with the raster package (Hijmans 2015) under the R software (R Core Team 2014). Variables related to Human activity included human density (Oak Ridge National Laboratory (2008) and the Global Radiance Calibrated Nighttime Lights (NOAA's National Geophysical Data Center (2015)). The values of these variables per UTM cell were extracted with the Zonal Statistics plugin in QGIS 2.6 (QGIS Development Team, 2015).

To build the distribution model of Golden Jackal we used the favorability function described in Real et al. (2006), a GLM that computes local variations in species presence probability in relation to overall species prevalence. With the favorability function, thresholds for all models are levelled according to the species prevalence in each area. A favorability value of 0.5 means that presence of the species is as probable as its prevalence in the corresponding study area, i.e., neutral environmental favorability.

Regressions were performed using forward–backward stepwise variable selection (Pearce and Ferrier 2000) using Akaike's Information Criterion (AIC). Any non-significant variables left in the model after this procedure were removed. Stepwise selection is commonly used in distribution modelling (Araújo et al. 2005; Bulluck et al. 2006; Arntzen 2006) as it maximizes predictive efficiency in an objective and

reproducible way and is a useful and effective tool when the individual importance of each variable is not known a priori (Hosmer and Lemeshow 2000). This methodology is implemented in a software package, fuzzySim (Barbosa 2015), which allows a variety of methods for converting (multiple) species presence/absence data into continuous, fuzzy surfaces, including inverse distance to presence raised to any power, trend surface analysis of any given degree and generalized linear models based on presence–absence. Such models have been successfully used for several species before (Barbosa et al. 2009; Barbosa et al. 2010).

Table 2 - Environmental variables used to model the distributions of the Golden Jackal in Europe and Middle East and their positive (+) or negative (-) influence.

Type of variable	Variable	Influence (+/-)	Source
Climate	Annual Mean Temperature	+	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Mean Diurnal Range (Mean of monthly (max temp - min temp))	+	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Isothermality (* 100)	-	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Temperature Seasonality (standard deviation *100)	-	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Max Temperature of Warmest Month	-	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Min Temperature of Coldest Month	+	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Temperature Annual Range (BIO5-BIO6)		<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Mean Temperature of Wettest Quarter	+	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Mean Temperature of Driest Quarter		<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Mean Temperature of Warmest Quarter	+	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Mean Temperature of Coldest Quarter	-	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Annual Precipitation		<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Precipitation of Wettest Month	-	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Precipitation of Driest Month	+	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Precipitation Seasonality (Coefficient of Variation)	-	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Precipitation of Wettest Quarter	+	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Precipitation of Driest Quarter	-	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Precipitation of Warmest Quarter		<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Climate	Precipitation of Coldest Quarter	+	<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Topographic	Altitude		<a href="http://www.worldclim.org/bioclim">http://www.worldclim.org/bioclim</a>
Human	Night Light	-	<a href="ftp://ftp.ngdc.noaa.gov/DMSp/web_data/x_rad_cal/rad_cal.tar">ftp://ftp.ngdc.noaa.gov/DMSp/web_data/x_rad_cal/rad_cal.tar</a>
Human	Human population		<a href="http://web.ornl.gov/sci/landscan/">http://web.ornl.gov/sci/landscan/</a>

## 2.2. Genetic analyses

### 2.2.1. Samples

A total of 224 tissue samples of golden jackal (animals founded dead by shot or runover, for example) were obtained by a network of collaborators across most jackal range in Europe and Middle East (Figure 6) comprising the following countries: Germany (1), Austria (3), Slovenia (4), Hungary (15), Croatia (31), Serbia (49), Bulgaria (19), Romania (5), Macedonia (4), Greece (10), Latvia (9), Estonia (7), Georgia (6), Azerbaijan (1), Iran (42), Israel (15) and Turkey (3). All tissue samples were preserved in ethanol at room temperature until DNA extraction.

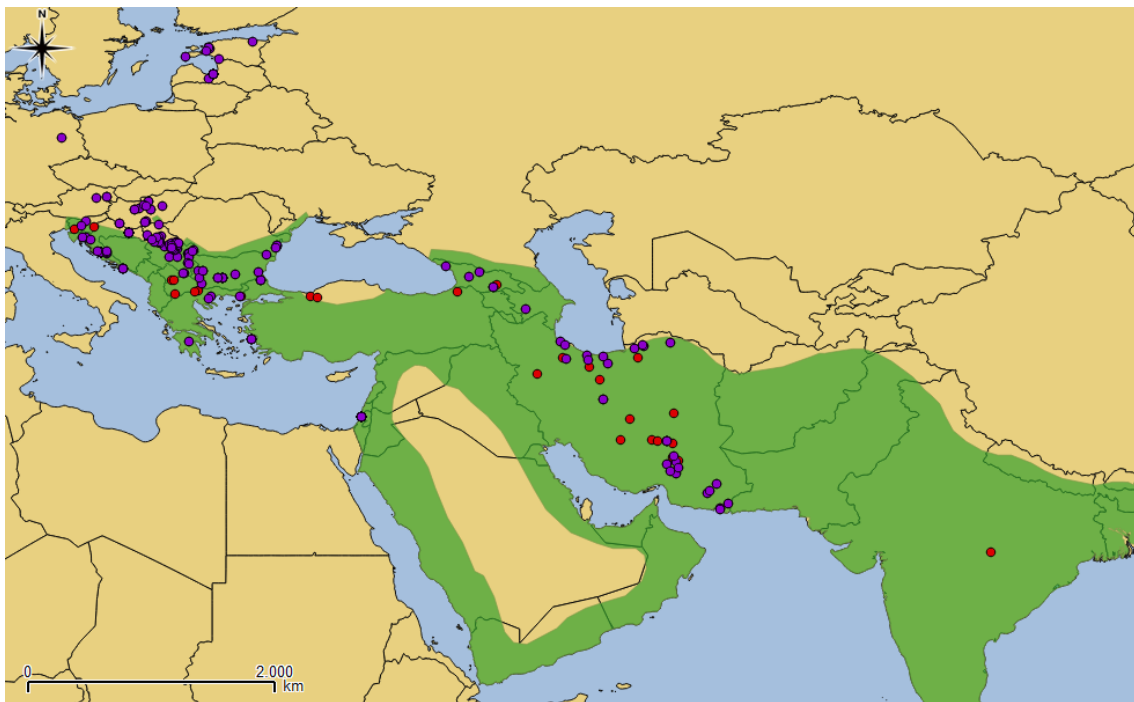


Figure 6 - Location of the samples available for this study and the geographic distribution of Eurasian golden jackal according to the IUCN (<http://www.iucnredlist.org/>). Purple dots – successful samples in DNA extraction and microsatellites genotyping. Red dots – samples that failed DNA extraction or genotyping.

### 2.2.2. DNA extraction

The DNA of tissue samples was extracted using QIAGEN's EasySpin Kit, following an adapted protocol for tissue samples (available in Annex A3). The success of DNA extraction was assessed by electrophoresis on 0.8% agarose gels stained with Gel Red, in TBE 0.5x buffer, run for 10 to 15 minutes at 300V. Further visualization

through UV radiation was achieved in a Biorad Universal Hood II Quantity One 4.4.0. According to the estimated DNA concentration, the samples were appropriately diluted in ultrapure water to allow proper amplification PCR and stored at - 20 °C, where they remained throughout the work laboratory.

### **2.2.3. Microsatellite amplification and genotyping**

In this study we used 43 autosomal microsatellites developed for canids, summarized in the Annex A4 and already optimized in the lab by Godinho et al. (2011, 2015). PCR reactions were performed in four multiplex reactions (MS1, MS2, MS3 - Supplementary Material in Annex A5) at a final volume of 10 $\mu$ L, with 5 $\mu$ L of Qiagen Master Mix, 1 $\mu$ L of DNA, 1 $\mu$ L of the primer mix and 3 $\mu$ L of pure water and in another multiplex reaction (Finnzymes) at final volume of 10 $\mu$ L, with 5 $\mu$ L of Fusion Master Mix, 1 $\mu$ L of DNA, 3 $\mu$ L of the primer mix and 1 $\mu$ L of pure water. For multiplex MS1, MS2 and MS3 touchdown PCR reactions were conducted. For MS1 the PCR profile was the following: initial denaturation at 95°C for 15 minutes; 20 cycles with a denaturation step at 95°C for 30 seconds, annealing at 58°C for 45 seconds and extension at 72°C for 45 seconds; 15 cycles of 95°C for 30 seconds, 56°C for 45 seconds, 72°C for 30 seconds; 10 cycles of 95°C for 30 seconds, 53°C for 45 seconds, 72°C for 30 seconds, followed by a final elongation step at 60°C for 30 minutes. For MS2, the PCR was made with an initial denaturation at 95°C for 15 minutes; 35 cycles with a denaturation step at 95°C for 30 seconds, annealing at 56°C for 45 seconds and extension at 72°C for 45 seconds; 8 cycles of 95°C for 30 seconds, 53°C for 45 seconds, 72°C for 30 seconds, followed by a final elongation step at 60°C for 30 minutes. Finnzymes multiplex, a commercial kit, reactions were performed according to the manufacturer's instructions. For MS3, PCR profile was the following: initial denaturation at 95°C for 15 minutes; 7 cycles with a denaturation step at 95°C for 30 seconds (decreasing 0.5°C each cycle), annealing at 60°C for 45 seconds and extension at 72°C for 45 seconds; 22 cycles of 95°C for 30 seconds, 57°C for 45 seconds, 72°C for 30 seconds; 8 cycles of 95°C for 30 seconds, 53°C for 45 seconds, 72°C for 30 seconds, followed by a final elongation step at 60°C for 30 minutes. Amplifications were performed in Biorad T100 Thermal Cyclers, and a negative control was used in each PCR to monitor for possible contaminations. After the amplification, PCR products were accessed by electrophoresis on 2% agarose gel stained with Gel Red, using a mass DNA ladder (NZYDNA Ladder V), with further conditions being identical to the ones described for the extraction electrophoreses. PCR products were diluted, if necessary, according to

the observed quality in the agarose gels and were then separated by capillary electrophoresis on an automatic sequencer ABI3130xl Genetic Analyzer (AB Applied Biosystems), using 1µl of amplification product for 10µl of formamide 75-400 (-250) LIZ NEW size standard.

Microsatellite reading and scoring was performed in GENEMAPPER v4.0 (Applied Biosystems). Automated scoring was combined with manual checking to mitigate the amount of errors. Very sporadic suspicious genotypes were declared as missing data. Samples were PCR amplified up to three times in case of amplification failure.

## 2.2.4. Statistical analyses

Samples and loci presenting 40% or more of missing data were discarded from the analysis. A final dataset was created with 184 samples from Asia and Europe, representing 82% of the initial sampling.

Bayesian clustering and admixture analyses were performed in STRUCTURE v2.3.4 (Pritchard *et al.* 2000), using admixture model with correlated frequencies and no priors for putative populations. Ten independent simulations were performed for each K (1 to 8), with  $1.0 \times 10^6$  MCMC interactions and a burn-in of  $5.0 \times 10^5$  interactions. Population information was not considered in this analysis in order to infer the most probable number of genetic clusters without a priori definition of populations. The results were uploaded to the STRUCTURE HARVESTER 0.6.94 (Earl and vonHoldt 2012) in order to identify the most likely number of genetic clusters (K) according to the highest value of  $\Delta K$  (Evanno *et al.* 2005). To visualize patterns of genetic differentiation among individuals, a principal coordinate analyses (PCoA) was performed using GenALEx v6.5 (Peakall and Smouse 2006). A second dataset with 142 samples from Europe was created and bayesian clustering and admixture analyses were performed in STRUCTURE v2.3.4, to compare our results with other results from literature. The spatial locations of genetic clusters were analysed further by landscape genetic analyses in GENELAND 4.0.3 (Guillot *et al.* 2005), with  $10^6$  MCMC iterations (thinning = 100 and post-process burn-in = 50 iterations).

Genetic diversity was examined for nine populations defined from results of STRUCTURE and by geographical coherence with the existence of historic-present records and recent distribution trends: Baltics (Latvia and Estonia), Samos Island



(Greece), Dalmatia (coastal Croatia), Central Europe (Germany, Austria, Slovenia, Hungary and western of Serbia), South Europe (Eastern Serbia, Bulgaria, Romania and Greece), Caucasus (Azerbaijan, Georgia and Northwestern Iran), Northeastern Iran, Baluchistan (Southern Iran) and Israel. Deviations from Hardy-Weinberg equilibrium and tests for linkage disequilibrium across all populations and loci were performed in GENEPOP v4.2 on the web (Raymond and Rousset 1995; Rousset 2008). Genalex 6.5 (Peakall and Smouse 2006, 2012) was used to calculate genetic diversity index such as observed ( $H_o$ ) and expected heterozygosity ( $H_e$ ), mean number of private alleles per locus and  $F_{ST}$  pairwise analyses between populations.

### 3. Results

#### 3.1. Available knowledge on golden jackal distribution

A total of 86 publications were found with references to golden jackal presence in its entire range, from which 55 were from Asia and 33 from Europe. Considering the year of publication, there is a clear trend towards an increase of available literature with time, which is noticed in both continents, being 73% of publications dated after 2000 (Fig. 7A). Regarding publication type, in Europe most of publications on jackals are found in reports or nonscientific journals, such as books, reports and websites; (67%), whereas in Asia 58% of publications were in Scientific Citation Index (SCI) journals (Fig. 7B). Only four reviewed publications, all of them in Europe, were concerning distribution atlases at a large (e.g. above country level) or continental scale resulting from a detailed and systematic survey to detect jackal presence.

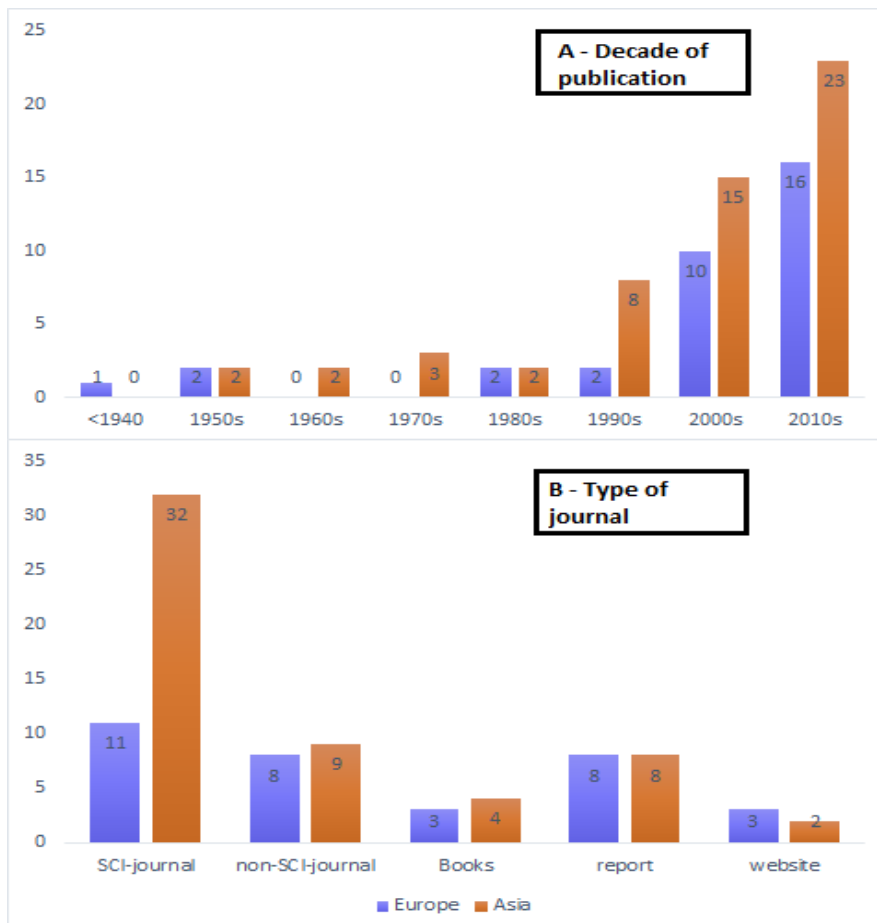


Figure 7 – Number of bibliographic reports per decade of publication (A) and per type of publication (B)

### 3.2. Distribution patterns and trends

Based in bibliographic search, a total number of 1842 presence records of Eurasian Golden Jackal were obtained across their entire range in Europe and Asia. In general, until 1980, we found more records from Asia than Europe, occurring a significant increase of records between 1980 and 2010 in both continents, and more recently, there are more records of golden jackal in Europe than in Asia (Fig. 8).

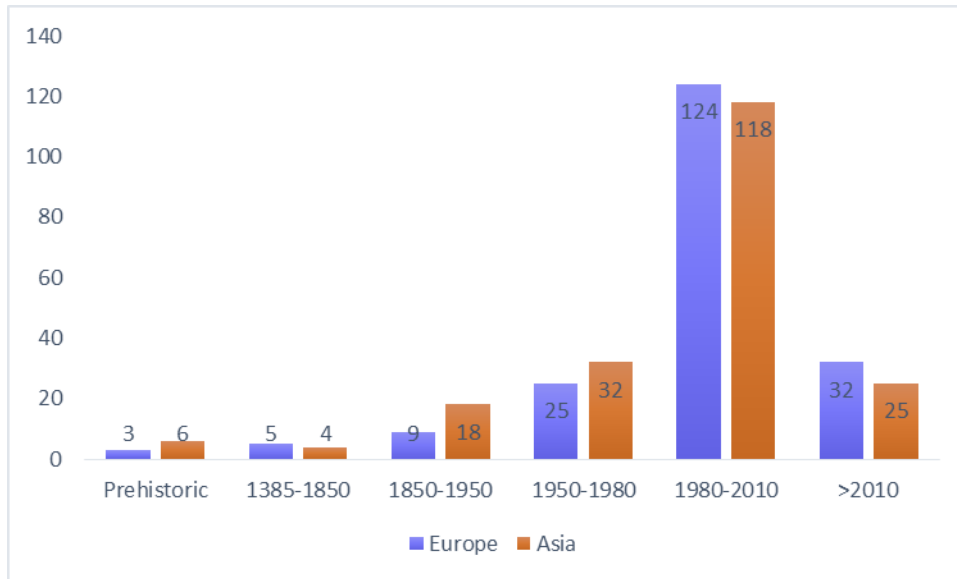


Figure 8 - Number of presence records per time periods

Presence records were analysed in two main time periods: prehistoric records (Last postglacial, Holocene, Neolithic and Bronze Age), ranging from 20,000 years ago to 2,600 years ago (N=9 records) and historic records, ranging from 1384 (XIV<sup>th</sup> century) to present times (N=1833 records) (Fig. 9). Prehistoric records of jackals were found in Europe and Middle East. The oldest fossil record is from Ksar Akil (Palestine), dating from late postglacial, approximately 20.000 years ago. From lower Holocene, more precisely around 11.700 years ago, it was found a fossil in Hvar Island (Croatia). Also from Holocene, four more fossils were found in Mousterian Cave (West Iran), Apsheron Peninsula (Azerbaijan), Saraibulakh ridge and Tskhali-Tsiteli gorge (Armenia). In Greece, golden jackal's remains were found in Delphi and Kitsos, dating from Neolithic (10.200 – 4.000 years ago). More recently, from Bronze Age (3.300 – 2.600 years old), a fossil was found in Bisotun Cave, near Behistun, West Iran.

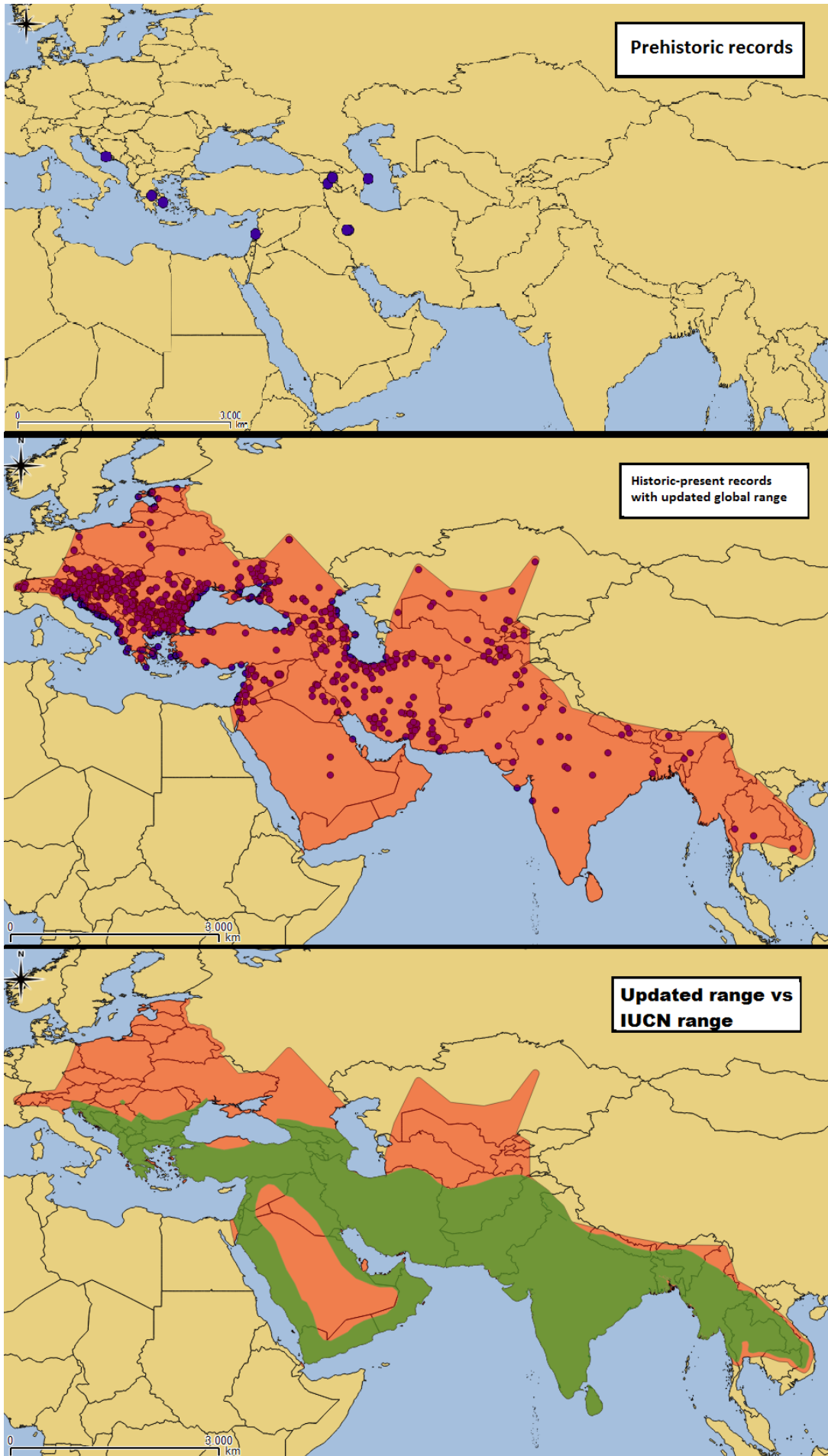


Figure 9 - Presence records of golden jackals compiled for Europe and Asia in two time periods: prehistoric times (A) and historic-present times (B) with shaded area that represents the updated range distribution of golden jackal. C - Comparison between IUCN global range (green) and Updated global range (red) of Eurasian golden jackal

At least one presence record was obtained per country within golden jackal known range (Fig. 9 and Annex 2). However, a much higher number of known records were obtained in Europe (N=1250) than in Asia (N=592), reflecting the amount of available literature and knowledge in jackal distribution. Europe, Iran and Caucasus are the regions where more information on jackal distribution was obtained. Our bibliographic search allowed to detect the presence of golden jackals in wide new areas located north from the previously known geographic distribution of the species according to IUCN, including most of Central and Northeast Europe (Switzerland, Italy, Germany, Austria, Czech Republic, Slovakia, Hungary, Romania, Moldavia, Ukraine, Poland, Belarus, Latvia, Estonia), northern Black sea and Caucasus (Ukraine, Russia) and central Asia (Turkmenistan, Uzbekistan, Kazakhstan and Tajikistan). The 1842 presence records allowed to map an updated global range of Eurasian golden jackal with 1713.95 km<sup>2</sup>, increasing about 72% of IUCN global range (Fig. 9 and 10). In Europe the area of presence of golden jackal increases up to five times of IUCN global range and, in Asia, increases 49%.

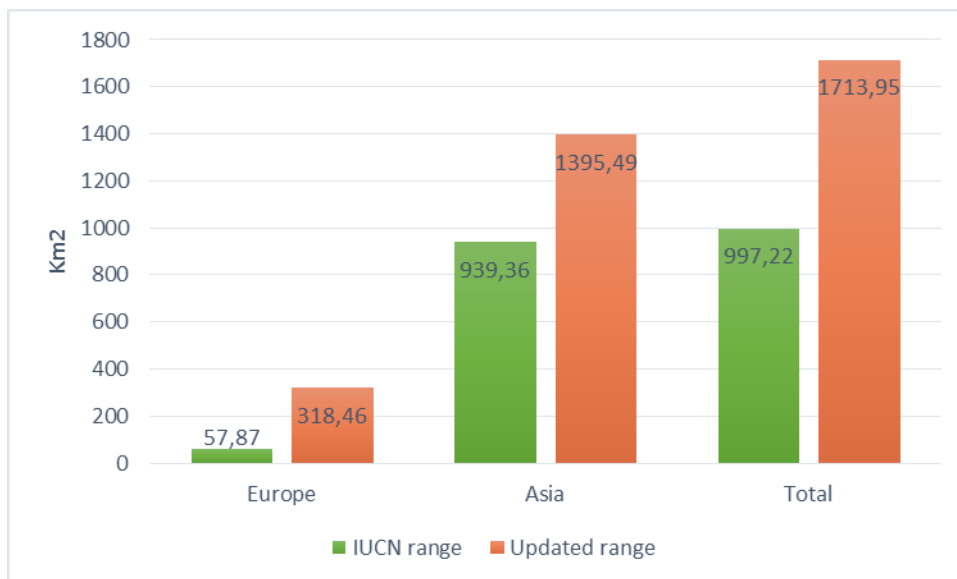


Figure 10 - Updated and IUCN distribution area of golden jackal for the total global range, and for European and Asian ranges, separately.

The distribution trend of golden jackal was evaluated in Europe, based on records represented in 50x50 km grid square across several time intervals (Fig. 11). Jackals are known from pre-historic times only from three records in Dalmatia (coastal Croatia) and southern Greece. Between 1384 and 1850, seven jackal records were obtained from Dalmatia, the Panonian Basin (Hungary), and around the Black sea (Bulgaria and South of Russia). After 1850 until 1950, 24 records were obtained roughly in the same areas and after 1950 up to 1980, 80 records were obtained,

suggesting the expansion along the plains adjacent to Danube River and in coastal areas of Greece and Albania. Between 1980 and 2010, 453 records of golden jackal show the expansion across coast of Mediterranean Sea and Black Sea, as well as until Germany, Slovakia and Austria in the North. After 2010, 334 records comprise the expansion of the species until Switzerland, in the West, and Latvia and Estonia, in the North. Comparing our results with IUCN distribution range of golden jackal, we notice the existence of records outside the area indicated by IUCN for the species occurrence.

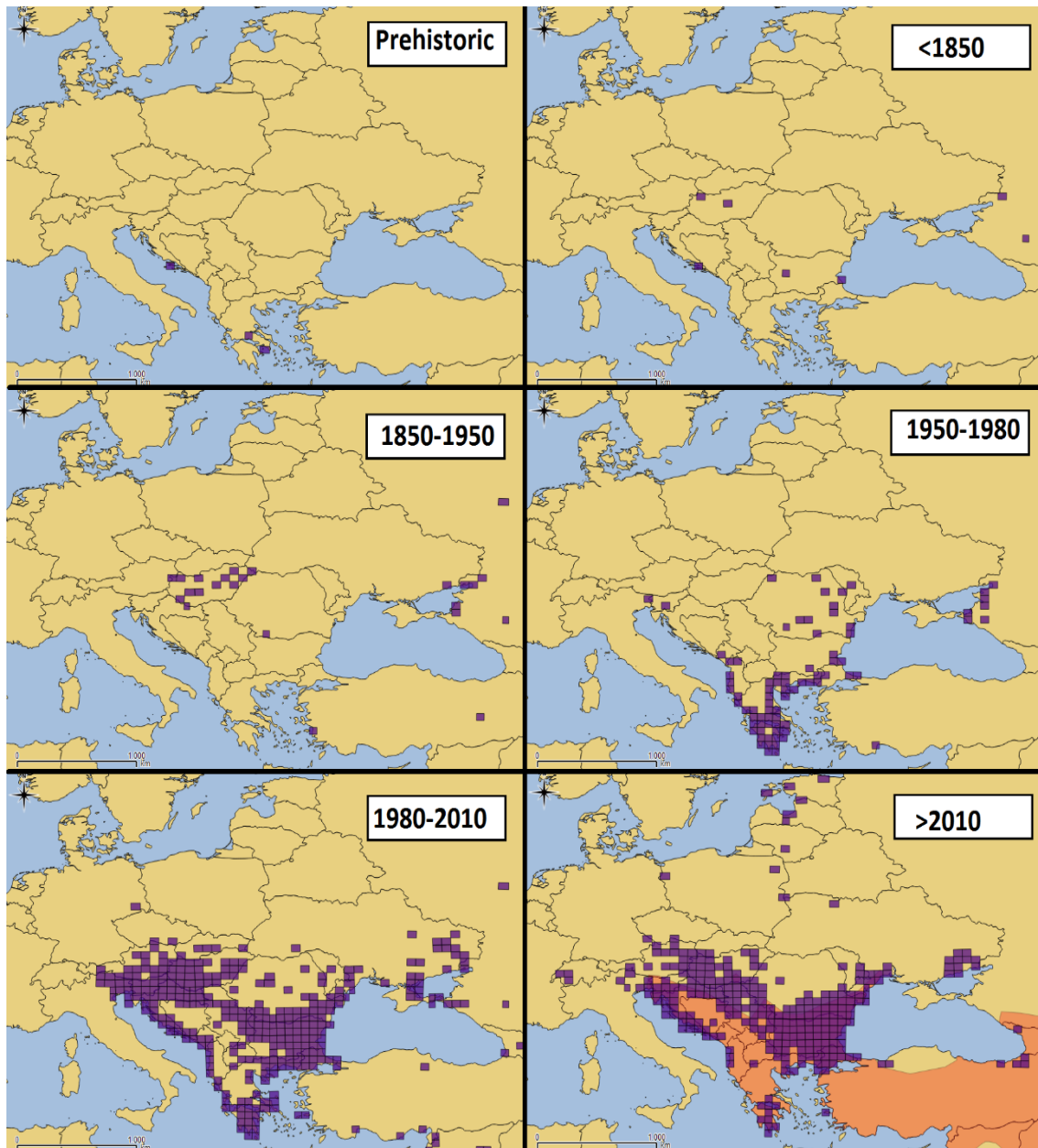


Figure 11 - Distribution of golden jackals in Europe represented in 50x50 km grid squares, across several time periods. A - Prehistoric records. B - Records 1384 until 1850. C - Records between 1850 and 1950. D - Records between 1950 and 1980. E - Records between 1980 and 2010. F - Records after 2010. Shaded area represents the known geographic distribution of golden jackal according to the IUCN (<http://www.iucnredlist.org/>)

### 3.2.1. Potential distribution model

The map of environmental favourability values for Golden Jackal in Europe and Asia is represented in Fig. 12. In general the Asian continent shows lower values of environmental favourability in comparison with Europe. According to the map, both north and south of the Black Sea, that is, both areas of connectivity between Europe and Asia, seem environmentally appropriate for the migration of golden jackals. The high levels of environmental favourability in Central and Western Europe show that the recent expansion of jackals is plausible, as well as possible expansion to the south coast of Scandinavia, where the species is not currently recorded present. The model we analyzed showed good predictive capacity, showing the AUC equal to 0.835.

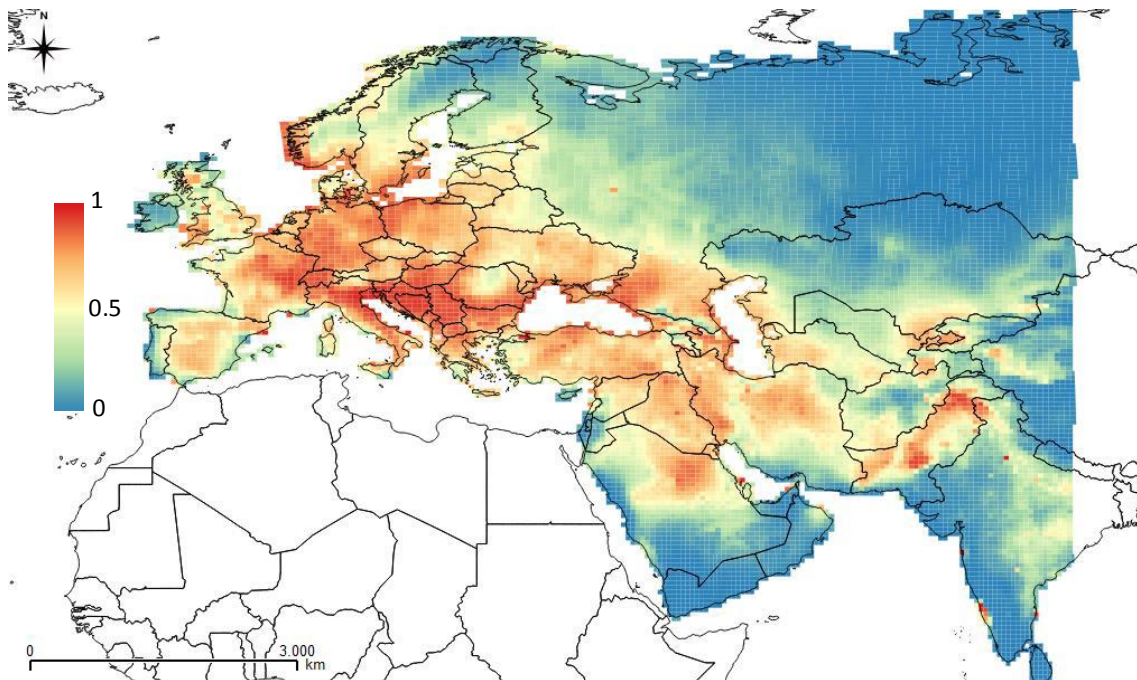


Figure 12 - Potential distribution of Golden Jackal according to an environmental favourability model based on records at a scale of 50 x 50-km UTM grid.

### 3.3. Genetic structure of Golden Jackal populations

Genetic clustering analyses using STRUCTURE showed, according to the Evanno Method, that the most likely number of clusters found in the dataset is two ( $k=2$ ; Annex A6). These two clusters are constituted by Europe and Asia individuals (Fig. 13). Samples from Latvia and Estonia exhibit some degree of genetic admixture between Asia and Europe, as it also happens with samples from Caucasus. However, Baltic golden jackals have a higher component of European genome while in the Caucasus individuals are more related to Asian ones. Clusters can be well defined using Structure until  $k=7$  (Fig. 13). In this analysis the seven clusters are the following: Levant (Israel), South eastern Iran, North of Iran and Caucasus, Baltics, Dalmatia (Coastal Croatia), core range in Europe (central Europe and Greece) and Samos Island (Greece). Based on geographical coherence together with existence of historic-present records and recent distribution trends, we considered nine populations in the following analyzes, separating the core range in Europe in Central and Southern Europe, and in Asia in Caucasus and Northeast Iran.

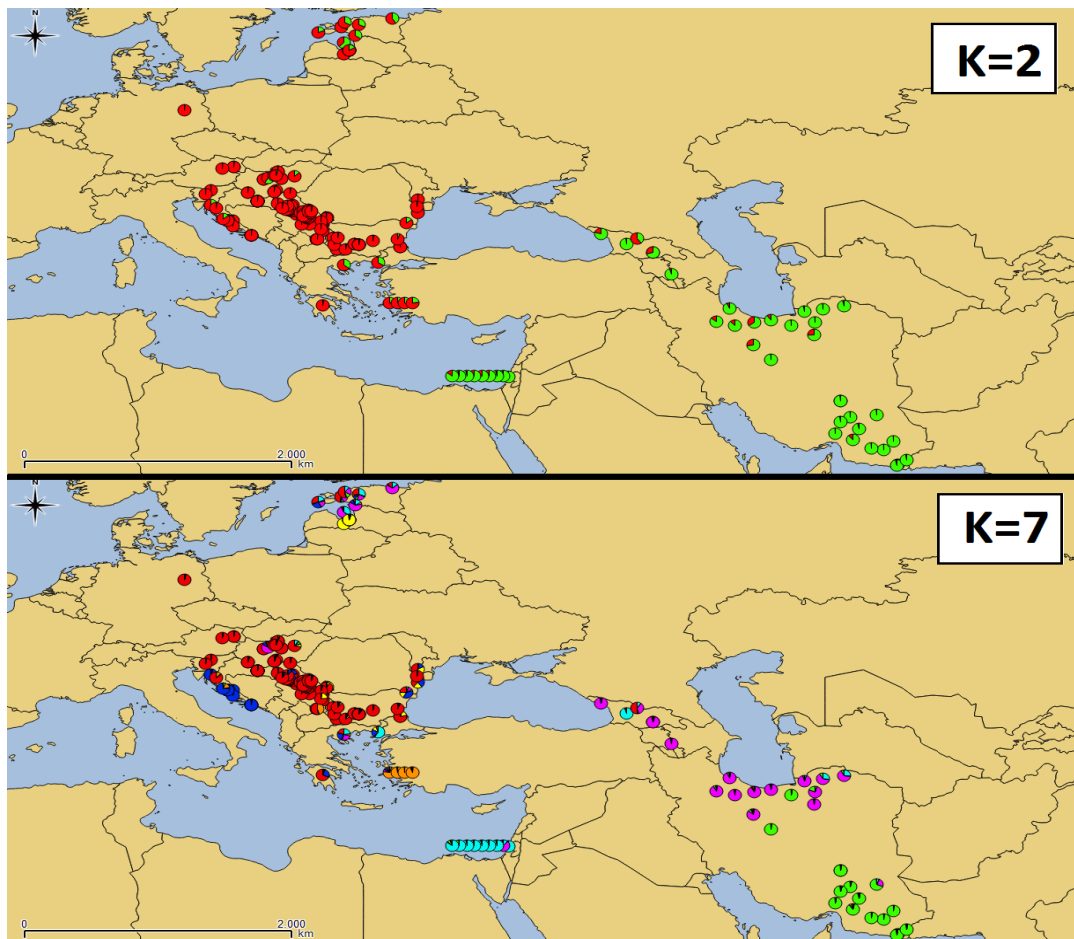


Figure 13 - Distribution of samples according to the STRUCTURE results for  $K=2$ (A) and  $k=7$ (B).



The use of prior sampling location information in GENELAND (Fig. 14) yield two geographical clusters (mean of log posterior density of the model = -12948.34, the best values over ten independent runs), confirming the genetic distinction of golden jackals from Europe and Asia, such as in Structure.

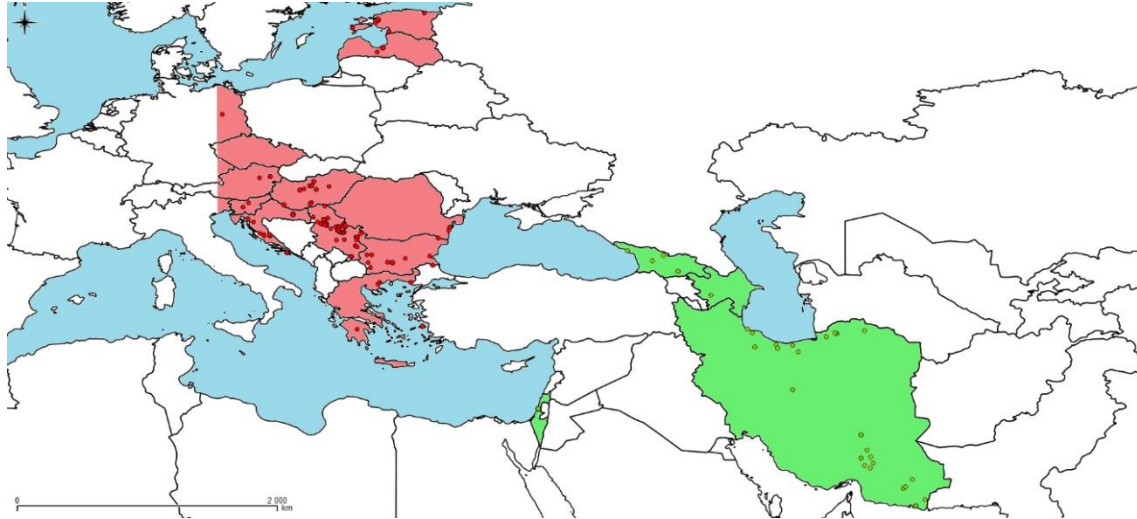


Figure 14 - GENELAND modal population for study area. Dots represents the samples.

The PCoA (Fig. 15) also supports STRUCTURE results, showing a separation of the same two clusters, European and Asian samples, and it is noticeable that European samples are more related between each other than Asian samples. Samos Island appeared isolated from others European populations and Asian populations. In general, all samples show small genetic distances between them.

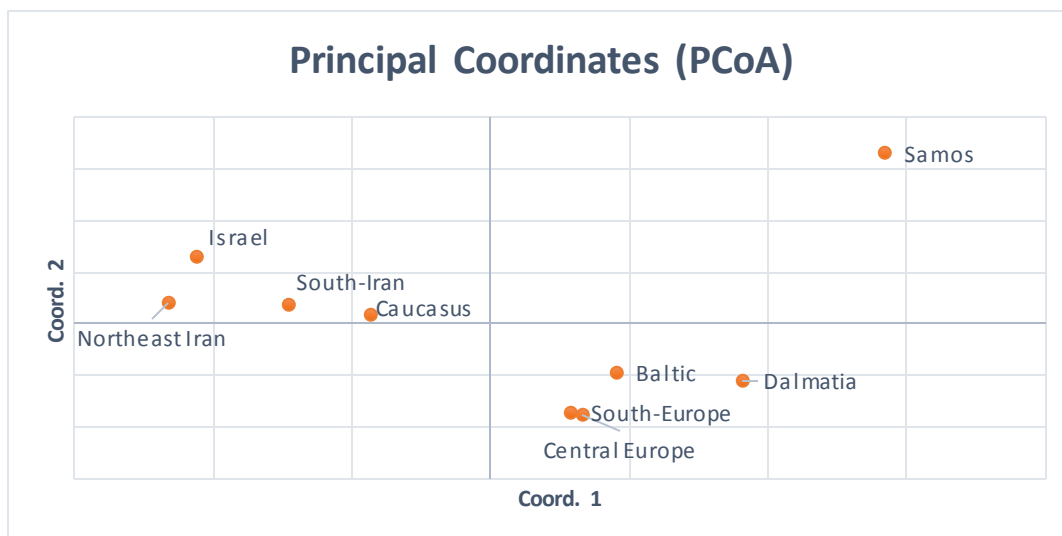


Figure 15 - Principal coordinate analyses (PCoA) for nine populations of Golden Jackal using a set of 43 microsatellites.

This pattern is also shown by pairwise  $F_{ST}$  (Table 3), for which genetic differentiation varied between 0.010 and 0.181. The greatest values of genetic differentiation appeared between Samos Island and all the other populations ( $0.131 < F_{ST} < 0.181$ ). The least differentiated groups in our dataset ( $F_{ST} = 0.010$ ) are Central and Southern Europe. Populations from Europe, except Samos Island, displayed low genetic differentiation between them ( $0.010 < F_{ST} < 0.062$ ), and the same occurs for Asian populations ( $0.038 < F_{ST} < 0.070$ ).

Table 3 - Pairwise  $F_{ST}$  analysis for nine golden jackal populations.

	Dalmatia	Central Europe	South Europe	Samos	Baltic	Caucasus	Northeast Iran	South Iran	Israel
Dalmatia	0.000								
Central Europe	0.062	0.000							
South Europe	0.061	0.010	0.000						
Samos	0.140	0.142	0.140	0.000					
Baltic	0.054	0.038	0.032	0.131	0.000				
Caucasus	0.084	0.053	0.056	0.132	0.055	0.000			
Northeast Iran	0.127	0.096	0.093	0.181	0.091	0.051	0.000		
South Iran	0.106	0.078	0.081	0.142	0.073	0.038	0.064	0.000	
Israel	0.118	0.076	0.072	0.159	0.066	0.048	0.070	0.056	0.000

The genetic clustering analyses of European samples (dataset 2) showed approximately the same clusters evidenced above for all data together. Populations from Dalmatia, Samos Island and Baltics appeared separated, while the rest of European populations constitute the same cluster (Fig. 16). Nevertheless, some admixture among groups is perceivable, and remarkably, the most northern and the most southern individuals in Europe cluster together.

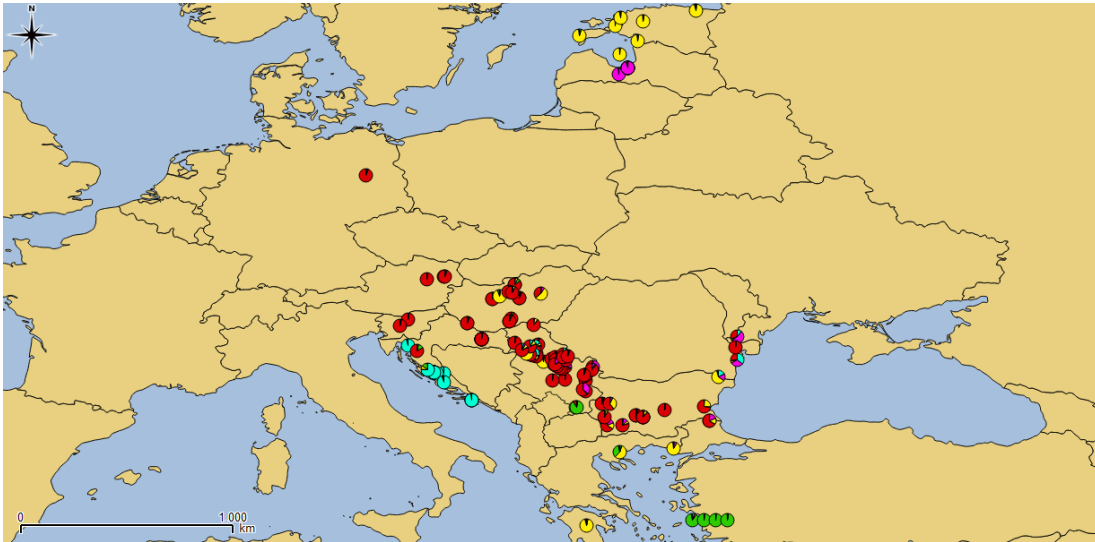


Figure 16 – Geographical distribution of European samples for the Bayesian assignment of individuals to five clusters (k=5) using STRUCTURE.

### 3.4. Genetic diversity

The nine populations established above showed no evidence for linkage disequilibrium and some deviations from Hardy-Weinberg equilibrium distributed by different populations (INU055 and REN169D01 in Dalmatia; AHT111-G, C9.173, AHT121, AHTk211, REN169D01, C20.446, CPH2 and REN64E19 in Central Europe; AHT111-G, REN169D01, C20.446 and CPH2 in South Europe; and C9.173 in Caucasus). These HW deviations were not highly significant, so all loci were used in subsequent analysis.

Genetic diversity patterns have a clear geographical trend, being in general Asian populations much more diverse than European ones. The highest expected heterozygosity was exhibited by Baluchistan in southern Iran ( $H_e = 0.67$ ), while the population of Samos Island, in Greece, presented the lowest expected heterozygosity ( $H_e = 0.35$ ) (Fig. 17). In general, European populations present low values of  $H_e$ , always below 0.52. Caucasus, Northeast Iran and Israel also show high values of expected heterozygosity, all above 0.60. Baluchistan population is also exhibiting the highest mean number of private alleles ( $P_a = 9.74$ ), whereas the lowest values for this measure are found in Southern Europe ( $P_a = 2.10$ ). All European populations present mean number of private alleles below 3.2, while Asian populations exhibited values above 4.4. In general, the Baltic population exhibits intermediate values between Asian and European populations. Remarkably, the golden jackal in Samos Island shows the

lowest level of heterozygosity but the highest value of private alleles among European populations.

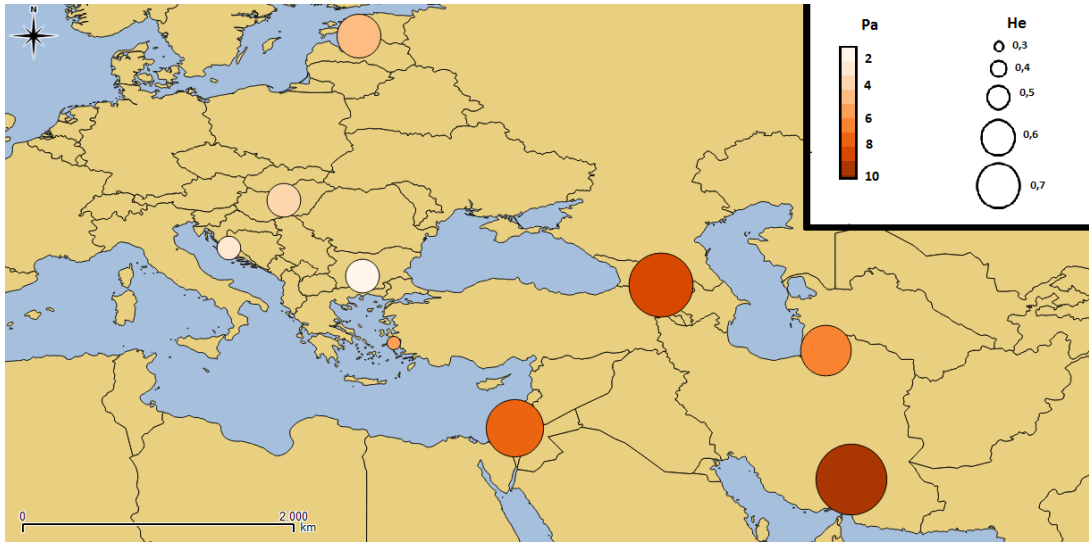


Figure 17 – Geographic distribution of genetic diversity of golden jackal through nine populations (He: Expected heterozygosity; Pa: Mean number of private alleles). Values of expected heterozygosity and percentage of private alleles are specified in Annex A7. Calculations were performed over 43 microsatellites.

## 4. Discussion

This study combines, for the first time, a vast compilation of presence records of Eurasian golden jackal to assess global range, distribution trends, and potential distribution with the genetic analyses using microsatellites to assess genetic structure and diversity in Europe and Middle East. Thus our results contribute to a breakthrough for the biological knowledge of the species and for its efficient management and conservation. This study is expected to be a reference for future research and management involving the Eurasian golden jackal, and, in particular, for an update of the IUCN global range.

### 4.1. Transcontinental patterns: Range extant and connectivity between Europe and Asia

Our study increases the IUCN global range of Eurasian golden jackal in about 72%, that is presently outdated essentially due to the recent expansion of the species and probably also because of the dispersion of literature in regional works available frequently in less visible journals or grey literature. This new global range has about 1,714 km<sup>2</sup> and it can be considered in future global assessments by IUCN. Our potential distribution model identifies the new areas of occurrence as higher favorable for golden jackal, however the huge clusters of observation in Southeast Europe may overestimate Europe in detriment of Asia. Indeed, various areas of Asia, such as Arabian Peninsula and India, appear as less favorable but in the literature are documented with jackals presence. In fact, in India and Southeast Asia there should be more records if taking into account is the concern in literature about the abundance of jackal in this region (Jaeger *et al.* 2007; Debnath and Choudhury 2013). The presence records further north in Central Asia and in North Thailand are ancient (1988 and 1997, respectively), and this may denote regression of these populations at present. The same may be also occurring in Arabian Peninsula and Turkey, with deficient literature perspective, as it happens in Levant (Cohen *et al.* 2013). As a matter of fact, the presence records and potential distribution model show that jackals in Asia do not have a continuous range, suggesting regression of populations. In Europe, this increase in the distribution area of golden jackal agrees with a well documented expansion (Arnold *et al.* 2012; Trouwborst *et al.* 2015), and now we can quantify and detail temporally.

Between 1990 until present, the global range of golden jackal in Europe increase up to almost five times the IUCN global range, which was made in 2008.

Golden jackals population structure analysis suggests a clear split in two populations, one in Asia and the other in Europe with little signs of gene flow between continents. This result combined with the depauperated genetic diversity and the very low number of private alleles exhibited by European populations, makes us to hypothesize that populations in Europe represent expansion events from Asia. We can find similar patterns of structuring associated with loss of diversity in other mammalian species, such as the Eurasian lynx (Ratkiewicz *et al.* 2014) and the brown bear (Kopatz *et al.* 2014). Northern Greece and Baltic individuals share with Asia a remarkable component of its genome, which is not present along the Balkans and central Europe. These are clear signs of introgression and should represent recent gene flow between the continents. Remarkably, Caucasian golden jackals show also an admixed genome with European and Asiatic component, consistent with their position between the two regions, but are clearly more associated with Iran and Israel individuals than with European ones (Fig. 13). This is corroborated by the principal component analysis in which two groups representing the same two populations are individualized (Fig 15). The genetic diversity of Asian populations of golden jackal is much higher than in European populations, suggesting that the species might have originated in Asia and expanding to Europe. However, the fossil record does not clarify this argument because paleontological studies often mention *Canis* sp. (Vershechagin 1959; Heptner and Naumov 1996). The highest values of genetic diversity occur in Baluchistan (Southeast Iran), suggesting that this might be nearer of the origin of the species, although there is a complete absence of bibliographic records of the species in fossil remains in the area. Future genetic evaluation of samples from India and Southeast Asia would greatly help to clarify this scenario.

The split of individuals in seven populations further allow us to particularly perceive gene flow among golden jackals from Israel, Caucasus and the north of Greece, suggesting that these populations have been recently connected. Routes of expansion to Europe probably occur either through the south of Black sea to southern Europe and through the north of the Black sea to northern Europe. The IUCN range suggests a continuity distribution in south of Black Sea, through Turkey, but actually there are few records in this area and our distribution model showed that it is less favorable for golden jackal. It would be very interesting to add genetic results with some individuals from Turkey to test our predictions. Our results of both continued presence and gene flow are also compatible with a corridor from Caucasus to Northern

Europe through the north of the Black sea. Our distribution trends maps suggest that the golden jackal has been expanding, since 1980, in north of Black sea, the potential distribution model shows highly favorable in this area, and the genetic results of Baltic population agree with this assumption due to genetic admixture of populations from Europe and Caucasus. The Caucasus region has been considered an important glacial refugee for temperate species of plants and animals, alongside with the Iberian, Apennine and Balkan refugia in Europe (Milne and Abbott 2002; Weiss and Ferrand 2007; Tarkhnishvili *et al.* 2012), and this possibility for the golden jackal may also be relevant. Additionally, and despite the splitting with geographical coherence when higher partitions of the data are imposed, both Caucasian and European populations show remarkable low levels of differentiation within them.

## **4.2. European patterns: from past to future**

The golden jackal inhabits in Europe since up to several thousand years. Pre-historic fossil records dating back to the Holocene (11.700 years ago) and Neolithic (10.200-4000 years ago) demonstrate that this species inhabited the Dalmatia (coastal Croatia) and southern Greece, apparently originating from Asian populations (Malez 1984; Sommer and Benecke, 2005). Considering the period between the Middle Ages and the first half of 20th century, were found golden jackal records in Panonian Basin, along the Danube river and East of Black Sea, suggesting a new expansion from Asia to Europe (Spasov 1989; Toth *et al.* 2009; Rozenko and Volokh 2010; Banea *et al.* 2012). The records until mid-1980 confirm the presence of jackal in these same areas denoting some expansion along the plains adjacent to Danube River and in the East of Black Sea. According to the records, there is apparently an expansion of the species in Greece, but this is an artifact because the jackal already inhabited in Greece before 1950 but was not documented their specific areas of presence. However, it is documented a relict population of golden jackal in Greece which suffered a decline between 1970 and 1990 (Karandinos and Parasch 1992; Giannatos and Ioannidis 1989, 1991; Giannatos *et al.* 2005). On the last two decades of the 20th century there were the first systematic studies focused on golden jackal allowing to know the distribution in Europe with greater precision (Krystufek *et al.* 1997; Szabó *et al.* 2009; Lapini *et al.* 2011; Arnold *et al.* 2012; Stoyanov 2012; Trouwborst *et al.* 2015). Such studies confirmed the presence of jackals in the aforementioned areas and showing connectivity between the main areas of occurrence within Europe: coastal Mediterranean, Panonian basin and along the Danube valley. Increasingly in the last

10 years, the populations of golden jackal expand to West and North East Europe. In fact, it is speculated that the jackal has already arrived in Denmark due to confirmation of DNA samples from an animal that was trapped and killed by a vehicle, in Jutland this summer (<http://www.thelocal.dk/20150911/first-european-jackal-spotted-in-denmark>). However, this individual was a male without testicles, which may indicate that the animal was held in captivity. Therefore, based on our species distribution model, it is expected that this expansion to Central Europe continues in the near future, mainly due to habitat suitability, particularly in Germany and eastern France.

These detailed data of distribution patterns complemented by genetic results helped to confirm and reinforce this population expansion, allowing valuable insights into the history of the golden jackals in Europe. However, we found in Europe two other genetic clusters apart from the main population. These are located in the Samos Island and in the Dalmatian region, and may represent remnants of older colonization events from Asia, being presently relict populations. This is particularly supported to Samos Island where a high number of private alleles was observed, although with a low heterozygosity value, expected due to geographic isolation of the island and, despite close proximity to mainland (about 18km), suggests an absence of gene flow with populations in Turkey. The European core, Panonian Basin and along Danube river, comprises the main population in Europe (red population in Fig. 13) with higher genetic diversity than the coastal populations, which seems the main source of the recent expansion towards West (Switzerland, Austria and Germany) and Northeast (Latvia and Estonia). Another interesting front of expansion is located in Italy and Slovenia, where populations from European core and Dalmatia meet. In future work it would be interesting to evaluate, with a fine-scale sampling, which is the source of jackals that are colonizing this region to understand if they result from the expansion of both or only one of these populations. The literature suggests that the Dalmatia population are the source of population of Italian and Slovenian jackals (Fabbri *et al.* 2013) but in this work we have no genetic evidence of that assumption. The results of Fabbri *et al.* (2013) show a differentiation of Bulgarian individuals from those in Serbia and in Italy, but in fact our results do not support such division among European samples. However, our results agree with those of Fabbri *et al.* (2013) considering the weak population subdivision between European populations, as well as the separation of the Dalmatia from other populations of Europe. Both works show the same values of expected heterozygosity for Dalmatia and Serbia populations ( $He=0.43$  and  $He=0.51$ , respectively), while in our work Zachos *et al.* (2009) found a very low genetic variability for Serbia population ( $He = 0.33$ ).



### **4.3. Management implications: is golden jackal a recent invader or a relict species?**

Society in general and the scientific community, in particular, has shown a minor interest in the golden jackal for which, comparing to the wolf, with morphological and ecological similarities and even sharing a great part of the distribution range, are much less published studies. This scientific lack hampers the correct management of the species that, in general, it is targeted by large persecution by man, resulting in population depletions with genetic consequences, as we can see in Israel. In Europe, jackal has become the most popular predator for hunting, due to its skin. Furthermore, golden jackal are defined, in most countries of Europe, as alien or invasive species (Trouwborst *et al.* 2015). Alien species is a species introduced outside its natural past or present distribution (According to a definition adopted by the CBD Conference of the Parties (COP) – CBD COP Decision VI/23, 2002). In other words, introduction is defined as the movement by human agency, indirect or direct, of an alien species outside of its natural range. As noted above, subfossil records denote golden jackal presence in parts of Europe for thousands of years. Therefore, as the recent and ongoing range expansion of the golden jackal in Europe is apparently not the result of active introduction by humans, but as a result of natural spread, possibly aided by milder winter conditions and reduced populations of wolves (Stratford 2015), so golden jackal cannot be considered an alien species in Europe. However, the Lithuanian, Estonian and Latvian governments regarded the species as alien and allowed unlimited lethal removal with the aim of eradicating the species (Trouwborst *et al.* 2015), which resulted in several animals being shot between 2013 and 2015. Evidence from studies of golden jackals elsewhere in Europe suggests that golden jackals will have no significant overall impact on the species that they are likely to encounter in Baltic region, except red fox (Macdonald 1987; Spassov 1989; Kryštufek and Tvrtkovic 1990; Giannatos 2004; Scheinin *et al.* 2006). With golden jackal and red fox having almost identical diets, any potential effects of predation by golden jackals are also likely to be mitigated by displacement of red foxes in areas settled by golden jackals (Stratford 2015). It should also be noted that if golden jackal is accepted as a species occurring as a result of natural range expansion, that is not human introduction, then the question of impact on the environment is secondary. All environments are in a state of flux, some species decreasing in numbers and range and some expanding. Therefore, the Estonian, Latvian and Lithuanian authorities should treat the jackals within their

respective boundaries as being subject to the regime of Annex V, which covers Animal and plant species of community interest whose taking in the wild and exploitation may be subject to management measures (Stratford 2015; Trouwborst *et al.* 2015).

Nevertheless, our results show that golden jackal is an intriguing kind with an interesting history associated with their populations, namely in Europe where there are relics populations from the Holocene, which currently have particular genetic structure and reduced genetic diversity, as we can see above in Dalmatia and Samos Island. The conservation of these populations is necessary, in contrast to other populations who are expanding. In Asia, various populations may also be on the decline, such as in Turkey, Levant, Arabian Peninsula and Southeast Asia, and it would be important perform regional assessments to access the status of populations.

## 5. Conclusions and future research

Our study allowed the update of the global range of Eurasian golden jackal, with a higher range extension that should be considered in future global assessments by IUCN. We documented the expansion of jackals in Europe, which in Asia is apparently in regression. The Asian continent may be the source of golden jackal's populations, due to higher genetic diversity, and it was found that European and Asian individuals constitute two different clusters with low gene flow between them. Nevertheless, our genetic results and distribution trends are in agreement with this connectivity between Asian and European populations, occurring both north and south of Black sea, with the appearance of genetic admixture in the Baltic region and North Greece. This expansion to Europe also occurred many years ago, once in Holocene and another in Middle Ages, how we can certify with the distribution trends and genetic characteristics of jackals from Dalmatia and Greece. This relict populations from Dalmatia and Samos Island should be considered as distinct management units and subject to protective measures. Taking into account the suitability of the habitat, it is expectable that the expansion of golden jackal in Europe continues, and with the expansion of individuals from Caucasus, will probably result in a significant contribution to the diversity and fitness of European populations.

In future, a higher sampling effort will be necessary in Turkey, Greece and Ukraine, in order to clarify the connectivity between Asian and European populations, as well as in Arabian Peninsula to understand if there is any regional a differentiated population, and also to assess if these individuals belong to the Eurasian golden jackal or are in fact African wolves. Also India and Southeast Asia should be included in this datasets to clarify the origin of the golden jackals. Studies using mitochondrial DNA and sexual chromosomes will also improve our understanding of golden jackal populations and their connectivity. More variables, such as variables related to soil, presence of wolf, agriculture and human activities, may improve the potential distribution model. In order to assess the relic populations of Dalmatia and Greece additional genetic, morphological and ecological studies should be performed in the future, and management and conservation of these populations should be rethought.



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## A1)

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## A2)

### Description of known information regarding the distribution of Eurasian golden jackal, at a country level

The number and description of presence records from golden jackals that were found in our literature review are discriminated, by alphabetic order of countries, in the following paragraphs:

#### **Afghanistan (8 presence records)**

Golden jackal were reported in Nuristan province, in eastern Afghanistan by Hassinger (1973) and Stevens *et al.* (2011). Stevens *et al.* identified a canid as a jackal using direct sightings, scat identification and camera-trap photographs, in 2009. Hassinger also found golden jackals in Nuristan province, in Natural History expeditions in 1960s. It is estimated that the jackal takes up more areas in Afghanistan due to the presence of records at the borders with neighboring countries

#### **Albania (10 presence records)**

The presence of golden jackal in Albania is shown only along the coast in 1990s, near the towns of Konispol, Fier and Mamuras by Krystufek *et al.* (1997). A discontinuous distribution in the coastal lowland, in localities Divjaka, Pisha, Poro, Butrint and Vrina, were suggested by Puzanov and Mitrushki (1955) and Koçi (1961), in 1960's. In the presence, it is possible that golden jackal inhabits the coastal areas of Albania, but there are a lack of information in this country.

#### **Armenia (3 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). In 1952, holocene bones of golden jackal were mentioned from close by the Sakazhia cave in Tskhali-Tsiteli gorge by Vershechagin (1959). In study of the present vertebrate fauna of the Saraibulakh (Urtskii) ridge, Dal' (1940) collected and identified bones of jackal from 2000-4000 years. Golden jackal's range was reported along the Araks valley to Oktemberjansk region, a little east of Erevan by Heptner and Naumov (1996). It is plausible that other areas of Armenia have jackals.

#### **Austria (9 presence records)**

The first golden jackal were mentioned in Styria in 1987 by Humer (2006). During the following years, vagrants were sighted, shot and found dead in Carinthia, Styria and Burgenland, mentioned by Arnold *et al.* (2012). Between 1996 and 2006, several records in Burgenland, Carinthia, Scheiblingkirchen, Styria and Wienerwald, were mentioned. The first breeding record of golden jackal from Austria was proved in 2007 by the observation of three cubs in the Austrian part of the National Park Neusiedler See-Seewinkel/Ferto-Hanság in Burgenland by Herzig-Straschil (2008). We have two samples of golden jackal founded in Wiener Neudorf, from 2012, and one sample founded in Freiland in 2013.

#### **Azerbaijan (26 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Bones of golden jackals were mentioned in Apsheron peninsula, from Holocene, by Vershechagin (1959) and Heptner Naumov (1996). More recently, records in Apsheron peninsula, along Alazan and Araks rivers, Aris, Babadag, Khachmas, Lenkoran, Sar Island, Shemakha, Shirvan, Talysh and Zakatal, were mentioned. Such as Armenia, it is estimated that the jackal inhabits in most areas of Azerbaijan, particularly along the coast.

#### **Bangladesh (2 presence record)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackals are locally common in Bangladesh, but only one study was conducted in Mirzapur and Ishurdi by Jaeger (2007). Bangladesh is bordered by India, so should have more abundant population of jackals.

### **Belarus (1 presence record)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackal record was published in the news online very close to the Polish border by Trouwborst *et al.* (2015). It's expected that in following years vagrant animals will be reported in Belarus, due to recent records in Latvia and Estonia.

### **Bhutan (2 presence record)**

In Bhutan there are two articles mentioned jackals. A study in Chitwan National Park in 2011, that used intensive camera trapping to record a presence of golden jackals made by Thapa *et al.* (2013), and a report from Heptner and Naumov (1996). Such as Bangladesh, Bhutan borders with India and it is possible that have more groups of jackals.

### **Bulgaria (124 presence records)**

A fossil of golden jackal was mentioned in Strandzha Mountain in Middle Age and a vagrant in Sofia in 1384 by Spassov (1989). During the 1950s was founded small numbers of jackals in south of Burgas and in the Strandzha Mountain. In recent years, golden jackals appeared to occupy almost all of Bulgaria, except in places with high altitudes, namely in mountainous regions of south-western and central Bulgaria. High densities of populations are situated in southeastern Bulgaria, from the Kamchia river basin and the Gulf of Burgas to the Turkish border to the south and from the Black Sea Coast to the Stara Zagora region to the west; northeastern Bulgaria, in the region between Dobrudja and Tutrakan and the central part of northern Bulgaria (the central region of the Bulgarian Danubian plain).

### **Cambodia (1 presence record)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackals were founded when intensive camera-trapping were conducted in the east of Phnom Prich Wildlife Sanctuary, Mondulhiri province, between December 2008 and August 2009, by Gray and Phan (2011).

### **Croatia (291 presence records)**

A fossil of golden jackals from Lower Holocene were mentioned by Malez (1984) and two records from 1491 in Korcula and Peljesac Peninsula were mentioned by Vuletic and Vukasovic (1908). Since 1980 to present, golden jackals were reported in Badija, Istria, Korcula, Krk, Mljet, Olipa, Pag Island, Peljesac Peninsula, Premuda, Ravni Kotari, Sipan and Vir Island by Krystufek *et al.* (1997), Krofel (2007, 2008) and Arnold *et al.* (2012). With the recent expansions, golden jackals seems to occupy all coastal Croatia, the border with Slovenia, northern part of the country and the border with Serbia.

### **Czech Republic (3 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). One jackal was mentioned in 2006, close to a road in southern Moravia. With recent expansions of golden jackal population, it is possible that Czech Republic be settled in the coming years by populations of Slovakia and Austria.

### **Estonia (15 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). We didn't find any report of golden jackals from Estonia in literature, but we have 7 confirmed samples of golden jackal. These samples are from Lihula, Ida-Virumaa, Parnumaa, Martna, Massu, Taristu and Tuudi. These vagrants appear mostly in coastal areas of the country.

### **Georgia (15 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackals were reported in Batumi, Kutais, Mtskheth and Tbilisi by Heptner and Naumov (1996). We have 6 samples from this country, more specifically in black sea coast, Asureti, Imereti, Tetrtskaro and Racha. Georgia is a crucial corridor between Asia and Europe therefore, it is possible that more regions are inhabited by jackals.

### **Germany (4 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). From Germany, a male was mentioned in southern Brandenburg in 1996 by Arnold *et al.* (2012), and four pictures of golden

jackal were confirmed in Bavarian Forest by Weingard *et al.* (2012). With recent expansion to Italy and Switzerland, probably more animals will appear in Germany in following years.

#### **Greece (47 presence records)**

Sub-fossil records of golden jackals from Neolithic were mentioned in Kitsos and Delphi by Sommer and Benecke (2005). More recently, the golden jackal's population in Greece is more scattered than in 1980s. In this decade, we could find jackals in Evros, Fokida, Halkidiki, Peloponnesos, Samos, Serres and Vistonida-Nestos, but couldn't find in Corfu, Ikaria, Kephallinia, Kythira, Leukas, Skiros and Thasos by Krystufek *et al.* (1997) and Arnold *et al.* (2012), where jackals inhabited in 1980s

#### **Hungary (124 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden Jackals reports were mentioned in Hedervar, Kunadacs, Tiszalök, Tiszadada, Tyukod, Dunantul, Tizantul and Derecske by Toth *et al.* (2009) between 1831 and 1942. From 1980s and 1990s, we found reports from Baranya, Paks, Somogy, Bács-Kiskun, Hajdu-Biar, Lakocsa, Dunantul and Tizantul mentioned by Szabo *et al.* (2009), Toth *et al.* (2009) and Arnold *et al.* (2012). In recent years, golden jackals were reported in Bács-Kiskun, Baranya and Somogy by Arnold *et al.* (2012). Jackals should be distributed throughout Hungary, due to expansion from Serbia, Romania and Bulgaria.

#### **India (16 presence records)**

In India, the golden jackals is a historic inhabitant, but only more recently were published articles that mentioned this species. Since 1988 until 2013, we found reports in Keoladeo National Park, Sariska Tiger Reserve, Great Indian Bustard Sanctuary, Gir National Park and Sanctuary, Kutch, Maharashtra, Rajasthan, Haryana, Jammu, Kashmir, Madhya Pradesh, Kolkata and Cachar. In June 2015, golden jackals were mentioned in Van Vihar National Park, Madhya Pradesh by Prerna *et al.* (2015). There is a lack of information in this country because it is estimated that golden jackals inhabits in all regions of India and is very abundant.

#### **Iran (136 presence records)**

Two fossils were founded in Iran that remains to prehistoric records. One in Mousterian Cave from Holocene and another from Bisotun cave from Bronze Age mentioned by Vershechagen (1959). Since 1876 until 1991, golden jackals were reported in Baluchistan, Bandar-i-Gaz, Behshahr, Caspian Coast, Chandyr, Damghan, Darband-e-Ravar, Elbruz mountain, Fars, Gorgan, Guilan, Kerman, Khuzistan, Lar, Qazvin, Seistan, Semnan, Shiraz and Tehran Lay (1967), Karami *et al.* (2008) and Heptner and Naumov (1996). Golden jackal are present along Elbruz Mountain, in north of the country, in western and south part of Iran. We have many confirmed samples from Iran, more specifically from Anbar Abad, Jiroft, Sistan, Fars, Kerman, Sanandaj-Kurdestan, Guilan, Khorasan, Qazvin, Mazandaran, Esfahan, Baluchistan, Semnan, Amol, Ardjan, Gonbad e Kavos.

#### **Iraq (12 presence records)**

Golden jackals were reported in Amara, Baghdad, Basra, Diyala, Hilla, Kut al Imara, Qalat Luqait, Shahraban, Shuaiba and Tigris by Hatt (1959). It is possible that golden jackals inhabits in more regions in Iraq due to abundant number of individuals in Iran, Syria and Jordan.

#### **Israel (19 presence records)**

Studies with golden jackals in Israel are scarce. A study about cattle predation by golden jackals in the Golan Heights was published by Yom-Tov *et al.* (1995). More recently jackals were mentioned in Golan, Galilee, Negev and Arava by a study in Jewish National Found by Borkowski *et al.* (2011) and a genetic characterization of populations of golden jackal in Israel made by Cohen *et al.* (2013). It is plausible that golden jackals are more abundant in Israel.

#### **Italy (45 presence records)**

In 1980s, firsts vagrants appears in Trentino and Udini, but only in 2000's that populations of golden jackal were reported in Julian, Treviso, Veneto, Venezia, Bolzano, Carnian Pre-Alps, Friuli and Tyrol by Lapini (2011). In 2015, was mentioned a vagrant in

Latisana and an established population in Alto Adige. Golden jackals may have appeared in North Italy from Slovenia and Austria.

**Jordan (10 presence records)**

Jackals were abundant in Jordan in the XIX century, but started to decline in the first half of the XX century, due to the continuous eradication of feral dogs and wolves by poisoning, to habitat destruction and also perhaps to competition with the red fox. Some records and bone remains were collected from Azraq Nature Reserve, Inbeh, Wadi Rum and Northern end of Dead Sea.

**Khazakistan (6 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackals were recorded in Chimkent, Kazalinsk, Lenger, Sary-su, Betpak-Dala, Emba and Karaganda by Heptner and Naumov (1996). In this region of Asia there is a lack of information, but with this information from Heptner and Naumov we can see reports of jackals well distributed across the country, therefore it is possible that golden jackals inhabit more regions in Khazakistan.

**Latvia (11 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). There are no reports of golden jackals in Latvia, but we have samples from Jelgava that can be vagrants that appeared in the coastal area. As in Estonia, it's possible that more records of golden jackals are documented in following years in Latvia.

**Lithuania (1 presence record)**

Range country not listed in Sillero-Zubiri *et al.* (2004). In recent years, a vagrant was mentioned near Kaunas by Trouwborst *et al.* (2015). As various jackal's samples were collected in Latvia and Estonia, it is estimated that more jackals are present in Lithuania.

**Macedonia (9 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackal became extinct in Macedonia during the first half of the 1960s, due to eradication that occurs in that time. Since 1980 and 2000, various vagrants were seen and samples were collected from Skopje, Zhelino and Dojran. It is estimated that recently the jackal is present in Macedonia, especially on the border with Bulgaria and Serbia, due to the abundance of this species in these countries.

**Moldavia (2 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). In 1954, jackals were reported near Piatra Neamt and Focsani in Moldavia by Angelescu (2004). In the 1990's, jackals were hunting in Husi. As the populations of Romania are spreading can be expected that more specimens possible inhabit Moldavia.

**Montenegro (2 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). In the south, near the Albanian border at the coast, jackals have been mentioned, in Ada Island by Schneider-Jacoby (2004). Jackals were mentioned killed on the road between Tivat and the village of Dub in 2002 and 2003 by Sackl *et al.* (2006). It is estimated that jackals inhabit coastal areas of Montenegro due to abundant animals in Croatia and coastal areas of Albania.

**Myanmar (1 presence record)**

In Southeast Asia, the information of golden jackal distribution is scarce, but a golden jackal was examined in Putao, with the animal reportedly killed in the forests east of the Mai Hka River reported by Rabinowitz and Khaing (1998). While this species appears to be present but not common in Myanmar.

**Nepal (3 presence records)**

A study about the interaction of golden jackals in human dominated landscapes in Manaslu Conservation Area and the report of Heptner and Naumov (1996) are the unique references of this species in Nepal.



#### **Pakistan (8 presence records)**

Golden jackals are very common in Pakistan. The presence of jackal's population were mentioned in Punjab by Maan and Chaundhry (2001). Later, the plains of Sind, Punjab, Balochistan and Khyber Pakhtoonkhwa (KPK) provinces were mentioned as locals with presence of golden jackal by Madmood *et al.* (2013). Also can found jackals in Chagai desert and Potohar. As the neighboring countries are India and Iran, jackals should be very abundant in Pakistan.

#### **Palestine (1 presence record)**

Range country not listed in Sillero-Zubiri *et al.* (2004). It is estimated that there are jackals in Palestine, due to the abundance of this species in neighboring countries. However, in literature, only one reference mentioned jackals and refers to a fossil found in Ksar Akil from late postglacial.

#### **Poland (4 presence reports)**

Range country not listed in Sillero-Zubiri *et al.* (2004). In Poland, vagrant animals were seen in northeastern part of the country by Trouwborst *et al.* (2015).

#### **Romania (110 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). The first report of golden jackals in Romania was in 1929, during a hunting session in Dolj County. In 1954, jackals were recorded near Calarasi, Comana, Craiova, Jijia, Nisipari, Piatra Neamt and Prundu. Between 1963 and 1996, jackals appeared in Gaesti, Berveni, Voronet, Stefanesti, Niculitel, Brasov, Bucuresti, Craiova, Sutesti, Turnu Severin, Walachian and Dobrogea, mentioned by Banea *et al.* (2012). In this century, jackals inhabits the southeastern part of Romania, nominally in Calarasi, Braila, Chilia, Cricau, Letea, Periprava, Sulina, Toboliu, Bihor, Arges, Cheveresu Mare, Mozacu, Niculitel, Pischia, Alba, Calarasi, Dobrogea, Giurgiu, Lupilor and Timis. With recent expansion, jackals moved to southeast of Romania and border with Hungary.

#### **Russia (66 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackals inhabits in Russia predominantly in Caucasus, between Black and Dead Sea. In first half of XIX century were reported the existence of jackals in Don, Ergeni, Stavropol, Volga, and Taganrog by Heptner and Naumov (1996) and, in final of XIX century, jackals inhabited Stavropol and Tambov, mentioned by Rozenko and Volokh (2010). In XX century, jackals were mentioned in Grozny, Kura, Mozdok, Sunzha, Terek, Timashevsk, Beryuzyak, Crimea, Dagestan, Gunib, Kizlyar, Kuban, Kuma, Makhachkala, Novorossiisk, Psebai, Stavropol, Sunzha and Tambov by Vershechagen (1959), Heptner and Naumov (1996) and Rozenko and Volokh (2010).

#### **Serbia (148 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). In 1980's, two established populations, one in the plain of Srem and another in Negotin, northeast Serbia, were reported by Milenkovic (1987). In the beginning of the century, jackals were mentioned in Fruska Gora, Negotin, Smederevo, Srem, Veliko Gradiste, Branicevo, Podunavlje and Svilajnac. At present, jackals inhabits almost an entire country. Southeastern part of the country is the only region with no reports of golden jackals.

#### **Slovakia (8 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Between 1989 and 2001, four jackals were shot near Cierna, Tisou and Lucenec. In 2008, it is possible that unconfirmed sighting of a first jackal were recorded from central Slovakia, in Banska Bystrica. With recent expansion of jackals to north, it's possible that more jackals are documented in following years.

#### **Slovenia (44 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). In Slovenia, jackals were been reported since 1952, in Kobarid and Ljubljansko barje, mentioned by Brelih (1955). In 2005, a golden jackal was shot in Savinja Valley, mentioned by Krofel and Potocnik (2008), and in 2008, the first established group of jackals in Ljubljansko barje was reported by Krofel (2009). In 2012, various jackals were seen near Kobaric and Idrsko, and new data confirm that at least one territorial family was formed in the Bovec valley, according Mihelic and Krofel (2012).

### **Switzerland (3 presence reports)**

Range country not listed in Sillero-Zubiri *et al.* (2004). In the end of 2011, pictures of golden jackals were reported in Bern, Freiburg and Waadt, confirming the first reports of golden jackals in Switzerland.

### **Syria (25 presence records)**

Between 1984 and 1994, many reports of golden jackals were mentioned in Syria. Jackals were mentioned in Al Bilaas, Alawit Mountains, As Sa'an, Idlib, Lattakia, Salamieh, Tadmor and Douma by Bunaian *et al.* (2001). More recently, jackals were mentioned in Palmyra and Deir ez-Zor by Masseti *et al.* (2009).

### **Tajikistan (14 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackals were mentioned along Amu-Darya river, in Chubek, Dushanbe, Gissar, Kafirnigan, Kanibadam, Kisyl-su, Kulyab, Kurgan-Tyub, Ordzhonikidzeabad, Pendzhikent, Ramit, Vakhsh and Varzob by Heptner and Naumov (1996). In 2010, the existence of jackals in Danghara was reported by Ostrowski (2010)

### **Thailand (2 presence records)**

In Thailand, the presence of golden jackal were reported in Khao Yai National Park and Thung Yai Naresuan, respectively, by Jenks *et al.* (2011) and Chutipong *et al.* (2014).

### **Turkey (22 presence records)**

The first record of golden jackal in Turkey was in 1895, in Efez. In 1960, Antalya was mentioned as a place with golden jackal's groups by Kaya and Raynal (2000). Since 1995 until 2007, various jackals were seen in Akyatan, Edirne, Ganos Mts, Lake Gala, Istanbul, Goksu, Yaylacik Research Forest and National Park of Termessos, mentioned by Brown and MacDonalds (1995), Krystufek *et al.* (1997), Akcinar *et al.* (2005), Johnson *et al.* (2005) Can and Togan (2009) Marinis and Masseti (2009) and Un *et al.* (2009). After 2010, a group of jackals were mentioned in Artvin, Rize, Trabzon and Yusufeli by Ucarli (2011) and Ambarli and Bilgin (2012). We also confirmed golden jackal's presence in Artvin, Duzce and Bolu, with the confirmation of genetic sample. The information available about golden jackal's distribution in Turkey is very scarce, it is thought that jackals inhabit an entire country.

### **Turkmenistan (8 presence records)**

In this part of Asia there is a lack of information. Amu-Darya and Atrek rivers, Tedzhen, Chikishlyar, Kopet-Dag, Kugitangtau, Murgab and Sumbar were mentioned by Heptner and Naumov (1996) with confirmed presence of golden jackal.

### **Ukraine (51 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackals are reported in south of Ukraine, more specifically in Mariupol, Krasnodar, Kerchensky Strait, Tuzla Spit, Chernovtsy, Donetsk, Biletskiivsky Plavni and Zaporizhja by Rozenko and Volokh (2010). After 2010, jackals appeared in Odessa and Olevsk, in north of the country mentioned by Arnold *et al.* (2012) and Zila (2014). Jackals are present in southern part of the country, but some vagrant appears in North and Eastern part in borderer with Russia.

### **Uzbequistan (14 presence records)**

Range country not listed in Sillero-Zubiri *et al.* (2004). Golden jackals are reported in Termez by Volozhenikov (1972) and in Syrdaria by Taryannikov (1974). In 1980's, jackals were seen in Aydarsk, Amu-Darya river, Baisun, Bukhara, Fergana, Samarkand, Surkhan-Dar'ya, Tashkent and Ustyurt by Ishunin (1980) and Heptner and Nuamov (1996).

### **A3)**

## **EasySpin protocol of the Genomic DNA Microplate Tissue Kit**

1. Cut up 30mg tissue and place in Eppendorf tube.
2. Add 300µl of ACL solution (Animal Cell Lysis solution) to Eppendorf tube and 20 µl Proteinase K.
3. Incubate in shaking water bath at 55°C overnight, for tissue is completely lysed.
4. Cool to room temperature. Vortex for 20 seconds and centrifuge 14000 rpm for 5 minutes.
5. Pipette 300µl of supernatant into an EasySpin 96-Well plate (if pellet not visible, repeat previous step) and add 300µl of AB solution. Seal, mix by occasionally inverting plate and keep for 2 minutes.
6. Centrifuge at 4000 rpm for 2 minutes with a rotor for microtiter plates. Discard the flow-through.
7. Add 500µl Wash solution to each well of 96-Well Plate and spin at 8000 rpm for 1 minute.
8. Discard flow-through and place EasySpin 96-Well Plate back to the same Deep Well collection plate.
9. Add 500µl Wash solution to each well of the EasySpin 96-Well Plate, spin at 8000 rpm for 1 minute. Discard the flow-through and spin once more at 14000 rpm for 5 minutes to remove residual amount of Wash solution.
10. Transfer the EasySpin 96-Well Plate to a 96-Well storage plate. Add 50µl Elution Buffer to the EasySpin 96-Well Plate; incubate at 55° for 10 minutes.
11. Centrifuge at 14000 rpm for 5 minutes.

## A4)

### List of the 43 analyzed microsatellite loci along with repeat motif, allele range, multiplex and reference.

Name	Repeat	Allele Range	Multiplex	Reference
AHT103	Di	71-89	MS2	Holmes et al. 1995
AHT111	Di	72-92	MS2	Holmes et al. 1993
AHT121	Di	74-118	Finnzymes	Holmes et al. 1995
AHT137	Di	128-160	Finnzymes	Holmes et al. 1995
AHT171	Di	216-240	Finnzymes	Breen et al. 2001
AHT260	Compound	229-265	Finnzymes	Breen et al. 2001
AHTk211	Di	82-98	Finnzymes	Lingaas et al. 1997
C04.140	Di	132-160	MS2	Ostrander et al. 1993
C08.410	Di	95-125	MS3	Ostrander et al. 1995
C08.618	Di	188-208	MS3	Ostrander et al. 1995
C09.474	Di	111-133	MS3	Ostrander et al. 1995
C13.758	Di	220-244	MS2	Mellersh et al. 1997
C14.866	Di	221-257	MS2	Mellersh et al. 1997
C20.253	Di	95-125	MS2	Ostrander et al. 1993
C20.446	Di	173-201	MS3	Ostrander et al. 1995
C27.442	Di	158-172	MS1	Ostrander et al. 1995
C09.173	Di	100-118	MS2	Ostrander et al. 1993
CPH05	Di	95-131	MS3	Fredholm and Wintero 1995
CPH14	Di	185-205	MS2	Fredholm and Wintero 1995
CPH02	Di	87-113	MS3	Fredholm and Wintero 1995
CPH09	Di	133-163	MS3	Fredholm and Wintero 1995
CXX.459	Di	141-167	MS3	Ostrander et al. 1995
FH2001	Tetra	123-155	MS2	Francisco et al. 1996
FH2010	Tetra	216-240	MS1	Francisco et al. 1996
FH2054	Tetra	121-181	Finnzymes	Francisco et al. 1996
FH2079	Tetra	246-292	MS1	Francisco et al. 1996
FH2161	Tetra	228-260	MS3	Francisco et al. 1996
FH2848	Di	224-244	Finnzymes	Breen et al. 2001
INRA21	Di	87-103	Finnzymes	Mariat et al. 1996
INU005	Di	104-136	Finnzymes	Finnzymes, Inc

INU030	Di	136-156	Finnzymes	Finnzymes, Inc
INU055	Di	196-210	Finnzymes	Finnzymes, Inc
PEZ3	Tri	106-147	MS1	Neff et al. 1999
PEZ5	Tetra	95-119	MS1	Neff et al. 1999
PEZ1	Tetra	99-131	MS1	Neff et al. 1999
REN162C04	Di	189-215	Finnzymes	Guyon et al. 2003
REN169D01	Di	192-220	Finnzymes	Guyon et al. 2003
REN169O18	Di	145-171	Finnzymes	Guyon et al. 2003
REN247M23	Di	263-283	Finnzymes	Guyon et al. 2003
REN54P11	Di	223-245	Finnzymes	Guyon et al. 2003
REN64E19	Di	132-181	MS3	Breen et al. 2001
VWF	Hexa	138-192	MS2	Shibuya et al. 1994

### **Cited literature for the 43 analyzed microsatellite loci**

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## A5)

### PCR programs for the microsatellite multiplexes

#### MS1

Amplification Step	Temperature (°C)	Duration	Number of Cycles
<b>Initial denaturation</b>	95°	15min	1
<b>Denaturation</b>	95°	30seg	20
<b>Anneling</b>	58°-56° (Touchdown -0.1°C)	45seg	
<b>Extension</b>	72°	45seg	
<b>Denaturation</b>	95°	30seg	
<b>Anneling</b>	56°	45seg	15
<b>Extension</b>	72°	45seg	
<b>Denaturation</b>	95°	30seg	
<b>Anneling</b>	53°	45seg	10
<b>Extension</b>	72°	45seg	
<b>Final Extension</b>	60°	30min	

#### MS2

Amplification Step	Temperature (°C)	Duration	Number of Cycles
<b>Initial denaturation</b>	95°	15min	1
<b>Denaturation</b>	95°	30seg	35
<b>Anneling</b>	56°	45seg	
<b>Extension</b>	72°	45seg	
<b>Denaturation</b>	95°	30seg	
<b>Anneling</b>	53°	45seg	8
<b>Extension</b>	72°	45seg	
<b>Final Extension</b>	60°	30min	

#### Finnzymes

Amplification Step	Temperature (°C)	Duration	Number of Cycles
<b>Initial denaturation</b>	98°	3min	1
<b>Denaturation</b>	98°	15seg	40
<b>Anneling</b>	56°	1min,15seg	
<b>Extension</b>	72°	45seg	
<b>Final Extension</b>	72°	5min	1

**MS3**

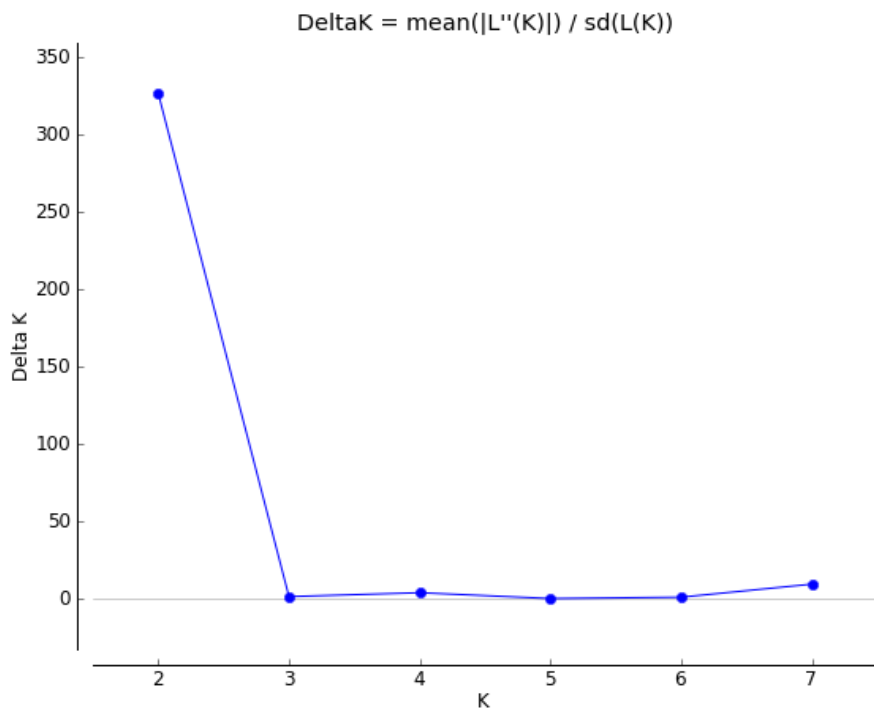
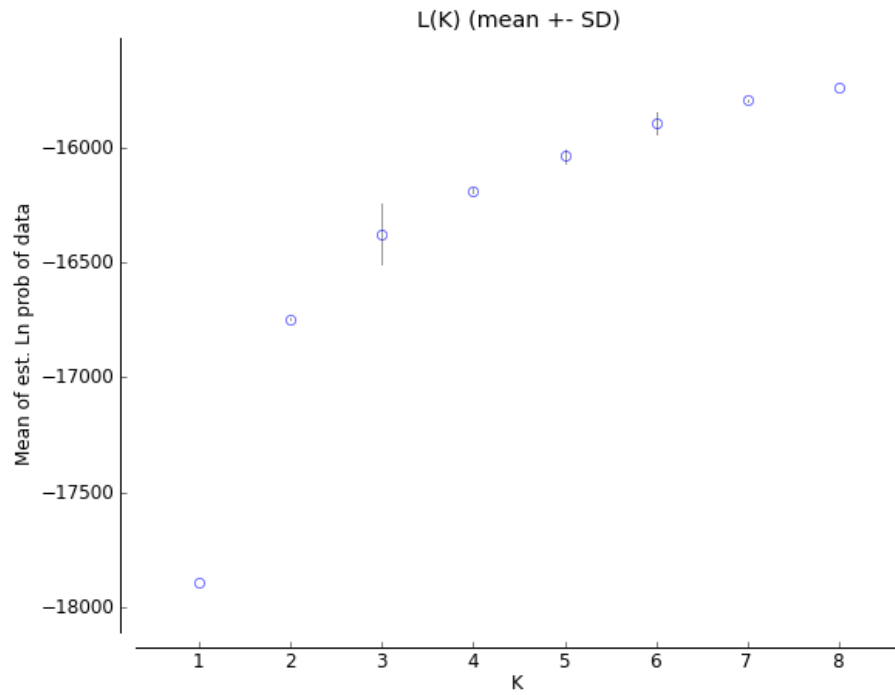
Amplification Step	Temperature (°C)	Duration	Number of Cicles
<b>Initial denaturation</b>	95°	15min	1
<b>Denaturation</b>	95°	30seg	7
<b>Anneling</b>	60°-57° (Touchdown -0.5°C)	45seg	
<b>Extension</b>	72°	45seg	
<b>Denaturation</b>	95°	30seg	22
<b>Anneling</b>	57°	45seg	
<b>Extension</b>	72°	45seg	
<b>Denaturation</b>	95°	30seg	8
<b>Anneling</b>	53°	45seg	
<b>Extension</b>	72°	45seg	
<b>Final Extension</b>	60°	30min	1



## A6)

### Graphical output of the Structure Harvester results for L(k) and Delta K

#### L(K)



**A7)**

**Values of observed and expected heterozygosity and percentage of private alleles**

Pop	Ho	He	Na	Pa	%Pa
Samos	0,4457	0,3546	94	3	3,1915
Croatia-Cc	0,3978	0,4280	141	3	2,1277
Center Eu	0,4712	0,4977	204	6	2,9412
South Euro	0,5007	0,5042	190	4	2,1053
Baltic	0,5471	0,5155	159	5	3,1447
Iran North	0,6105	0,5894	159	7	4,4025
Israel	0,6276	0,6150	180	11	6,1111
Caucasus	0,6231	0,6456	229	19	8,2969
Iran South	0,6942	0,6749	236	23	9,7458