# Genetic polymorphism and structure of grey wolf (*Canis lupus*) populations in Eurasia

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## Introduction

While the grey wolf would be a top predator in most of Eurasia's terrestrial ecosystems, hunting and environmental transformations have contributed to a strong reduction or eradication of populations over much of this area. Today's protection strategies depend on knowledge of population genetic variability and structure, and our analysis of those characteristics presented here draws on a unique sample set that can offer a comprehensive view of this. Indeed, as far as we know, this is the first nuclear-based genetic study of wolf populations to encompass the Eurasian continent.
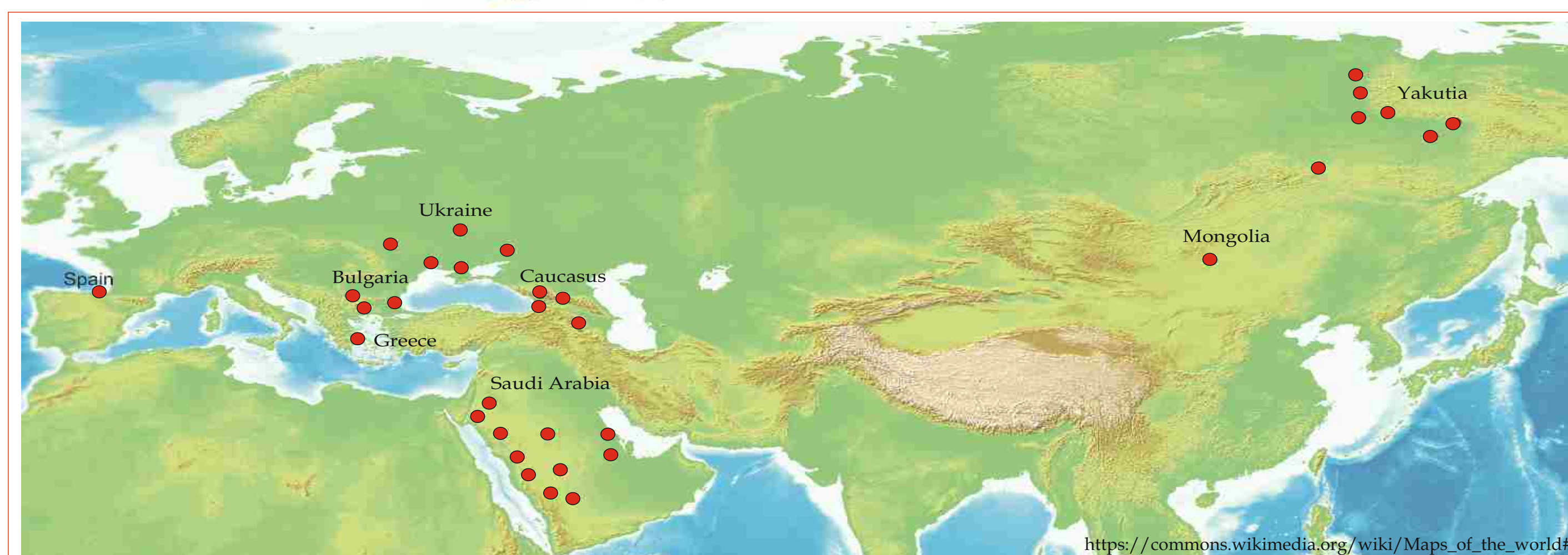

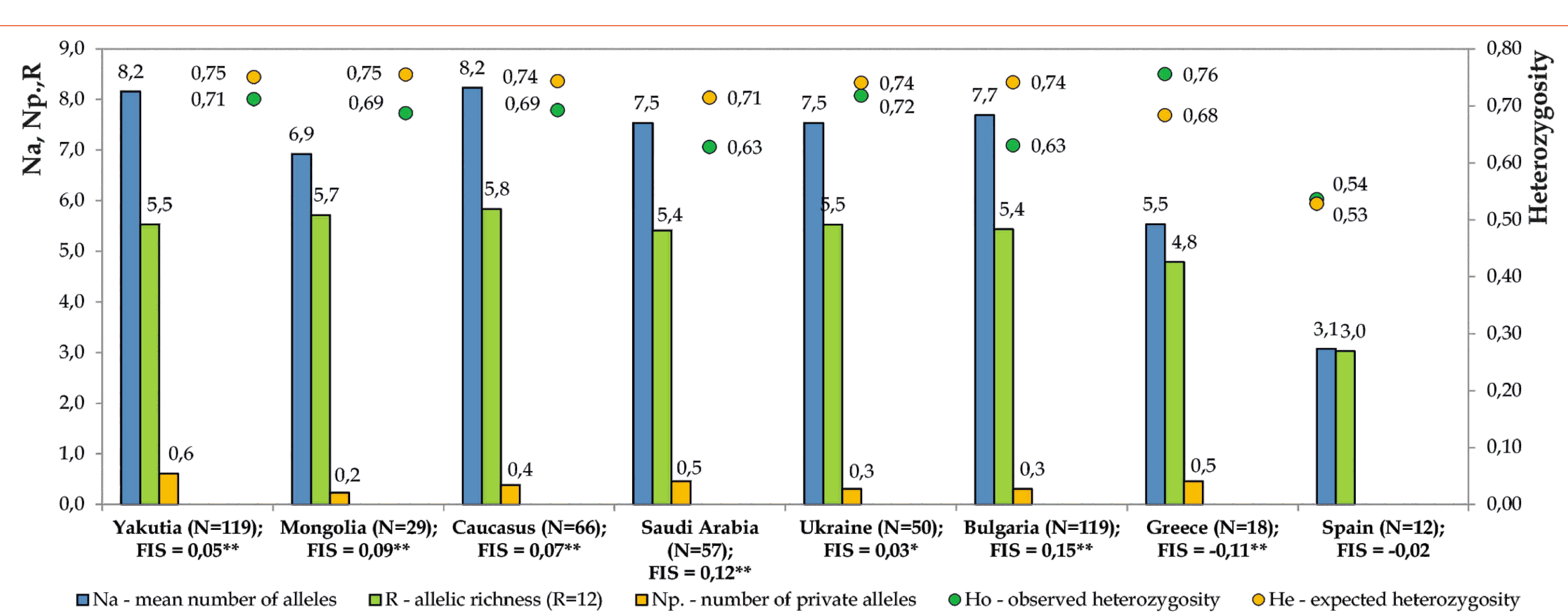**Fig. 1.** Schematic representation of the locations at which wolf populations were sampled.


**Fig. 2.** Polymorphism indices of the studied wolves populations (N=470).

## Results

⊙ allelic diversity and heterozygosity values were similar between wolf populations, except among Spain's wolves, in which they are much lower (Fig. 2)
⊙ a hierarchical structure detected was found to be conditioned geographically (Fig. 3A&B)
⊙ studied populations of wolves showed clear separation from those of golden jackals or free-living dogs (Fig. 3A)
⊙ genetic-distance values between wolves ranged from small ($F_{ST}<0.05$) to large ($F_{ST}>0.15$), but this was generally seen to be consistent with populations' geographical distributions
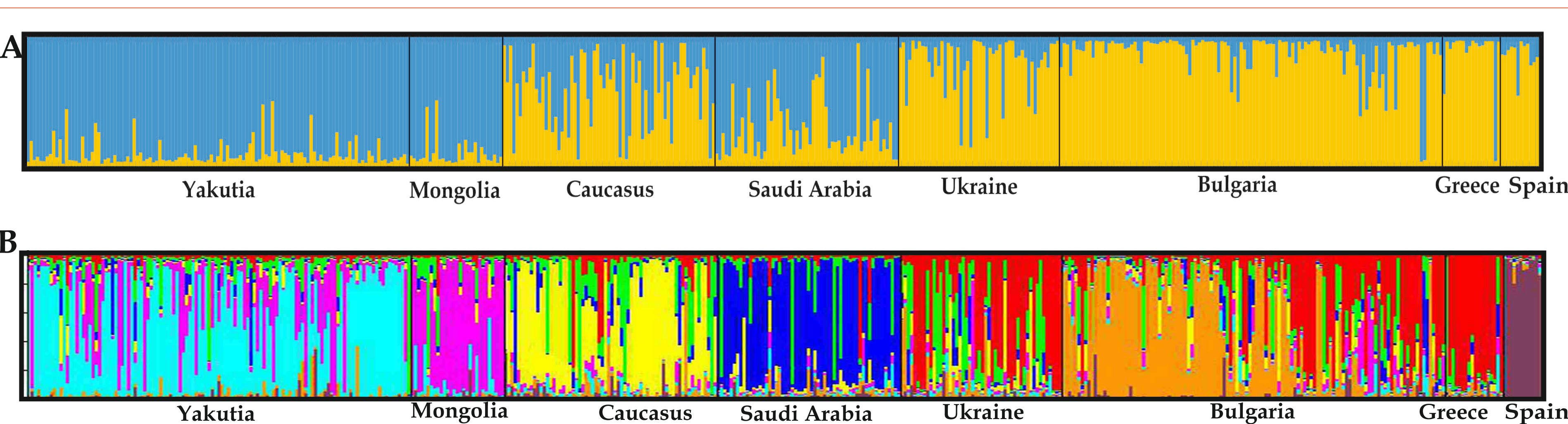

**Fig. 3A and B.** Admixture analyses performed using STRUCTURE v.2.3.4 with optimal ΔK = 2 (A) and equalling the population number used in the study — ΔK = 8 (B). Each individual is represented by a vertical bar. Colours of the bars represent the proportion of the membership coefficient (Q) characterising pre-defined clusters.


**Fig. 4.** Principal coordinates analysis (PCoA) of pairwise genetic distance ($F_{ST}$) values between the populations as pre-defined. (A) genetic distances values between wolves (N=470) and the reference populations of free-living dogs (N=80) and jackals (N=47); (B) genetic distance values between wolf populations other than in Spain.

## Discussion

The studied wolves populations:

⊙ are characterised by an exceptional situation in Spain (in relation to otherwise moderate and comparable genetic diversity), because wolves there are much less polymorphic and the populations are anyway known for being small and genetically bottlenecked [1].
⊙ split at higher levels of differentiation into Eastern and Western genetic groups, albeit with Caucasian and Saudi Arabian populations showing features of admixture.
⊙ split into distinct clusters at lower levels, except in the case of a Bulgarian population presenting substructure [3].
⊙ manifest low to moderate genetic distances indicative of extensive continental-scale connectivity, except in the small and isolated Spanish population [1], as well as in the newly-established Greek population, which most probably experienced a founder effect also visible in lower values for polymorphism.

**Tab. 1.** Genetic distance ($F_{ST}$) values between the studied wolf populations, with all values achieving statistical significance. (P<0.05).

| $F_{ST}$ | Yakutia | Mongolia | Caucasus | Saudi Arabia | Ukraine | Bulgaria | Greece |
|---|---|---|---|---|---|---|---|
| Mongolia | 0.04 | | | | | | |
| Caucasus | 0.06 | 0.07 | | | | | |
| Saudi Arabia | 0.07 | 0.08 | 0.05 | | | | |
| Ukraine | 0.06 | 0.07 | 0.04 | 0.06 | | | |
| Bulgaria | 0.05 | 0.08 | 0.04 | 0.06 | 0.03 | | |
| Greece | 0.08 | 0.12 | 0.10 | 0.12 | 0.07 | 0.05 | |
| Spain | 0.16 | 0.16 | 0.15 | 0.18 | 0.15 | 0.15 | 0.20 |

## Materials and Methods

Wolf samples were obtained in 2012–2017 from several geographical sites located in the six regions (Fig. 1) of: Yakutia, northern Russia (N=144), Mongolia (N=29), Saudi Arabia (N=85), Ukraine (N=52), The Caucasus (N=2) and Greece (N=72). The samples collected in Greece were non-invasive (faeces), while all others were of muscle or skin tissue. All samples were genotyped at 14 microsatellite loci [1-3], with one locus excluded from the final analysis. The dataset obtained was then augmented by 119 Bulgarian, 64 Caucasian and 12 Spanish sample genotypes from previous studies [1,2,3] (collected in the 2000-2012 period). Analysis for the same set of loci was likewise carried out for reference samples of free-living dogs (*C. l. familiaris*), N=80, representing the 7 regions of China (N=8), Mongolia (N=15), Russia (N=9), The Caucasus (N=15), Ukraine (N=9), Bulgaria (N=9) and Saudi Arabia (N=15) [4]; as well as golden jackals (*C. aureus*), N=47, from the four regions of The Caucasus (N=12), Hungary + Ukraine (N=10), Romania (N=9) and The Balkans (N=16) [5]. With the non-invasive samples at least two genotypes were obtained in the case of heterozygotes and three for homozygotes at each locus. Possible matches between obtained profiles for the non-invasive samples were checked.
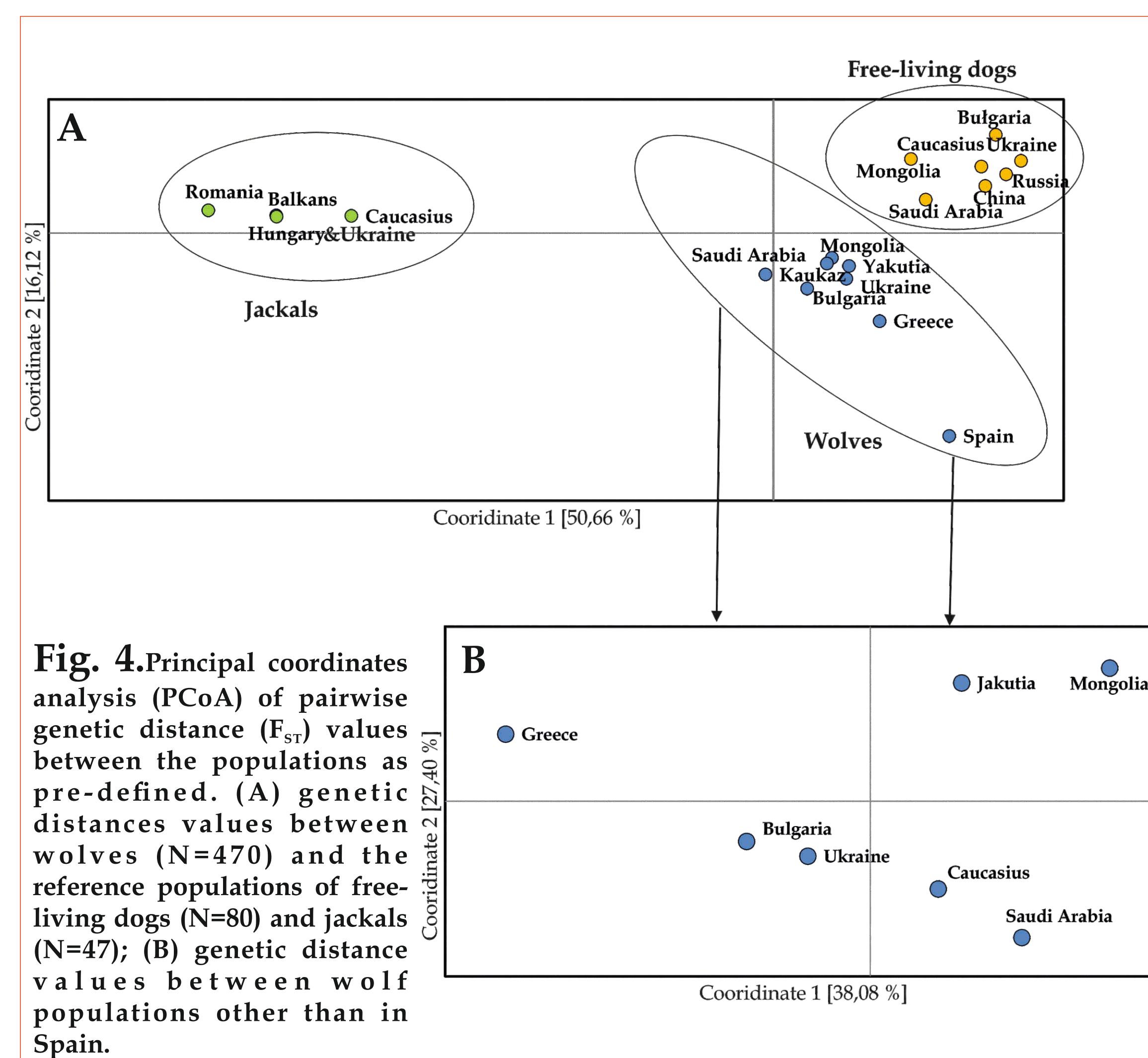
## Literature
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