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Genetic diversity of *Galeodes* populations (Arachnida:Solifugae) on Bolkar Mountains

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Abstract

In this study, genetic diversity levels of populations of *Galeodes*, distributing The Bolkar Mountains in Türkiye, was investigated using RAPD-pcr markers. A total of 29 samples (*Galeodes graecus*, *G. lycaonis, G. toelgi, Galeodes sp*. 1 and *Galeodes sp*. 2) were collected from 20 different localities and were analysed. *Gnosippus sp*. was evaluate as outgroup. It was calculated that the genetic diversity level was very high in *G. toelgi* and *G. graecus* spreading on the southern slopes, while this value reached the highest level in *G. lycaonis* on the northern slopes. This value was observed at the lowest level in *Galeodes sp*. 1 and *Galeodes sp*. 2. It was determined that the taxa which are spreading on the northern and southern slopes, were grouped on separate lines on the UPGMA trees created using the genetic distance values of Nei.

Keywords: Bolkar Mountains, *Galeodes*, RAPD-pcr, Solifugae

Introduction

Arachnid taxa spread in almost all terrestrial habitats (1, 2). The current diversity of solpugid taxa, descriptions have started since 1772, represented by 12 family, 141 genus, and over the 1111 species (3, 4). *Galeodes* includes the most polytypic taxon compared to other solpugid taxa. It members are distributed troughout Africa, Asia and Southeast Europea (5). The majority of formerly studies on solifugae taxonomy based on definitions that including small numbers of samples or only type specimen. Because of such reasons, the taxonomic status of solpugid species remains complex (6). Compared to morphological studies, there are very few studies on the molecular systematics. While some of these studies related to evolutionary relationships within Arachnida (7, 8, 9, 10, 11, 12), the rest of articles carried out for a small

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number of populations with distribution in palearctic and nearctic regions (13, 14, 15, 16).

Türkiye *Galeodes* fauna is composed of 21 species according to recent scientific records (17, 5, 18, 19). However, these scientific records and status of types include some problems, for example homonym, synonym, diagnostic mistakes etc. There are no studies on solving these problems and on the expression of genetic diversity with molecular markers.

The Bolkar Mountains where located in the south of Türkiye, is part of the Middle Taurus Mountain Ranges with high biodiversity, have many endemic species, also bearing barrier effect between the Mediterranean and Iran Turan phytogeographic regions (20, 21). Considering these unique geographical features, it was estimated that the galeodids living in the Bolkar Mountains may have been genetically differentiated. With this perspective, the hypothesize was tested by galeodid taxa distributed on the two slopes of the Bolkar Mountains may have different levels of mitochondrial genomic differentiation depending on their adaptations. The aim of this study is to investigate the genetic differences between galeodid taxa distributed in the Bolkar Mountains and to contribute to the Solifugae taxonomy by using molecular markers.

Materials and Methods

Total of 30 solpugid samples were collected from 20 localities on Bolkar Mountains between the years 2009 and 2011 (Figure 1). 29 of samples belonging to the genus *Galeodes* and an outgroup belonging to the genus Gnosippus were analyzed by random amplified polimorphic DNA technique (RAPD-pcr). 10 primers were used for *Galeodes toelgi* (n= 10), *G. graecus* (n=10), G. lycaonis (n=3), *Galeodes sp*. 1 (n=3), *Galeodes sp*. 2 (n=3) and outgroup *Gnosippus sp*. (n=1) DNA isolation was performed by adapting the procedures of Gaubert and Zenatello (22) and Üstünbaş et al. (23) from tissue pieces taken from the coxa and trochanter of the samples stored in alcohol (70%). For the PCR cycle, Kaya and Neale (24) and Üstünbaş et al. (23), it was adapted by making changes in the program. Pre-denaturation: 1 minute at 95 ºC; Denaturation: 1 minute at 94 ºC; Binding: 1 minute at 36 ºC; Elongation: 2 minutes at 72 ºC; Final elongation: 2 minutes at 72 ºC. The data set was created by giving the values of "1 or 0" to the bands formed as a result of agarose gel electrophoresis. UPGMA trees were drawn using POPGENE 3.1 and NTSYS package program.

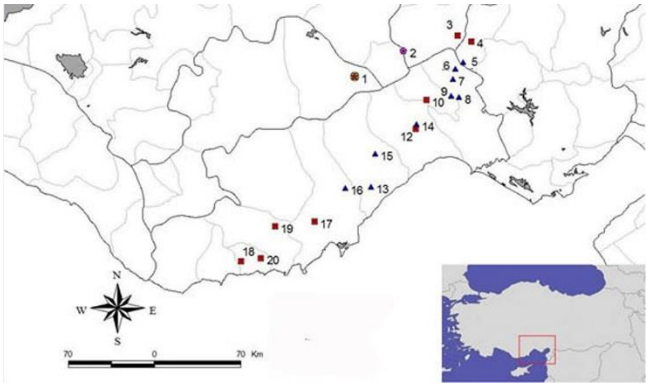

Figure 1. Sampling localities on Bolkar Mountains: 1: Karaman, 2: Konya, 3: Niğde, 4-5: Adana, 6-20: Mersin

Results

A total of 97 alleles were identified for all taxa in the 10 different RAPD primers. In the analysis performed by including the outgroup, 94 (96.91%) polymorphic loci were detected. 81 (83.51%) of polymorphic loci are belong to only genus *Galeodes*.

The lowest level of genetic differentiation value (Shannon index (I) 0.0407, H= 0.0275; Pn= 7; %P= 7.22) among the five galeodid taxa was calculated for *Galeodes sp*. 1. Although the average allele counts, polymorphic loci number and polymorphic loci percentages observed at the similar values in G. graecus and G. lycaonis (Na= 1.43; Pn= 42; %P= 43.30), but the effective allele number of G. lycaonis (Ne=1.34) is higher than that of G. graecus (Ne= 1.25). G. lycaonis has the highest genetic diversity value and Shannon index was found as 0.2654.

Table 1. Genetic diversity values of taxa

Population	N	Na	Ne	Pn	% P	H	I
Galeodes toelgi	10	1.2784	1.1720	27	27.84	0.0983	0.1463
	SDs	0.4505	0.3281			0.1765	0.2537
Galeodes graecus	10	1.4330	1.2472	42	43.30	0.1435	0.2157
	SDs	0.4981	0.3575			0.1940	0.2776
Galeodes lycaonis	3	1.4330	1.3369	42	43.30	0.1841	0.2654
	SDs	0.4981	0.4208			0.2198	0.3119
Galeodes sp. 1	3	1.0722	1.0471	7	7.22	0.0275	0.0407
	SDs	0.2601	0.1838			0.1021	0.1493
Galeodes sp. 2	3	1.0928	1.0722	9	9.28	0.0395	0.0569
	SDs	0.2916	0.2393			0.1270	0.1812
Gnosippus sp.	1	1	1	0	0	0	0
	SDs	0	0			0	0

Na: Average observed alleles number, Ne: Number of expected alleles (Kimura and Crow (1978)), Pn: Number of polymorphic loci, %P: Percentage of polymorphic loci, H: Average heterozygosity of Nei (1973), I: Shannon index , SDs: Standard deviation

G. graecus (Pn=42, The genetic diversity values of G. toelgi (Pn=27, %P=27.84, H=0.0983, I=0.1463) is lower than that of G. graecus (Pn=42, %P=43.30, H=0.1435 and I=0.2157). Genetic diversity values of *Galeodes sp*. 1 and *Galeodes sp. 2* (Pn=7, %P=7.22, H=0.0275, I=0.0407; Pn=9, %P=9.28, H=0.0395, I=0,0569 accordingly) shared lowest rates than that of others (Table 1).

Table 2. Nei's (26) genetic similarity (upper diagonal) and genetic distance (lower diagonal) values

Population	1	2	3	4	5	6
Galeodes toelgi	****	0,956	0,870	0,687	0,7235	0,4967
Galeodes graecus	0,044	****	0,870	0,708	0,7479	0,5274
Galeodes lycaonis	0,138	0,1382	****	0,7325	0,7657	0,478
Galeodes sp. 1	0,374	0,3451	0,311	****	0,768	0,5611
Galeodes sp. 2	0,323	0,290	0,267	0,263	****	0,5998
Gnosippus sp.	0,699	0,639	0,738	0,5778	0,5112	****

Based on RAPD data, Nei's (25) Hs (Expected mean heterozygosity within Populations), Ht (Total expected heterozygosity) and Gst (Genetic differentiation index) values were calculated. It was observed that Ht=0.3225, Hs=0.0821, Gst=0.7453 for six taxa. Genetic diversity of *Galeodes* were detected as Ht=0.2656, Hs=0.0986, Gst=0.6289. Number of polymorphic loci was 81, representing 83.51% of all loci.

Unbiased genetic distance and genetic similarity values were calculated In the POPGENE 1.32 package program, according to Nei (26). The genetic distance value between G. toelgi and G. graecus species was calculated as 0.044 and the genetic similarity value as 0.957. These two taxa were thought to be closely related species. However, it was observed that G. lycaonis had almost the same genetic distance (0.139 and 0.138) and similarity values (0.870 and 0.871) to G. toelgi and G. graecus species. The genetic distance value between *Galeodes sp*. 1 and *Galeodes sp*. 2 was observed as 0.263 (Table 2).

Samples of five galeodid taxa were evaluated independently by NTSYS package program. UPGMA tree was constructed using the unbiased genetic distance values of Nei (26). It was observed that the specimens belonging to G.toelgi and G. graecus were groupped as a separate line from other species on the UPGMA. Samples of both species tended to mix with each other in small groups (Figure 2). However, populations of *G. lycaonis, Galeodes sp*. 1 ve *Galeodes sp*. 2 located as separate branch on UPGMA tree.

Discussion

Phylogenetic approaches based on genetic markers are the most reliable methods used in recent years to explain Solifugae biodiversity, intraspecific and interspecific relationships and their evolution (Masta et al 2008, Arabi et al. 2012). However, detailed

molecular studies on solpugids are insufficient and most of them were carried out on taxa distributed in Palearctic and Neartic regions. (13, 27, 14, 15, 16).

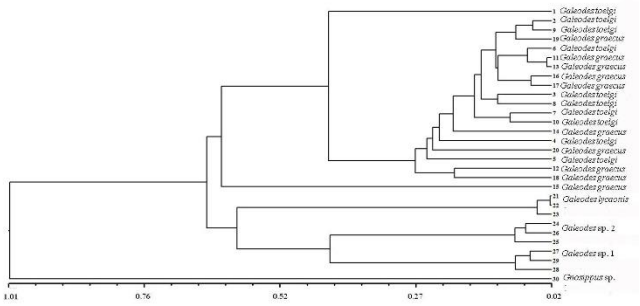

Figure 2. The UPGMA tree was created the unbiased genetic distance values, according to Nei (26).

Genetic relationships and moleculer taxonomy studies on solpugid fauna of Türkiye has been neglected for a long time. In this study, genetic diversity of galeodid fauna of Bolkar Mountains (*Galeodes toelgi, G. graecus, G. lycaonis, Galeodes sp. 1, Galeodes sp.* 2) researched using RAPD pcr analysis for the first time. The data obtained from the analysis showed that genetic diversity level was high in *Galeodes* toelgi and G. graecus, which spread throughout the Mediterranean on the southern slopes of the Bolkar Mountains. It was observed that genetic diversity was at the highest level in G. lycaonis, which spread on the northern slopes. *Galeodes sp.* 1 and *Galeodes sp.* 2 shared at the lowest levels (Table 1).

The distribution maps drawing for each taxon are examined, it was observed that G. toelgi and G. graecus on the southern slopes, G. lycaonis on the northern slopes have a wide distribution. This distributional pattern can be explain, affects allele frequency and may explain the higher rates on genetic diversity. It can be said that this distributional pattern affects allele frequency and may explain the high level of genetic diversity for these species. The low percentage polymorphic loci of *Galeodes sp.* 2, which distributes as sympatric population with *G. lycaonis* and covers a narrower area, is consistent with this view (Figure 1).

Twenty five samples belong to five taxa of the genus *Galeodes* groupped on coherent branches on the UPGMA tree. The samples of outgroup, Gnosippus sp. (Daesiidae), were diverged directly from the main line. the genus *Galeodes* was mainly divided into as two subgroups. When the taxa clustered in these subgroups are examined, one of the clusters was formed by a total of 20 samples of G. toelgi and G. graecus, which spread on the southern slopes of the Bolkar Mountains. On the other hand, the other three populations, G. lycaonis, *Galeodes sp.* 1 and *Galeodes sp.* 2, clustered on second branch and each population was grouped as separete subgroups spread on the northern slopes. In this case, it can be claim that taxa can form groups "living in the south and north" influenced by barrier effect of the Bolkar Mountains. It can be said that the geographical characters (soil structures, climate and vegetation, etc.) of the Mediterranean and Central Anatolian Regions may be effective on the evolutionary process of solpugid taxa (20, 21). Subgroups of G. toelgi and G. graecus, tended to mix on the UPGMA tree, but clustered in small groups (Figure 2). Although nineteen subgroups form small groups for both species, it can be said that there is a heterogeneous distribution in terms of locality.

Declaration of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

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