

DETERMINATION OF GENETIC RELATIONSHIPS AMONG THREE GROUND SPIDERS (ARANEAE: GNAPHOSIDAE) BY USING RAPD-PCR FROM TURKEY

Osman Seyyar*, Fahriye Sümer Ercan and Hakan Demir***

* Department of Biology, Faculty of Science and Art, Niğde University, Niğde, TURKEY. E-mail: ozyptila@gmail.com

** Department of Biology, Faculty of Science and Art, Bozok University, Yozgat, TURKEY.

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ABSTRACT: Random amplified polymorphic DNA (RAPD-PCR), was used to determine three Gnaphosidae species, in the study. RAPD-PCR was carried out by using fifty random primers each of them consisted of ten base pairs. Fourteen of these primers gave sufficiently clear and distinguishable bands. Forty eight samples that represented three Gnaphosidae species, *Drassodes lapidosus* Walckenaer, 1802, *Haplodrassus signifer* Koch, 1839 and *Nomisia ripariensis* Cambridge, 1872, were used for RAPD-PCR and they were identified by their molecular characteristics. Genetic polymorphism among these samples was recorded by UPGMA method. The aim of this study was to achieve genetic marker to clearly differentiate and determine the genetic variation of these three samples that collected from different locations of Turkey. Our results showed that RAPD-PCR is an effective, fast and simply alternative method for identification of the spider species.

KEY WORDS: *Drassodes lapidosus*, *Haplodrassus signifer*, *Nomisia ripariensis*, RAPD-PCR, genetic polymorphism

Many researchers have focused on phylogenetic and evolutionary studies of the Araneidae. Several methods have been used to describe the variation within a species of spiders. The common way of classification of spiders is based on morphological characteristics, particularly structural features of genitalia but morphology based identification of spiders such as web form, stabilimenta, genitalia, mating and predatory behaviour are problematic and time consuming (Scharff, 1997).

Gnaphosidae is one of the largest families of spiders in regard to numbers of species and genera. Up to now, it includes 2147 species and 121 genera worldwide (Platnick, 2015). According to literatures, this family is one of the most diverse and abundant families of ground spiders. They are nocturnal hunters that move very fast on the ground (Chatzaki, 2008; Seyyar et al., 2009).

The limitations of morphological, physiological and cytological markers for identifying the genetic diversity in many species have been overcome by the development of the DNA markers such as random amplified polymorphic DNAs. Polymerase chain reaction (PCR) technology allows for analysis of DNA polymorphism in many invertebrate organisms, especially systematically problematic species. RAPD is a PCR based technique that showed the genetic polymorphism by using random and short primers without any information about DNA sequences of target samples. This technique has advantages in high efficiency, easy detection, small usage of samples and relatively simple. RAPD analysis generates species specific banding profiles by using a single 10 base pair (bp) oligonucleotide primers. So, it has been widely used for systematic and genetic polymorphism studies of many organisms (Williams et al., 1990).

Also, molecular markers based on the PCR are confirmed as precious tools for ecological studies (Garipey et al., 2007). RAPD-PCR has been used for identification of spiders in recent years (Gurdebeke et al., 2000). Approximately, 44.000 spider species and subspecies are known relating to 112 families identified worldwide. Basically 46% and 1.5% of spider specifications are based on only one sex and juvenils, respectively (Platnick, 2015). For supporting the identification of known species, molecular based tecniques is most likely to be helpful for species diagnose of spiders.

Bond (2004), identified morphologically indistinguishable females of two reputed species of genus *Apomastus* by using molecular tecniques. Agnarsson (2010), used the ribosomal internal transcribed spacer ITS2 for the phylogenetic analysis of *Anelosimus* species, however it was found that ITS2 had an inadequate variation within species and among closely related species. Defining the genetic variability of Mexican populations of *Brachypelma vagans* Ausserer, 1875, seven ISSR (Inter Simple Sequence Repeat) primers were used and four of these primers gave sufficiently clear and reproducible bands (Machkour-M'rabet et al., 2009). Not only DNA markers, but also enzymatic studies were done by distinguishing the relationship between spider species. Twelve gene loci from ten enzymes of *Phidippus* species were analyzed by using poliacylamide gel electrophoresis (Terranova & Roach, 1987). Baert et al. (2008) investigated the allozyme characterization of *Hogna* species and they found a highly similar allele frequencies within species. Due to the results, Bond et al. (2001) suggested that morphology based identification was the most conservative approach. Consequently, the combination of morphological and molecular datas will allow the most accurate identification of species (Gibss, 2009).

The aim of our study is to evaluate RAPD-PCR as a molecular marker system for taxonomic studies of three spider species of the Gnaphosidae. We use this technique for getting inferences about phylogenetic relationship among *D. lapidosus*, *H. signifer* and *N. ripariensis*.

MATERIALS AND METHODS

Sample vouchering

Specimens were collected from Central and South of Turkey (Fig. 1). Also, some of samples were obtained from NUAM (Nigde University Museum of Arachnology) (Table 1).

DNA extraction and quality

Ethanol-preserved specimens were used for DNA extraction. Total genomic DNA was extracted from one or two legs of each spider specimen. Legs were removed with a clean forceps and rest of the body was stored at -20 °C as voucher specimen. Legs of each spider were placed in a 1.5 ml eppendorf tube and Qiagene DNeasy kit were used for extraction following the manufacturer's protocol. The concentration of the DNA obtained was determined by UV spectrophotometer, we quantified the concentration of DNA in 48 extracts (19 *D. Lapidus*, 19 *H. signifer* and 10 *N. ripariensis*).

RAPD-PCR Analysis

The RAPD analyses were performed using fifty oligomers obtained from Bio Basic Inc.. Fourteen of these primers gave sufficiently clear and distinguishable bands. The sequences of these oligomers are shown in Table 2. Samples were amplified with arbitrary primers in a total volume of 15µl and contained 2µl DNA template, 1.5µl PCR buffer (10X buffer with (NH₄)₂SO₄, Fermentas), 0.5µl dNTPs (10mM stock solution), 2µl random primer (10µM, Bio Basic Inc.), 0.25µl Taq

Polymerase (5 u/μl, Fermentas), 1.2μl MgCl₂ (25mM stock solution, Fermentas), 1.5μl BSA (10mg/ml) and 6.05μl of sterile distilled water. A negative control that contains water instead of template was included in each reaction set. The temperature profile for the RAPD-PCR was a pre-denaturing step of 2.5 min at 94 °C, followed by 35 cycles of 45 s at 94°C, 1.45 min at 35°C and 1.00 min at 72°C, with a final extension step of 7 min at 72°C. PCR amplification was carried out in a Thermal Cycler (Applied Biosystems). Following amplification, the PCR products were electrophoresed in a Tris-Asedic Acid-EDTA buffer by 1% agarose gel for 1.5h at 80V. The DNA was stained with ethidium bromide (0.1μg/ml) and the bands were photographed under UV light. The results were captured using Quantum- ST4 1100/20M image analysis system.

The banding patterns of the samples were scored for the presence (1) or absence (0) of each amplified band. Data were then converted to a distance matrix and a dendrogram was constructed with the UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) method (Sneath & Sokal, 1973), using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System) (version 2.0) (Rohlf, 1988).

RESULTS

In our study fifty random primers were selected for a band sharing analysis of 48 Gnaphosidae species that contain *D. lapidosus*, *H. signifer* and *N. ripariensis* from different localities of Turkey. Fourteen primers gave scorable bands and a total of 64 RAPD bands were scored. Fourty of these bands were polymorphic.

Genetic distances were calculated among 48 Gnaphosidae samples that represent *D. lapidosus*, *H. signifer* and *N. ripariensis* from the RAPD data by using NTSYS-pc (Numerical Taxonomy and Multiware Analysis System) (version 2.0) (ROHLF, 1988). While the lowest intra-species genetic distance was 40% between *H. signifer* and *N. ripariensis*, the highest was 73% between *H. signifer* and *D. lapidosus*.

According to the dendrogram that was constructed with the UPGMA, *D. lapidosus*, *H. signifer* and *N. ripariensis* were clearly seperated from each other (Fig. 2). It has been seen that there isn't any difference about genetic features between the population of *H. signifer* which was sampled from six different population and two areas, so this regional varieties hasn't caused huge differences on population. In the species of *N. ripariensis* which was sampled from different population in Mediterranean Region has been seen the same results. However when we examined the species of *D. lapidosus* which was sampled from six different population from Central Anatolia and Mediterranean Region, it has been seen that there are significant differences in the population of these two regions as different from other two species.

Especially the effectiveness of the elevation difference has been thought in this distinction. It was seen that the population living between 250-600 meters makes a group and the populations living over 1000 meters makes different groups according to the elevation difference (Table 1). It has been marked that especially the population of Melendiz Mountain in Niğde and the population of Erciyes Mountain in Kayseri are separated from each other significantly, and the population of Erciyes is more similar to the population of Mediterranean Region than the population of Melendiz Mountain. This situation showed that the gene flow between Central Anatolia and Mediterranean Region populations of *D. lapidosus* is on the highest point of Toros Mountains and this gene flow is

provided by tunnels in the North of Adana (Saimbeyli, Feke Kezan) and in Kahramanmaraş either than the tunnel of Gülek.

Moreover, it was examined that there are small differences between the main population and the samples taken from the same population of Central Anatolian Region especially the sample species of D11 (Melendiz Mountain in Niğde) and D15 (Erciyes Mountain in Kayseri) because of the elevation differences in mountainous regions.

DISCUSSION

Geographic features of an area that populations live have an effective role on the occurrence of the genetical differences between species. RAPD-PCR is a powerful and technically accessible tool for clarifying the systematics of closely related and uncharacterized species (Wilkerson et al., 1993).

In recent years, DNA markers have widely used in molecular studies of genetic relatedness, phylogeny and population dynamics (Loxdale & Lushai, 1998). RAPD technique has been successfully used to detect genetic variation and for taxonomic studies. This technique doesn't require any prior information about specific sequences, so it became a useful method for classification studies.

Many researchers used RAPD-PCR for determining genetic variation among species; Black et al. (1992) differentiated four aphid species by RAPD-PCR. Chapini et al. (1999) determined four genetically distinct *Anagrus* species by same method. Using RAPD markers Aljanabi et al. (1998) showed genetic variability in stink bug egg parasitoids. Genetic polymorphism in two *Trichogramma* species were detected by RAPD-PCR (Ercan et al., 2012). A'hara et al. (1998), determined RAPD profiling of tree spider species, *Lepthyphantes tenuis* Blackwall, 1852, *Enoplognatha ovata* Clerk, 1757 and *Clubiona reclusa* Cambridge, 1863, members of the Linyphiidae, Theridiidae and Clubionidae, respectively. They demonstrated that this technique can easily used and gaved repeatable results for arachnological studies.

In our study, samples of ground spiders, taken from Central Anatolia and Mediterranean region are evaluated, it was indicated that the population of *H. signifer* and *N. ripariensis* were spread out in both region homogeneously. Regional differences didn't cause so big differences on populations, however the population of *D. lapidosus* was separated from the population of Central Anatolia and Mediterranean Region significantly and this separation was mainly the result of elevation differences.

Our results supported the usage of RAPD-PCR for detecting the genetic variability of different ground spiders. This method was used for the first time in systematic studies on the spider in Turkey.

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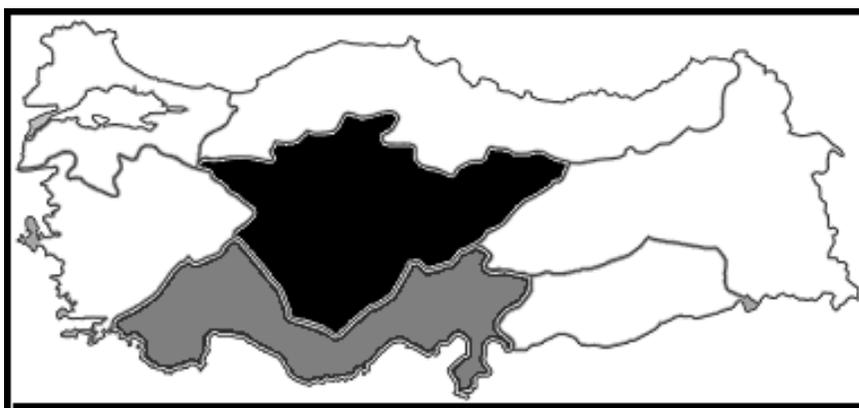


Figure 1. Study area. Black: Central Anatolia Region, Grey: Mediterranean Region.

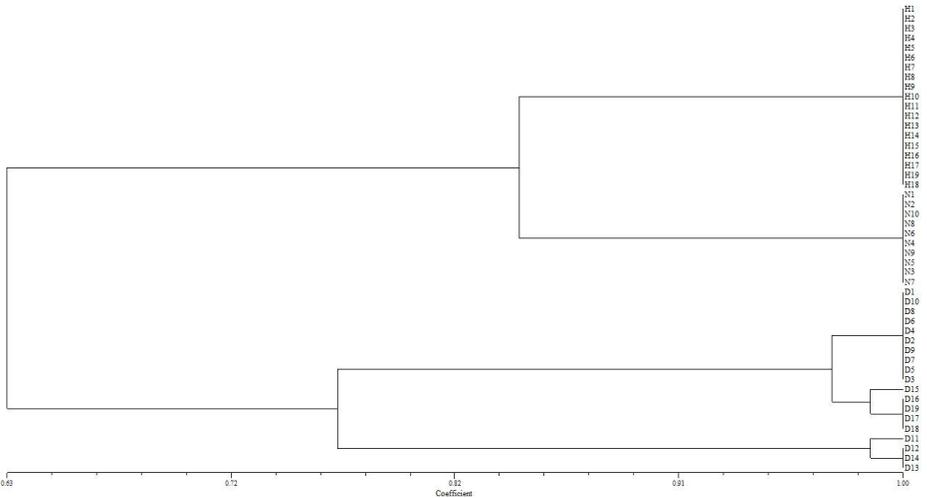


Figure 2. Phylogenetic tree constructed on the basis of UPGMA among three spider species, *D. lapidosus*, *H. signifer* and *N. ripariensis*, using data of RAPD-PCR.

Table 1. Location knowledges of species.

Code	Species	Region in Turkey	Altitude (m)	Location
D 1	<i>D. lapidosus</i>	Mediterranean	587	Osmaniye Boğaz plateau
D2				
D3				
D4				
D5	<i>D. lapidosus</i>	Mediterranean	252	Mersin, Çamlıyayla
D6				
D7				
D8	<i>D. lapidosus</i>	Mediterranean	611	Kahramanmaraş, Andırın
D9				
D10				
D11	<i>D. lapidosus</i>	Central Anatolia	1534	Niğde, Melendiz mountains
D12				
D13				
D14				
D15	<i>D. lapidosus</i>	Central Anatolia	1765	Kayseri, Erciyes Mountain
D16				
D17				
D18	<i>D. lapidosus</i>	Central Anatolia	1146	Konya, Seydişehir
D19				
D 1	<i>H. signifer</i>	Mediterranean	1581	Kahramanmaraş, Göksun, Püren Pass, Andırın
D2				
D3				
D4				
D5	<i>H. signifer</i>	Mediterranean	652	Kilis, Polateli
D6				
D7				
D8	<i>H. signifer</i>	Mediterranean	823	Adana, Pozantı, Belededik
D9				
D10				

D11	<i>H. signifer</i>	Central Anatolia	1534	Niğde, Melendiz Mountains
D12				
D13				
D14				
D15	<i>H. signifer</i>	Central Anatolia	1208	Konya, Akseki
D16				
D17				
D18	<i>H. signifer</i>	Central Anatolia	1052	Kayseri, Erkilet
D19				
N1	<i>N. ripariensis</i>	Mediterranean	917	Kahramanmaraş, Pazarçık
N2				
N3				
N4				
N5	<i>N. ripariensis</i>	Mediterranean	598	Mersin, Çamalan
N6				
N7				
N8	<i>N. ripariensis</i>	Mediterranean	450	Hatay, Hassa, Akbez
N9				
N10				

Table 2. RAPD primers gaved polymorphic bands in RAPD-PCR of spider species.

No	Sequence (5'→3')	%GC
S34	TCT GTG CTG G	60
S39	CAA ACG TCG G	60
R4	CTG ATC GCG G	70
R5	CAG GCC CTT C	70
R6	GTT GCG ATC C	60
R8	AGC GTC TGT G	60
R12	TAC GTA CGT C	50
R17	TTG ACA GTA A	50
R26	CCG ATA CCA G	60
R28	CGA TCA GCT C	60
R39	GCT AGA ATC G	50
R43	CGC TCG TCG T	70
R47	GAT AGG GAT G	50
R49	CAG CTG GCT C	70