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Molecular diversity and phylogeography of *Andricus curtisii* (Hymenoptera, Cynipidae) in Turkey

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ABSTRACT

A 433-bp fragment of the mitochondrial cytochrome *b* gene and the entire nuclear ITS2 region were sequenced in an oak gall wasp species, *Andricus curtisii*, which represents 21 populations across its range in Turkey. Forty cytochrome *b* haplotypes and 63 ITS2 alleles were discovered among the 161 individuals studied. Remarkable genetic variation was detected in the species, with an average haplotype diversity (h) of 0.72 and 0.76 and an average nucleotide diversity (π) of 0.06 and 0.01 for the *cyt b* gene and the ITS region, respectively. Most populations were genetically distinct, possessing unique cytochrome *b* haplotypes. Maximum likelihood and Bayesian phylogenetic analyses and application of the haplotype network to both datasets revealed that the most basally located haplotypes/alleles were from the eastern part of Turkey. The detection of older sequences in the phylogenetic trees of the easterly located populations implied an eastern origin of the species. The application of a conventional insect mitochondrial DNA clock to the dataset suggested a splitting of ingroup haplotypes from the outgroup lineages predating the Pleistocene epoch and the formation of two major haplogroups that coincided with the oscillation of the early Pleistocene glaciations.

1. Introduction

In the last several decades, much information has accumulated regarding the phylogeographic patterns of species, and this evidence has revealed that current genetic structures are shaped by past or present factors (Avise, 2000). Among historical factors, past climatic fluctuations during the Pleistocene epoch have been widely recognized as influential factors in the current spatial distribution of species and in greatly altering the contemporary genetic variations of many Palearctic species (Hewitt, 2004).

Located in the Palearctic, Turkey is a biodiversity hotspot that has received considerable attention from a biogeographic point of view. Owing to its unique location at the crossroads of Europe, Asia and Africa, its biodiversity includes representative species of the Caucasus, Irano-Anatolian, and Mediterranean regions (Kosswig, 1955). In addition to its unique tectonic and paleoclimatic history, Turkey has diverse climates, ecosystems and microhabitats even within short geographic distances. Moreover, varied landscapes, including a wide elevation range and highlands that roughly divide Anatolia into eastern and western regions, are thought to have shaped the phylogeographic structure of many plant and animal taxa distributed in Turkey. The mountain line running from the northeast to the Mediterranean Taurus Mountains, known as the Anatolian Diagonal (Davis, 1971), has been shown to act as either a geographic barrier or a distribution corridor, thus contributing to the

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shaping of the composition of contemporary species in the southwest Palearctic (Çıplak et al., 1993). As a consequence, Turkey harbors many plant and animal species in addition to endemics and with respect to the species examined to date, Turkey harbors considerable genetic diversity compared to Europe (Şekercioğlu et al., 2011).

There is now growing phylogeographic evidence indicating that Anatolia played an important role in the recolonization of Europe, particularly after the Last Glacial Maximum (Korkmaz et al., 2014), and acted as a refugium during climatic oscillations (Çıplak et al., 2015). Several studies have pinpointed the significance of Anatolia, from which several oak gall wasp lineages spread westward, indicating an eastern origin from Turkey and Iran (Challis et al., 2007). Although the phylogeography of several Anatolian taxa supports its significance as an important source of species and genetic variation in European populations, detailed studies are still needed to elucidate the genetic variation present within the distribution range of species in Turkey. Hence, range distributions of lineages or genetic structuring of individual oak gall wasp species would shed additional light on the biogeography of Turkey.

Andricus curtisii (Müller, 1870), common to eastern Mediterranean, is an oak gall wasp species that has been reported from southern Italy to Iran (pers. comm. with George Melika). It is an obligate parasite, inducing its unilocular asexual generation galls on accessory buds on the trunk and well-established branches of *Quercus infectoria*. Adults emerge from the galls in late summer and early fall. This study aimed to i) reveal the intraspecies genetic variation across the distribution range of *A. curtisii* in Turkey, ii) assess the phylogeographic structure of *A. curtisii* in Turkey, and iii) investigate whether glaciations and topography influenced the present distribution of genetic variation among Turkish *A. curtisii* populations.

2. Materials and methods

2.1. Sampling and molecular procedures

Sampling locations were chosen to cover the entire distribution range of *A. curtisii* in Turkey. However, despite all efforts, either one or no gall specimens were found in some locations. In total, 161 individuals from 21 populations were collected between 2007 and 2013, covering much of the range of the species across Turkey (Table 1, Fig. 1). After rearing adults from the galls, extraction of the total genomic DNA was performed with commercial kits (DNeasy Tissue Kit, QIAGEN, Inc.) according to the manufacturer's instructions for insect DNA isolation. A 433-bp fragment of the mitochondrial cytochrome *b* (*cyt b*) gene was amplified using the CB1/CB2 primer pair (Jermin and Crozier, 1994), and the entire internal transcribed spacer 2 (ITS2) region was amplified using the ITS2-F/ITS2-R primer pair (Ji et al., 2003). All PCR experiments were performed using a PTC-200 DNA Engine thermal cycler (MJ Research). The amplification protocol for the *cyt b* fragment was performed as previously described by Stone et al. (2007). The PCR products were sent to a company (Macrogen) for purification and sequencing. To minimize PCR artifacts, ambiguities and base-calling errors, both strands were sequenced, and the haplotypes were deposited in GenBank (Accession numbers KT447045–KT447084 for the *cyt b* haplotypes and KT447085–KT447147 for the ITS2 alleles).

Table 1
Names of sampling sites with their coordinates, abbreviations, and number of sequenced individuals, haplotype (*h*) and nucleotide (π) diversity of each population shown for the *cyt b* gene and ITS2 region separately.

Population	Abbreviation	Coordinates	Sample size	*Cyt b*		ITS2	
				h	π	*h*	π
Siirt	SII	38° 13.78′N; 41° 75.79′E	10	0.5333 ± 0.0947	0.004927 ± 0.003378	0.8444 ± 0.1029	0.013841 ± 0.008212
Batman	BAT	38° 16.05′N; 41° 60.94′E	1	1.0000 ± 0.0000	0.000000 ± 0.000000	1.0000 ± 0.0000	0.000000 ± 0.000000
Bingöl	BIN	38° 92.85′N; 40° 36.79′E	5	1.0000 ± 0.1265	0.010624 ± 0.007325	0.6000 ± 0.1753	0.005955 ± 0.004516
Elazığ	ELA	38° 53.90′N; 38° 75.53′E	14	0.6703 ± 0.1262	0.006928 ± 0.004317	0.6923 ± 0.0652	0.011943 ± 0.006964
Erzincan	ERZ	39° 44.46′N; 38° 42.94′E	12	0.8788 ± 0.0751	0.011687 ± 0.006870	0.5909 ± 0.1079	0.007783 ± 0.004881
Malatya	MAL	38° 72.19′N; 38° 01.83′E	4	0.5000 ± 0.2652	0.004619 ± 0.003875	1.0000 ± 0.1768	0.024400 ± 0.016948
Adıyaman	ADI	37° 72.80′N; 37° 95.22′E	12	0.8636 ± 0.0786	0.011337 ± 0.006687	0.8333 ± 0.0691	0.020528 ± 0.011527
Sivas	SIV	39° 40.85′N; 38° 34.59′E	8	0.7500 ± 0.1391	0.006763 ± 0.004507	0.8571 ± 0.1083	0.017015 ± 0.010211
Kahramanmaraş	KAH	37° 46.76′N; 37° 44.10′E	8	0.7857 ± 0.1127	0.785700 ± 0.112700	0.9286 ± 0.0844	0.022687 ± 0.013316
Konya	KON	37° 85.40′N; 33° 80.04′E	10	0.6444 ± 0.1012	0.010675 ± 0.006468	0.7556 ± 0.1295	0.013455 ± 0.008007
Kırıkkale	KIR	40° 10.19′N; 33° 71.83′E	3	1.0000 ± 0.2722	0.013857 ± 0.011314	0.6667 ± 0.3143	0.001654 ± 0.002063
Ankara	ANK	40° 17.30′N; 31° 30.37′E	2	1.0000 ± 0.5000	0.018476 ± 0.019596	0.0000 ± 0.0000	0.000000 ± 0.000000
Isparta	ISP	37° 74.88′N; 31° 18.74′E	4	0.6667 ± 0.2041	0.009238 ± 0.006943	0.6667 ± 0.2041	0.001654 ± 0.001856
Eskişehir	ESK	39° 79.32′N; 30° 29.90′E	4	0.6667 ± 0.2041	0.001540 ± 0.001728	0.6667 ± 0.2041	0.016543 ± 0.011805
Afyon	AFY	38° 46.62′N; 30° 41.38′E	1	1.0000 ± 0.0000	0.000000 ± 0.000000	1.0000 ± 0.0000	0.000000 ± 0.000000
Kütahya	KUT	39° 52.19′N; 30° 05.66′E	12	0.6818 ± 0.1019	0.004584 ± 0.003130	0.9394 ± 0.0577	0.019024 ± 0.010746
Denizli	DEN	38° 35.48′N; 29° 74.00′E	14	0.5824 ± 0.1372	0.009796 ± 0.005802	0.4396 ± 0.1120	0.005454 ± 0.003597
Uşak	USK	38° 61.72′N; 29° 65.78′E	10	0.2000 ± 0.1541	0.004619 ± 0.003209	0.8444 ± 0.0796	0.011470 ± 0.006950
Manisa	MAN	39° 03.36′N; 27° 92.34′E	12	0.4091 ± 0.1333	0.000945 ± 0.001048	0.9545 ± 0.0569	0.008159 ± 0.005079
Çanakkale	CAN	39° 87.37′N; 26° 19.54′E	1	1.0000 ± 0.0000	0.000000 ± 0.000000	1.0000 ± 0.0000	0.000000 ± 0.000000
Edirne	EDI	41° 26.26′N; 26° 46.42′E	14	0.3846 ± 0.1494	0.3846 ± 0.14940000	0.7912 ± 0.0673	0.008971 ± 0.005431
Average	–	–	–	**0.7246 ± 0.0141**	**0.0619 ± 0.0170**	**0.7653 ± 0.1007**	**0.0100 ± 0.0062**